WITHIN-GENERATION AND TRANSGENERATIONAL THERMAL PLASTICITY IN COLD-ADAPTED SALMONIDS OF THE GENUS *SALVELINUS*

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ABSTRACT

Within-generation and Transgenerational Thermal Plasticity in Cold-Adapted Salmonids of the Genus Salvelinus

Chantelle M. Penney

Climate change is a major conservation concern, especially for many cold-adapted species. The rate of warming due to climate change will likely outpace adaptive responses, and many populations will likely need to rely on phenotypic plasticity to cope with environmental warming. It is currently unclear whether plasticity in physiological responses to warming will be sufficient to offset the negative consequences of chronic environmental warming in ectotherms. I studied within-generation and transgenerational plasticity in two cold-adapted species of fishes, lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*), following temperature acclimation. Adults of both species were acclimated to either cold or warm temperatures and offspring were generated using a fully factorial breeding design, whereby the family thermal histories included crosses made within each temperature treatment and bidirectional crosses between temperatures. Offspring families were subdivided into two groups and acclimated to either warm or cold temperatures, so that offspring thermal experience matched or mismatched that of one or both parents. Offspring metabolic rate and critical thermal maximum during an acute thermal challenge were measured for both species. Limited transgenerational plasticity was detected in both species, but had a lesser effect than within-generation

acclimation. In brook trout, the paternal contribution was greater than the maternal contribution. In lake trout, a mismatch in thermal acclimation, where the offspring were cold-acclimated but the parents warm-acclimated, resulted in elevated offspring metabolic rate without a corresponding increase in growth, suggesting that a mismatch in temperatures across generations could be detrimental to offspring. Using RNA-sequencing, transgenerational plasticity was linked to differential gene expression in the liver of lake trout offspring, in that genes were differentially expressed depending on the parental acclimation temperatures. Within-generation warm acclimation had the greatest effect on gene expression profile of offspring, with more genes differentially expressed under conditions of within-generation warm acclimation compared with transgenerational warm acclimation. Although it has been suggested that transgenerational plasticity may help to buffer the impact of warming due to climate change, my work implies that transgenerational plasticity, like within-generation plasticity, will be insufficient for these two species of cold-adapted salmonids to cope with climate change.

KEYWORDS

Brook trout, lake trout, climate change, thermal tolerance, phenotypic plasticity, transgenerational plasticity, acclimation

PREFACE

The data chapters in this thesis are written in the style of an academic paper. Chapters 2 and 3 have been published in *Conservation Physiology* (volume 9) and *Physiological and Biochemical Zoology* (volume 95), respectively. At the time of writing, Chapter 4 has been submitted for publication. The pronoun “we” is used in these three chapters because authorship of these published papers included multiple people, without whom the work would not have been possible.

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LIST OF ABBREVIATIONS

CTM……..……..Critical thermal maximum

MO_2…….…….Rate of oxygen consumption

O_2……...……Oxygen

TGP…….……..……Transgenerational plasticity

WGP……..Within-generation plasticity

Chapter 1: General Introduction

Anthropogenic climate change is altering environmental parameters all over the world (Malhi et al. 2020; Jane et al. 2021). Temperature has been dubbed the 'ecological master factor' given its influence on a species' geographical distribution, population size and behaviour (Brett 1971), so the rise in temperature associated with climate change can be a particularly powerful abiotic factor. Warming associated with climate change can have largescale, ecological ramifications, such as a disruption in food web dynamics due to phenological shifts, compromised reproductive success or recruitment, and increased heat-related mortality threatening the survival of populations worldwide (IPCC 2022; Guzzo and Blanchfield 2017; Pérez-Ruzafa et al. 2018). An increase in environmental temperature towards physiological limits can have profoundly detrimental impacts on individuals, leading to impaired immune function, growth and reproduction (Cook et al. 2004, Wang and Overgaard 2007). Habitat degradation and loss of biodiversity due to climate change will continue to adversely affect ecosystems, and human populations are not exempt from these impacts (IPCC 2022).

Responses to climate change

Biological responses to environmental changes can occur at multiple levels of organization, from communities and species down to individuals within populations, and can incorporate distributional shifts, behavioural, morphological and physiological plasticity, and evolutionary adaptation. Species or population-level responses could include range expansion to track suitable environmental temperatures, often resulting in poleward or altitudinal movements of populations (Chen et al. 2011; Comte et al. 2013;

IPCC 2022). Movements are largely driven at the individual level but can eventually result in population-wide shifts with individuals pushing the distribution's leading edge forward and individuals at the trailing edge potentially perishing (Lenoir et al. 2015). Most observations of range shifts have been made in Europe, with North American studies lagging behind (Lenoir et al. 2015), however, range shifts due to warming have been observed in Canada (Lynch et al. 2016). In Ontario, Canada, freshwater fishes have been experiencing both range contractions and range shifts with some predator species moving northwards at a rate of 11.6-83.6 km per decade (Wu et al. 2022).

Population-level adaptation to chronic environmental changes can occur through selection for traits that improve fitness under the altered conditions (Hoffmann and Sgro 2011; Comte and Olden 2017). This occurs over generational (Stockwell et al. 2003) or evolutionary timescales (Hoffman and Sgro 2011), as long as there is sufficient functional genetic variation present within a population upon which selection can act (Stockwell et al. 2003, Willi et al. 2006, Comte and Olden 2017). Contemporary evolution (on the scale of tens of generations to a few hundred years) may be possible for some populations (Stockwell et al. 2003), depending on standing genetic variation at genes underlying adaptive traits (Hoffmann and Sgro 2011). Populations with low genetic variation in fitness-related traits may need to rely on the introduction of new traits through genetic mutation or through gene flow from other populations, either naturally by immigration or through conservation management (Hoffmann and Sgro 2011). However, whether adaptation occurs is also contingent on local effective population size and whether the fitness differential is sufficient to confer a net benefit to individuals expressing the adaptive trait (Crozier and Hutchings 2014).

The rate of climate change is quickly outpacing the rate of adaptation for many populations (Willi et al. 2006, Comte and Olden 2017), and some species and populations will be limited in their ability to cope with climate change. For dispersal-restricted taxa such as freshwater fishes, distributional shifts are often not a viable option for tracking suitable habitat, especially for arctic and sub-arctic populations that are already near the northern limit of their habitat. Contemporary evolution is unlikely for populations of species with longer generation times, and geographically isolated populations do not have access to the potential benefit of gene flow (Stockwell et al. 2003; Hoffmann and Sgro 2011). Many populations will likely be limited to their existing physiological tolerances and adaptive genetic resources to cope with climate change, but plenty of uncertainty remains regarding if and how plasticity will aid populations in resisting the negative impacts of climate change (Hendry 2016; Donelson et al. 2018).

Plasticity within- and across generations

Phenotypic adjustments, including morphological, behavioural, or physiological changes, can aid survival in variable environments. This ability to express multiple phenotypes to suit environmental changes from a single genotype is referred to as phenotypic plasticity (Somero 2010; Schulte et al. 2011). These phenotypic adjustments can be achieved through up- or downregulation of genes, thus altering the amount of mRNA available for translation, or through epigenetic modification or alternative splicing (Best et al. 2018; Healy and Schulte 2019). Reversible plasticity (cf. developmental plasticity; Skúlason et al. 2019) can occur over relatively short time periods (minutes to weeks, depending on the organism), allowing an individual to maintain fitness when the

environment changes (Somero 2010; Bates and Morley 2020). For example, a 4-week acclimation period to warmer temperatures led to changes in the fatty acid composition of the inner mitochondrial membrane in the liver of killifish (*Fundulus heteroclitus*) allowing for maintenance of bioenergetic homeostasis in warmer temperatures (Chung et al. 2018).

Phenotypic plasticity is expected to have evolved in populations that experience environmental variation (Beaman et al. 2016; Hendry 2016), such as for those living in temperate (seasonal) latitudes. The capacity for trait plasticity does not always correlate with thermal habitat (Comte and Olden 2017), but a population's environmental history generally corresponds to their ability to respond phenotypically to environmental variability, especially when the cues that signal environmental change are reliable and the cost for production and maintenance of plasticity is low (Hendry 2016; Fox et al. 2019; Burton et al. 2022). Plasticity is generally thought to be beneficial (or adaptive) in allowing organisms to maintain fitness by expressing or chasing an optimal phenotype under changing environmental conditions. However, plastic responses may not always be adaptive or beneficial (Hendry 2016). Expressing a phenotype that does not match the environment could result in a fitness cost to the organism (Auld et al. 2010). Further, an organism may correctly anticipate the direction of the environmental change, but the required phenotypic adjustment could exceed the organism’s capacity to fully meet requirements for the new environment. In this case, the phenotypic change could lead to a waste of resources (e.g., energy) through chasing an optimum that is not fully achieved and that does not sufficiently benefit the organism in the new environment (Auld et al. 2010).

Plasticity can occur during an organism's lifetime (within-generation plasticity) or across multiple generations (transgenerational plasticity). Transgenerational plasticity (also referred to as TGP or transgenerational acclimatization/acclimation) describes a mode of non-genetic inheritance whereby plastic responses occur over multiple generations, meaning that the offspring's phenotypic response to an environmental change depends in part on the environmental experiences of its parents or grandparents (Bell and Hellmann 2019; Bonduriansky 2021). Transgenerational plasticity can include parental effects, such as egg provisioning, transfer of hormones or sperm cytoplasmic components (Crean and Bonduriansky, 2014; Jonsson and Jonsson 2016; Sopinka et al. 2017; Kekäläinen et al., 2018). Transgenerational plasticity can also occur through epigenetic control of gene expression whereby the offspring inherit epigenetic factors from their parents (Greenspoon and Spencer 2018). Epigenetics refers to the molecular factors that act on the genome, including modifications in histone configuration, DNA methylation, expression of microRNA or changes in chromatin structure (Hanson and Skinner 2016; Ord et al. 2020), each of which can be induced by changes in the environment. For example, temperature can have the effect of hyper-methylating genetic material in the ovaries and testes of fish (Fellous et al. 2021) and this can be carried over into the genome of the offspring (Jiang et al. 2013). This was shown recently in brook trout (*S. fontinalis*), where warm acclimation of parents increased the amount of differentially methylated regions in their offspring compared to within-generation warm acclimation of those offspring (Venney et al. 2020).

Transgenerational plasticity is predicted to evolve when the environment varies over a span of time that exceeds the life cycle of the organism, with a reliable cue that

accurately signals the offspring's (future) environment (Leimar and McNamara 2015; Lind and Spagopoulou 2018; Colicchio and Herman 2020). Transgenerational plasticity can persist after multiple generations (Cayuela et al. 2019; Lee et al. 2020) but can also wane over time if the stressor no longer persists (Burton et al. 2021). Transgenerational plasticity can be beneficial if parents correctly anticipate their offspring's environment and can precondition their offspring for those predicted environmental conditions (Bonduriansky et al. 2012; Beaman et al. 2016; Norouzitallab et al. 2019). However, the fitness benefit of transgenerational plasticity to successive generations needs to be balanced against the costs of maintaining the molecular machinery required for the process of transgenerational plasticity (Lind and Spagopoulou 2018). Like within-generation plasticity, transgenerational plasticity may not always be beneficial. Environmental mismatching across generations can be detrimental to the fitness of the offspring and the transgenerational response may rely on a complicated interaction of multiple factors (Guillaume et al. 2016; Harmon and Pfenning 2021). Furthermore, transgenerational effects may be stress-specific and a response to one stressor could reduce the ability of individuals to adequately respond to a different type of stressor (Burton et al. 2021).

Transgenerational plasticity: questions and evidence

The prevalence and importance of transgenerational plasticity is hotly debated, with four major questions often discussed: 1) How common is transgenerational plasticity? 2) How does transgenerational plasticity operate at different levels of organization? 3) What is the relationship between within-generation and

transgenerational plasticity? 4) Can transgenerational plasticity buffer the negative impacts of climate change? I discuss these in further detail in the following paragraphs.

How common is transgenerational plasticity?

Transgenerational plasticity has been observed in multiple studies, including responses to thermal stressors (see reviews by Bell and Hellman 2019; Yin et al. 2019). Studies suggest that beneficial transgenerational plasticity occurs more commonly in aquatic species than terrestrial species (Donelson et al. 2018; Rebolledo et al. 2023). With regards to temperature, studies have shown that transgenerational plasticity can be beneficial when changes in the parental and offspring environment trend in the same direction. For example, sheepshead minnow (*Cyprinodon variegatus*) offspring grew best at acclimation temperatures that matched those of their parents prior to egg laying (Salinas and Munch 2012). Warm acclimation of tropical damselfish (*Acanthochromis polycanthus*) parents had the effect of decreasing resting metabolic rates and increasing maximum metabolic rates in the offspring when parent and offspring environments matched (Donelson et al. 2012). Likewise, warm-acclimated three-spined sticklebacks (*Gasterosteus aculeatus*) from warm-acclimated mothers grew faster, had a slower rate of oxidative phosphorylation and less proton leak through the mitochondrial membrane at warm temperatures compared with warm-acclimated offspring of mothers acclimated to cooler temperatures (Shama et al. 2014). Aquatic vertebrate species used for transgenerational plasticity experiments are often short-lived, eurythermal or warm-adapted stenothermal fish, as exemplified by the above studies. Less attention has been

given to longer-lived, cool- or cold-adapted stenothermal fish (but see Venney et al. 2022; Houle et al. 2023).

How does transgenerational plasticity operate at different levels of organization?

Transgenerational plasticity is often measured at one level of biological organization. Previous studies have examined the physiological responses to transgenerational thermal acclimation at the whole animal level, including measures of growth and metabolic rate (Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014). At the transcriptomic level, RNA-sequencing has been used successfully to observe the functions that are under the influence of transgenerational effects, such as mitochondrial respiration, immune function and heat shock response (Veilleux et al. 2015; Shama et al. 2016; Bernal et al. 2018). The field of transgenerational plasticity stands to benefit from more comprehensive studies designed to observe transgenerational plasticity at multiple levels of organization (Baustista and Crespel 2021), for example, to link observations of transcriptomic and physiological responses (Veilleux et al. 2015; Shama et al. 2016).

What is the relationship between within-generation and transgenerational plasticity?

Literature suggests that transgenerational plasticity is contingent on within-generation plasticity; when within-generation plasticity occurs in a population, typically transgenerational plasticity does as well (Leimar and McNamara 2015). Uncertainties remain regarding the relative strength of these two forms of plasticity with respect to one another. For example, is a high degree of within-generation plasticity a precursor for

transgenerational plasticity, or are the two negatively correlated? Theoretical evidence suggests the latter and it is generally thought to depend on the strength and timing of the environmental change relative to generation time and/or offspring developmental stage (Leimar and McNamara 2015; Lind and Spagopoulou 2018; Colicchio and Herman 2020; Clement et al. 2023). It is also possible that the relative contribution of within- versus transgenerational plasticity to the offspring's phenotype can be modified according to shifts in microclimate over time (Wadgymar et al. 2018).

This question can be expanded to ask if within-generation plasticity can override transgenerational plasticity, or vice versa (Leimar and McNamara 2015; Shama 2017; Donelson et al. 2018). Transgenerational plasticity may be stronger or more important at earlier stages of offspring development, as environmental cues detected by the parents during gametogenesis/reproduction more closely represent the offspring's developmental environment (Leimar and McNamara 2015). If and when offspring can override parental effects/transgenerational plasticity is an active area of research with some reports showing that within-generation (offspring) plasticity can override parental effects (Shama et al. 2017) and others showing that maternal effects can override within-generation plasticity (Auge et al. 2017). These theories and findings highlight the importance of further studying how within- and transgenerational plasticity interact at different magnitudes of environmental instability, and comparing these findings across different developmental stages, populations, species and taxa.

Can transgenerational plasticity buffer the negative impacts of climate change?

At the population level, acclimatization (or acclimation, under simulated/lab settings) across generations could theoretically limit some of the detrimental impacts of extreme environmental change for long enough to allow evolutionary adaptation to occur (Bernatchez 2016; Smith et al. 2016). This is especially relevant for organisms that have low adaptive capacity due to limited standing genetic variation, or where the rate of environmental change exceeds mutations rates and the rate of selection (Willi et al. 2006; Meier et al. 2014). However, it is not yet clear whether transgenerational plasticity will actually benefit populations that are threatened by stressors that are new or enhanced by climate change, or under what conditions transgenerational plasticity will be adaptive or maladaptive (Uller et al. 2013; Donelson et al. 2018; Harmon and Pfenning 2021). Some have argued that transgenerational effects are too weak to meaningfully help maintain fitness in natural settings (Uller et al. 2013; Sánchez-Tójar et al. 2020). It is also possible that, for some populations, transgenerational plasticity may have historically been adaptive but now rendered neutral or maladaptive under the rapidly changing conditions brought on by climate change (Auge et al. 2017). Experiments simulating stressors associated with climate change are necessary to elucidate whether transgenerational plasticity will be an effective response to these stressors.

Lake trout and brook trout

Cold-adapted species are particularly vulnerable to the effects of climate change (Chu et al. 2005; Reist et al. 2006; Casselman 2008). While the continuing rise in global average temperature will impose ecological risks all over the planet (IPCC 2022), the

effects of climate change will not occur uniformly across all latitudes. On average, Canada has seen a temperature increase of approximately 1.7℃ in less than a century, with Arctic regions experiencing a temperature increase of approximately 2.3℃ (Zhang et al. 2019). In particular, freshwater lakes at higher latitudes in the province of Ontario have warmed at faster rates than lakes at lower latitudes (Wu et al. 2022). This disproportionate increase in temperature means that more northerly distributed fish, such as sensitive arctic and sub-arctic populations, experience a relatively larger degree of environmental warming. Thus, stenothermal organisms living in higher latitudes, such as lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*), are ideal model species for studying the response to climatic warming through within- and transgenerational plasticity given that they generally have a poor tolerance for warm temperatures (Beitinger and Bennett 2000; Evans 2007) and are vulnerable to climate change (Wu et al. 2022).

The lake trout is a cold-adapted salmonid distributed across northern North America, with the Great Lakes Region representing its southernmost native range (Muir et al. 2021). The distribution of lake trout is limited to post-glacial oligotrophic lakes, although some arctic populations can migrate towards the sea and tolerate mildly brackish water (Chavarie et al. 2021; Muir et al. 2021). Lake trout are considered to be an ice-age fish, having inhabited the lakes formed in North America during the Pleistocene by glacial scouring and meltwater (Wilson and Mandrak 2021). Their glacial legacy is reflected in their habitat preference today as they reside in well-oxygenated lakes with cool temperatures between 10-12°C (Edsall 2000; Martinez et al. 2009). Lake trout are long-lived (up to 60 years) and can take 4-7 years to reach sexual maturity with

populations in the Arctic growing larger, maturing later and living longer (Muir et al. 2021). In autumn, spawning fish deposit gametes in the spaces between coarse cobble and the young hatch in late winter and emerge in the spring. Lake trout are morphologically diverse showing interspecific variation in body shape, size and colouration depending on habitat niche (Chavarie et al. 2018), but they exhibit little variation in thermal tolerance and performance (Evans 2007; Kelly et al. 2018).

The brook trout is a sister species of the lake trout (Crête-Lafrenière et al. 2012; Esin and Markevich 2018). Brook trout are native to eastern North America, with southern populations limited to high altitude regions where suitable habitat exists (Power 1980). These fish are commonly found in brooks and streams, but some populations also reside in lakes (Power 1980; Smith and Ridgway 2019). Like other charrs, brook trout prefer cool (10-16°C), well-oxygenated freshwater habitats, however, the upper end of their preferred temperature range places them among the least temperature sensitive of the North American charrs (Esin and Markevich 2018; Kovach et al. 2019; Durhack et al. 2021). Brook trout will occupy different areas of their habitat depending on life stage and time of day (Biro et al. 2008; Smith and Ridgway 2019). In contrast to adults, juveniles feed in warmer water at shallow depths near shore and near the surface, and brook trout are known to forage in warmer water at night when prey are more abundant (Biro et al. 2008; Goyer et al. 2014).

Both lake trout and brook trout survived multiple glaciation and interglacial intervals over the last several million years, but whether they can withstand the predicted rapid habitat alterations due to climate change is an increasingly urgent question (Kovach et al. 2019). The rise in average global temperatures is shortening the time that lakes are

covered by ice, increasing lake surface temperatures, and prolonging the period of thermal stratification that is established in deep lakes during the summer (Lehman 2002; Reist et al. 2016; Guzzo and Blanchfield 2017). Lake trout rely on the hypolimnion as a thermal and oxygen refuge during summer, but climate change is threatening the availability of the cold, highly oxygenated conditions they require for survival (Casselman 2008; Reist et al. 2016; Guzzo & Blanchfield 2017). Similarly, stream temperatures are forecasted to increase by up to 1.6°C by the end of the century (Kovach et al. 2019), requiring stream-dwelling fish like brook trout to cope with warmer temperatures and associated lower oxygen levels. Lake-dwelling brook trout are experiencing range contractions (Wu et al. 2022) and smaller lakes can heat to temperatures over 20°C which can have negative effects, such as on reproduction (Warren et al. 2012; Smith et al. 2020). Given that populations of these charrs are not likely to have sufficient time to adapt to climate change, they may have to rely on thermal plasticity to cope with environmental warming (Stockwell et al. 2003; Comte and Olden 2017). Whether non-genetic inheritance of thermal experiences occurs in these fish is unclear.

Lake trout and brook trout differ with regards to variation in upper thermal tolerance: variation in thermal tolerance is limited among lake trout populations (McDermid et al 2013; Kelly et al. 2014) but has been observed among brook trout populations (Stitt et al. 2014). If both species are capable of transgenerational thermal acclimation, then I have an opportunity to examine whether a relationship exists between the capacity for transgenerational and within-generational plasticity. More specifically, is the magnitude of the transgenerational effect contingent on existing within-generational

variation; would transgenerational plasticity be more or less pronounced in brook trout? Additionally, these potential differences in acclimation ability will allow me to test whether within-generation plasticity can override transgenerational plasticity in juveniles (Shama et al. 2014; Leimar and McNamara 2015; Donelson et al. 2018).

Thesis objectives

In this thesis, I examine the transgenerational effect of elevated environmental temperature on the physiology of cold-adapted, stenothermal fish, using lake trout and brook trout as model species. How transgenerational plasticity fits within the scope of adaptive responses to environmental change is not well understood. It is not clear whether transgenerational plasticity will be beneficial for salmonid species facing the challenges of climate change. In either case, understanding how plasticity acts across generations will be important for scientists and policy-makers to fine-tune conservation efforts.

In the following chapters, I explore the metabolic and transcriptomic responses to an acute temperature increase following within-generation and transgenerational warm acclimation in lake trout and brook trout. In Chapter 2, I hypothesized that transgenerational plasticity occurs in lake trout and may allow these fish to cope with warmer environments. I also hypothesized that parents would additively contribute to transgenerational plasticity in their offspring. The predictions from these hypotheses were tested using respirometry to measure the metabolic rate of cold- and warm-acclimated lake trout offspring from factorial crosses of similarly acclimated adults. In Chapter 3, I used a parallel approach to test my hypotheses that both within- and transgenerational plasticity make significant contributions to brook trout responses to environmental

temperatures, and that transgenerational plasticity would enhance upper thermal tolerance. Chapter 4 investigates the transcriptomic responses of lake trout offspring following within- and transgenerational temperature acclimation. I hypothesized that genes associated with metabolism, growth and thermal stress/tolerance would be differentially expressed in juvenile lake trout depending on both the acclimation temperature of the offspring and the acclimation temperature of their parents. Finally, Chapter 5 summarizes the main findings of the within- and transgenerational responses of lake trout and brook trout to warming and synthesizes the results to infer the potential of populations of both of these species to cope with the anticipated warming due to climate change. It is crucial that we thoroughly understand how populations respond to the habitat disturbances brought on by climate change to better inform effective policies that are integral to helping us conserve ecosystems.

Chapter 2: Limited transgenerational effects of environmental temperatures on thermal performance of a cold-adapted salmonid

A version of this chapter has been published in Conservation Physiology.
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Abstract

The capacity of ectotherms to cope with rising temperatures associated with climate change is a significant conservation concern as the rate of warming is likely too rapid to allow for adaptative responses in many populations. Transgenerational plasticity, if present, could potentially buffer some of the negative impacts of warming on future generations. We examined transgenerational plasticity in lake trout to assess their inter-generational potential to cope with anticipated warming. We acclimated adult lake trout to cold (10°C) or warm (17°C) temperatures for several months, then bred them to produce offspring from parents within a temperature treatment (cold-acclimated and warm-acclimated parents) and between temperature treatments (i.e. reciprocal crosses). At the fry stage, offspring were also acclimated to cold (11°C) or warm (15°C) temperatures. Thermal performance was assessed by measuring their critical thermal maximum and the change in metabolic rate during an acute temperature challenge. From this dataset we also determined their resting and peak (highest achieved, thermally-induced) metabolic rate. There was little variation in offspring critical thermal maximum

or peak metabolic rate, although cold-acclimated offspring from warm-acclimated parents exhibited elevated resting metabolic rates without a corresponding increase in mass or condition factor, suggesting that transgenerational effects can be detrimental when parent and offspring environments mismatch. These results suggest that the limited transgenerational plasticity in thermal performance of lake trout is unlikely to significantly influence population responses to projected increases in environmental temperatures.

Introduction

Populations are being forced to respond to climate change as environmental temperatures continue to increase towards their viable limits (Hazen et al. 2013; Galbraith et al. 2014; Luo et al. 2015). Many species are resorting to migration and range shifts where movement to more suitable habitats is possible (e.g. freshwater fish: Chu et al. 2005; birds: VanDerWal et al. 2013; mussels: Inoue et al. 2017), but those that are unable to relocate will need to acclimatize or adapt to warmer conditions if they are to persist. Organisms with little phenotypic plasticity may not be able to acclimatize to projected climatic conditions (Somero 2010; Kelly et al. 2014), and the potential for rapid adaptive responses will be limited by the available standing genetic variation for traits under selection (Stockwell et al. 2003) and seems likely to be outpaced by rapidly changing environmental temperatures (Comte and Olden 2017). This may be particularly challenging for core metabolic process such as thermal physiology, as rapid adaptation would require existing variation at the many genes underlying these pathways (Willi et al. 2006). In particular, populations that are small, isolated, or have adapted to thermally stable habitats may be particularly vulnerable, as they are expected to have reduced standing genetic variation and less evolutionary potential (Willi et al. 2006; Meier et al. 2014). For species with long generation times, the rate of environmental change may also outpace the fixation of beneficial alleles (O'Grady et al. 2008; Visser 2008; Munday et al. 2013). Within-generation and transgenerational plasticity can potentially influence adaptation of populations to climate change by mitigating impacts of climate change-related stressors, providing more time for adaptation to occur (Bernatchez 2016; Smith et al. 2016).

Populations may be able to compensate for long-term changes in temperature by preconditioning their offspring for harsher environments (Yin et al. 2019) which may, over time, influence adaptation (Bonduriansky et al. 2012). This preconditioning can involve maternal and paternal (non-genetic) effects including nutrient provisioning of the eggs, transfer of hormones and other cytoplasmic components, and inheritance of epigenetic factors which can change the way genes are expressed (Deans and Maggert 2015; Charlesworth et al. 2017). This non-genetic inheritance can be observed through studies of transgenerational plasticity (TGP), which is a plastic response that occurs when the effects of the parent's environment appear in the offspring's phenotype (Bell and Hellmann 2019). Though the occurrence and impact of transgenerational/anticipatory effects are still under debate (Uller et al. 2013; Yin et al. 2019; Sánchez-Tójar et al. 2020), TGP has been shown to benefit some fish species when faced with environmental warming, including three-spined stickleback (Shama et al. 2014), sheepshead minnow (Salinas and Munch 2012), and tropical damselfish (Donelson et al. 2012; Munday et al. 2017). These fish are warm-adapted or eurythermal species, and it has not yet been confirmed whether TGP can benefit cold-adapted, stenothermal ectotherms. It is also unclear whether TGP is contingent on existing genetic variation which is relevant to populations that have adapted to cold, stable environments since they are likely to have experienced reduced genetic variation over time (Willi et al. 2006; Wilson 2017).

The lake trout (*Salvelinus namaycush*) is a cold-adapted, stenothermal salmonid (Martin and Olver 1980; Casselman 2008) under significant threat from climate change (Evans 2007; Guzzo and Blanchfield 2017). Populations of lake trout are restricted to northern oligotrophic lakes in North America, preferring temperatures between 10-12°C

(Edsall 2000; Martinez et al. 2009). Lake trout habitat is transforming due to climate change: lake surface temperatures are increasing, the length of time that lakes are covered by ice is shortening, and the extent of cool, highly-oxygenated refuges are becoming limited during the summer (Reist et al. 2016; Guzzo and Blanchfield 2017). These environmental changes already have an observable negative impact on lake trout as warmer temperatures at spawning reduces the survival of the fry at hatch (Casselman et al. 2002). Furthermore, evidence suggests that standing genetic variation is low for some populations of lake trout (Perrier et al. 2017), and there is little variation in the capacity for within-generation temperature acclimation within and among allopatric populations (McDermid et al. 2013; Kelly et al. 2014). The lake trout is an ideal model species to study whether TGP occurs in cold-adapted, stenothermal organisms, as their limited within-generation plasticity provides an opportunity to understand how TGP fits within the scope of possible thermal responses of organisms that are forced to cope with climate change.

We hypothesized that transgenerational plasticity occurs in lake trout, potentially enabling them to cope with warmer environments. Conversely, transgenerational plasticity may be limited or non-existent in lake trout, based on the species' narrow temperature preference and thermal habitat stability (Martin and Olver 1980; Evans 2007; Wilson and Mandrak 2004). To test these hypotheses, we acclimated hatchery-raised, adult lake trout to cold (optimal, 10°C) and warm (17°C) temperatures, then used a full factorial mating design to cross fish within a temperature treatment (cold-acclimated pairs and warm-acclimated pairs) and between temperature treatments (i.e. reciprocal crosses). Their offspring were also acclimated to cold (11°C) and warm (15°C) temperatures so

that offspring environments matched or mismatched that of their mother and/or father. This allowed us to observe transgenerational effects when offspring and parental environments matched and compare them with effects when temperature conditions differed between generations. Because the mothers and fathers were from matched or mismatched environments, this provided us with an opportunity to assess the relative parental contribution of the parents to offspring thermal performance. Given the evidence supporting anticipatory effects from both mothers and fathers (Marshall 2015; Shama et al. 2016), we hypothesized that parents would additively contribute to transgenerational plasticity if it occurred.

We looked for evidence of TGP in the offspring's upper thermal tolerance (measured as critical thermal maximum, CTM) and metabolic response (measured as the rate of O_2 consumption, MO_2) to an acute temperature increase. Our predictions were based on the evidence that standard metabolic rate in warm-acclimated adult lake trout would be elevated due to temperature acclimation (Kelly et al. 2014) and that this phenotype would be passed on to their offspring, predisposing them for a higher resting MO_2. We also predicted that peak (highest achieved, thermally-induced) MO_2 would increase in offspring with warm-acclimated parents, similar to the maximum metabolic rate findings of Donelson et al. (2012). When visualized as the response to an acute temperature increase (Fig. 2.1), we predicted an overall upward shift in the MO_2-temperature relationship of offspring from warm-acclimated parents compared to those from cold-acclimated parents. Lastly, offspring with warm-acclimated parents would also have a higher CTM compared to those from cold-acclimated parents. Provided that resources are not limited, a higher peak MO_2 and CTM would benefit the offspring

overall, allowing them to tolerate warmer environments, though the trade-off would be an increased resting MO_2 associated with a higher cost of living. If the hypothesis that the parents additively contribute to the transgenerational effect is correct, then the acclimation temperature of both parents will have an effect on the offspring's response.

Methods

These experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care. They have been approved by the Institutional Animal Care Committee of Trent University (Protocol # 24794) and the Ontario Ministry of Natural Resources and Forestry (OMNRF) Aquatic Animal Care Committee (Protocol # 136).

The strain of lake trout used in this experiment originated from Seneca Lake which is a glacial lake located in the Finger Lakes region in central New York state (42°41' N, 76°54' W). This strain has been kept in the OMNRF hatchery system for five generations since 1990 and has been maintained using rotational line crossing (Kincaid 1977) to maintain its original genetic diversity and reduce inbreeding (OMNRF Fish Culture Stocks Catalogue 2005).

Experimental design: Adult trout acclimation and breeding

Mature adult lake trout (age 8; 2.3-4.2 kg) were held at the OMNRF White Lake Fish Culture Station (Sharbot Lake, Ontario, Canada) where they were individually PIT tagged (Oregon RFID, Portland OR), divided into two groups (n = 8 and 9, mixed sex) and acclimated to two different temperatures (10 ± 0.5°C and 17 ± 0.5°C) beginning in

July, 2015, by increasing temperatures 1°C per day until target temperatures were reached. The lower target temperature was based on lake trout temperature requirements for spawning and the elevated temperature was chosen to exceed their typical range but remaining within physiological limits (Casselman 2008) with the aim of inducing a physiological stress response due to warming while attempting to avoid reproductive failure. Adults were housed in 1 x 1 x 6 m tanks that were covered with black tarpaulin to block out light. Temperatures were maintained by drawing water from above and below the thermocline in the hatchery's water source (White Lake) and mixing it as it was fed into the tanks where the fish were held. After September, the temperature of each tank was allowed to follow the seasonal cooling of the lake.

Beginning in October, offspring were produced by dry-spawning anaesthetized fish (anesthetic: 0.1 g L^{-1} MS-222; Aqua Life, Syndel Laboratories Ltd., B.C., Canada) where 140 mL of eggs were stripped from each female, divided evenly among 4 jars and fertilized by pipetting milt directly onto them. Families were produced by a full factorial 4x4 mating cross using two males and two females from each of the two temperature treatments (8 fish in total) so that resultant offspring were from parents who had been acclimated to either the same or different temperatures prior to spawning. This resulted in four offspring families from each of the four parental treatment groups (W♀xW♂, W♀xC♂, C♀xW♂ and C♀xC♂, where W refers to a warm-acclimated parent and C refers to a cold-acclimated parent) for a total of 16 families (Table A2.1). After fertilization, egg jars were kept cool and transported in coolers to the Codrington Fish Research Facility (Codrington, Ontario, Canada). Upon arrival, the eggs from each jar were placed in perforated steel boxes (9 x 9 x 7.5 cm, one family per box) which were kept in flow-

through tanks receiving freshwater at ambient temperature (5-6℃) and natural photoperiod under dim light. To eliminate the potential effects of developmental plasticity with temperature on the metabolism of the offspring, we reared all eggs under the same temperature and lighting conditions.

Experimental design: Offspring temperature acclimation

In March, when the fry reached the exogenous feeding stage, 14 individuals from each family were randomly selected, split into two groups of 7 and transferred into one of four larger (200 L) tanks. Each tank was separated into four sections to keep the families separate, however, due to space constraints two families were kept in each tank section where the offspring from the two families sharing a section were half-siblings by their father. The individuals would later be identified to family using microsatellite genotyping (appendix: Supplementary methods 2.2). Two tanks received a cold/optimal temperature (11°C) and the other two received a warm temperature (15°C) so that each family had 7 representatives acclimated to each temperature. The lower acclimation temperature was selected based on the optimal growth temperature for lake trout (Edsall 2002; Casselman 2008), and the warm temperature represents the potential warming in the Great Lakes region due to climate change by the end of the century (Hayhoe et al. 2010).

After transferring the fry to the larger tanks, we changed the water temperature at a rate of 1℃ per day until the target temperatures (11 and 15°C) were reached, and the fish were acclimated for 3-4 weeks before the experiments began. The fish were fed 5-6 times a day at 2-3% their mass, however, fish were fasted for at least 12 hours prior to

experimentation so that the physiological effects from recent feeding did not influence experimental results (Millidine et al. 2009).

Respirometry set up

To test for potential transgenerational effects of the parental environment on offspring physiology, we measured and compared the metabolic rate of offspring of parents acclimated to matched or mismatched temperature conditions. To do so, we first measured the metabolic rate of offspring as the rate of oxygen consumption (MO_2), using closed respirometry during an acute temperature increase ($+2°C \cdot h^{-1}$). From this dataset, we determined each individual offspring's resting rate of oxygen consumption (MO_2) and peak (thermally-induced) MO_2. The resting MO_2 was recorded as the MO_2 at the fish's acclimation temperature before temperature began to rise with the acute temperature challenge, and the peak MO_2 was recorded as the highest, thermally-induced MO_2 achieved during the trial. We distinguish peak MO_2 from maximum MO_2 (reported for exhaustive exercise protocols) because highest MO_2 observed due to temperature may not necessarily represent the absolute maximum rate possible for each offspring. For this reason, we do not calculate aerobic scope. To determine the upper thermal tolerance in the offspring, we measured the critical thermal maximum (CTM) which is the highest temperature that can be tolerated by the fish. This was recorded as the temperature at which the fish lost equilibrium as temperature increased, identified as a loss of dorsoventral orientation with the inability to right itself after 5-10 seconds.

Respirometers consisted of custom-built glass cylinders (8 cm diameter x 4.5 cm height, 226 cm^3 volume) sealed at one end and fitted with an acrylic lid. Each lid had an

inlet and outlet valve to allow water to flow through the chambers using a submersible pump that circulated water through the respirometers at 4.5 L min^{-1}. The valves were situated on either side of a fitting that held a dissolved oxygen probe (model DO-BTA, Vernier Software and Technology, OR, USA) in place. The respirometers were contained in clear plastic tubs (two respirometers per tub) atop two side-by-side stir plates so that each respirometer was positioned over a stir plate. A magnetic stir bar in each respirometer was set to spin at approximately 60 RPM to keep water circulating in the chamber and a perforated stainless-steel grid separated the fish from the stir bar. The containers received aerated freshwater from a source tank that was temperature controlled using three 500W titanium heaters (model TH-0500, Finnex, IL, USA) with digital temperature controllers (model HC 810M, Finnex). The plastic tubs were covered in a sheet of thin, black plastic to minimize visual disturbance to the fish.

Respirometry protocol and determining critical thermal maximum

The night before the experiment, eight fish were individually transferred into separate respirometers where they received a continuous flow of fresh water maintained at their acclimation temperature and were left to adjust to the experimental apparatus overnight. Resting MO_2 was measured the following morning. MO_2 measurements were collected by manually switching off the pumps that circulated water through the respirometers and closing the input and output valves to create a closed system. The stir bar kept water moving past the oxygen probe which was connected to a Lab Pro (Vernier Software and Technology) interfaced with LoggerPro software (version 3.8.6; Vernier Software and Technology) so that the reduction in oxygen concentration could be

recorded. Measurement of MO_2 began after a 30 second wait period, then the drop in O_2 was recorded for 10 minutes, after which the valves were opened to allow fresh water to flush the chamber until the next oxygen consumption measurement was made (approximately 30 minutes). We observed the activity of the fish during each trial and an MO_2 value was excluded from the analysis if a fish was active during the measurement period.

After measurement of resting MO_2, fish were subjected to an acute temperature challenge of +2°C per hour by raising the temperature of the water in the source tank that fed the tubs housing the respirometers. We chose this rate to be consistent with previous studies that measured metabolic rate via oxygen consumption in related species (Penney et al. 2014). We measured MO_2, for 10 minutes, at every 1°C increase until the fish lost equilibrium which was observed when the fish could no longer maintain an upright position in the respirometer chamber, and this was recorded as the CTM for that fish. At this point, the focal fish was quickly removed from the chamber and euthanized in a bath of 0.3 g L^{-1} of tricaine methanesulfonate (MS-222; Aqua Life, Syndel Laboratories Ltd., B.C., Canada). The focal fish was blotted dry on a paper towel so that mass (measured to the nearest 0.1 g using a digital balance scale) and fork length (measured to the nearest 1 mm using digital calipers) could be measured, and a caudal fin tissue sample (~0.25 cm^2) was preserved in 95% ethanol for subsequent genotyping to identify offspring individuals to their respective family (see appendix: Supplementary methods 2.2).

The oxygen saturation of the water in the source tank and respirometers was continuously monitored throughout each trial. Oxygen saturation was 6.5-7.5 mg L^{-1} at the start of the measurement period; O_2 saturation by the end of the measurement period

varied depending on the temperature during the acute temperature challenge, it ranged 4.5-5.5 mg L^{-1}. The measurement period was shortened if oxygen concentration in the respirometers began to approach the critical limit of 3.5 mg O_2 L^{-1} to attempt to minimize hypoxia-related responses in the fish (Doudoroff and Shumway 1970; Cook et al. 2018). Also, if the oxygen saturation levels in the source tank began to drop due to higher temperatures, then oxygen was supplemented to the source tank water with a tank of compressed O_2 and diffuser. Hyperoxia did not occur and O_2 supplementation did not influence temperature during the experiment.

Calculations and statistical analysis

Reduction in oxygen concentration was recorded as mg O_2 L^{-1} min^{-1}, and the rate of oxygen consumption (MO_2) was calculated using the following formula,

$$MO_2 = \frac{(Rate\ of\ decline\ in\ [O_2])(V_R - V_F)\ x\ 60}{h}$$

where *(Rate of decline in [O_2])* is the decline in water oxygen concentration during the 10-minute measurement period, V_R is the volume (L) of the respirometer, V_F is the volume of the fish (L) and h is the time in hours.

Condition factor was calculated as:

$$Condition\ factor = \frac{mass}{(fork\ length)^3}\ x\ 100$$

To explore factors that contributed to variation in body mass and condition factor we used the software JMP 13 (v. 18.1). Statistical analyses of the MO_2 during the temperature challenge, and the resting and peak MO_2 were conducted using R (v. 3.5.2)

with the ‘MuMIn’ (Barton 2019), ‘lme4’ (Bates et al. 2015), and ‘mgcv’ (Wood 2011) packages. The level of significance was set to 0.05 in all analyses, and all model assumptions (linearity, homogeneity of variance, sample independence, and residual normality) were confirmed with Shapiro-Wilk W, Levene’s, and Brown-Forsythe tests. In some cases, our response variable appeared non-normally distributed (according to Shapiro-Wilk W tests), however, we still opted for parametric tests as our selected analytical approaches are not highly sensitive to non-normality (Glass et al. 1972; Harwell et al. 1992; Lix et al. 1996; Bodden et al. 2017; Senduran et al. 2018) and depend more on homogeneity of variance instead. Lastly, we present MO_2 using two terms: mass-adjusted and mass-specific. The mass-adjusted values are derived from the GLMM which includes whole animal rates with mass as a covariate and are the values on which the statistical analysis was performed. The mass-specific values are the MO_2 divided by mass and we include mass-specific values to present the MO_2 data in a manner consistent and comparable with previous studies that include respirometry in fish.

The complexity of the experimental design (large number of fixed effects and interaction terms) and logistic limitations on sample size (number of independent crosses and available rearing space) prevented using conventional statistical analyses to assess the relative contributions of within- and transgenerational plasticity. We tested a large number of fixed effects to determine how offspring upper thermal tolerance and metabolic rate changed with offspring acclimation temperature (within-generation plasticity), parental acclimation temperature (transgenerational plasticity) and their potential interactions. The list of fixed effects was further expanded by splitting parental acclimation temperature into maternal and paternal components to assess the relative

parental contributions. Along with the interaction terms, this unavoidably gave rise to a complex global model.

To test for effects of maternal, paternal and offspring acclimation temperatures on offspring condition factor and mass we used two separate general linear mixed effects models (GLMM) in JMP, with mass and condition factor as Gaussian-distributed response variables. These models both included offspring acclimation temperature (cold or warm) and parent acclimation temperature as fixed effect predictors, where parents were treated as a single explanatory variable with mother and father acclimation temperature combined and represented as one of four fixed effects: C♀xC♂, C♀xW♂, W♀xC♂ or W♀xW♂ (C = cold acclimation and W = warm acclimation). To test for whether parental acclimation temperature yielded differential effects on mass and condition of offspring reared in cold or warm water, an interaction between offspring and parental treatment group was also included as a fixed effect predictor. Degree days was included as a random intercept to control for effects of age on mass and condition, since the experiment lasted approximately five weeks and most of the cold-acclimated offspring were tested in the first half of the experimental period. Here, degree days were calculated for each fish as the cumulative temperature experienced above 0°C (Chezik et al. 2013; Cook et al. 2018) until the day of the experiment. Finally, offspring identity (ID) and parental IDs (ID_M and ID_F) were also included as random intercepts to account for statistical non-independence between offspring that were sired or dammed from the same parents.

To test the effect of maternal, paternal and offspring acclimation temperature on the metabolic (MO_2) response of the offspring to an acute temperature challenge, we

again used a GLMM, using the 'nlme' package in R (Pinheiro et al. 2019) to permit correction for temporal autocorrelation. In this model, MO_2 was used as a Gaussian-distributed response variable, with acute challenge temperature (T_a; continuous variable), offspring acclimation temperature (T_O; cold and warm), and acclimation temperatures of the mothers (T_M; cold and warm) and fathers (T_F; cold and warm) as fixed effect predictors, along with all possible interactions between these terms. Additionally, *Mass* (fixed term) was included in the model as a continuous predictor because metabolic rate scales with mass and the warm-acclimated offspring grew heavier than cold-acclimated ones. Similar to our previous models, both mother and father ID (ID_M and ID_F) were included as random intercepts to account for relatedness among offspring, and offspring ID was included as a random intercept to control for statistical non-independence between measurements drawn from the same individual.

Because the relationship between MO_2 and acute temperature challenge was curvilinear and could not be predicted by a simple polynomial function (i.e. with relatively low degree), we first modeled the relationship between MO_2 and acute challenge temperature alone using a cubic regression spline in a general additive model (GAM), using three knots to appropriately capture the shape of the relationship while avoiding over-fitting. Predicted MO_2 at each challenge temperature was extracted for each offspring and used in place of acute challenge temperature within our GLMM to account for the variation in the response variable (observed MO_2) due to acute challenge temperature. This approach permitted us to: 1) remove the complex, curvilinear relationship between MO_2 and acute challenge temperature, 2) test whether the remaining variation in MO_2 (i.e., that not explained by acute challenge temperature) can be

explained by the other terms in the GLMM, and 3) include multi-level interactions between a previously non-linear predictor (acute challenge temperature) and additional factorial predictors, which cannot be accomplished simply with current additive models. Finally, to account for heterogeneity of variance in MO_2 across acute challenge temperature (and detected across predicted MO_2), and to correct for autocorrelation between measurements drawn at adjacent time-points, we weighted our model by acute challenge temperature, and included a type I autoregressive correlation structure, with an estimated ρ of 0.397.

An effect of parental acclimation temperature on the resting MO_2, peak MO_2 and CTM of offspring were analyzed using three independent linear mixed models in R, each with the 'lme4' package (Bates et al. 2015). Here, we first sought to include mother acclimation temperature (T_M), father acclimation temperature (T_F), and offspring acclimation temperature (T_O) as fixed effect predictors, with all possible interactions between each of these factors, along with offspring *mass* as a covariate. Unfortunately, however, our total number of observations per experimental group ($\bar{n}$ = 21.125 ± s.d. = 2.642; total n = 157) were too few to support a robust approach such as an ANOVA to test the individual effects of each predictor (π = 0.448; for expected relationships with weak explanatory capacity; Cohen's $f^2 \cong 0.05$; as tested using the 'pwr' package in r; Champely *et al*., 2018). We therefore used an Akaike Information Criterion (AIC) approach to identify which models best explained the variation in the data while avoiding over-parameterization, using the previously described global model, including mother ID (ID_M) and father ID (ID_F) as random intercepts to account for relatedness among offspring. The best models were considered as those with a $\Delta AIC \leq 2$ as recommended

by Burnham and Anderson (2002) where the ΔAIC was calculated as the difference in the AIC value of a given model versus the top model (i.e. the model with the lowest AIC value). We also calculated the evidence ratio (ER) and Akaike weight (Wi) of each model iteration. The ER is the likelihood that the top model is the best supporting model compared to another model, and the Wi is the weight (or proportion) of evidence that a given model best explains the variation in the data (Burnham and Anderson 2002). These metrics were used to compare the best models and to observe common parameters among these models.

Results

Mass and condition factor

On average, warm-acclimated offspring were nearly twice as heavy compared with cold-acclimated offspring (least squares means: 4.28 ± 0.14 g versus 2.76 ± 0.13 g; GLMM: $F_{1,21.28} = 93.58$, $p < 0.001$; Table 2.1). Offspring mass did not differ among parental groupings (C♀xC♂, C♀xW♂, W♀xC♂, W♀xW♂) (GLMM: $F_{3,3.63} = 1.08$, $p = 0.45$), but there was an interaction between offspring acclimation and parental group (GLMM: $F_{3,46.53} = 4.23$, $p = 0.01$).

Warm-acclimated offspring had significantly higher body condition than cold-acclimated offspring with means of 0.93 ± 0.01 vs. 0.89 ± 0.01 (Table 2.1), respectively (GLMM: $F_{1,20.47} = 38.67$, $p < 0.001$). Offspring condition factor was not affected by parent acclimation temperatures (GLMM: $F_{3,3.23} = 1.83$, $p = 0.31$) nor by the interaction between offspring acclimation and parental group (GLMM: $F_{3,2.07}$, $p = 0.12$).

Offspring metabolic rate with an acute temperature increase

For the effect of the acute temperature challenge on offspring metabolic rate, there was an increase in offspring mass-adjusted MO_2 with increasing body mass (*Mass*: t = 10.66, p < 0.001, Table 2.2). Offspring MO_2 also increased with challenge temperature (GAMM: T_a: t = 17.58, p < 0.001). Offspring acclimation temperature (T_O) had a significant effect on MO_2 with warm-acclimated offspring having a higher MO_2 (T_O: t = -3.40, p < 0.001, Table 2.2). Neither maternal nor paternal acclimation temperature in isolation was strong enough to influence offspring MO_2 (T_M: t = 2.12, p = 0.068; T_F: t = 1.22, p = 0.222; Table 2.2).

While the interaction between offspring and maternal acclimation temperature was not significant ($T_O \cdot T_M$: t = -1.66, p = 0.097), the interaction between offspring and paternal acclimation temperature did influence MO_2 ($T_O \cdot T_F$: t = -3.42, p < 0.001). There was no significant interaction between mother and father acclimation temperature on the offspring's metabolic response ($T_M \cdot T_F$: t = 1.01, p = 0.312). Significant two-way interactions occurred between T_a and T_O (t = 2.61, p = 0.009) demonstrating that some remaining variation in MO_2 that was not explained by challenge temperature could be explained by offspring acclimation temperature; more specifically, offspring reared at warm temperatures appeared to respond differently to the thermal challenges than did cold-acclimated offspring. The acclimation temperature of the parents interacted significantly with the acute temperature challenge to determine the offspring's MO_2 ($T_a \cdot T_M$: t = -4.34, p < 0.001; $T_a \cdot T_F$: t = -3.39, p < 0.001; Table 2.2). The metabolic response of the offspring also depended on the complex interaction between offspring acclimation temperature, parental (maternal or paternal) acclimation temperature and challenge

temperature ($T_a \cdot T_O \cdot T_M$, t = 2.34, p = 0.019; $T_a \cdot T_O \cdot T_F$, t = 4.46, p < 0.001; Table 2.1). No other main effect interactions were significant (Table 2.2).

To visually explore maternal and paternal influences on offspring MO_2, we plotted the mass-specific MO_2 for offspring from different parental combinations against the acute temperature challenge (Fig. 2.2). We did not perform a statistical analysis on the mass-specific values because the GLMM (previously described) tested MO_2 while accounting for mass in the model. Qualitatively, the cold-acclimated offspring from warm-acclimated parents (W♀xW♂) had a higher metabolic rate at the beginning of the temperature challenge compared with the other parental acclimation groups (C♀xC♂, C♀xW♂, W♀xC♂; Fig. 2.2A) indicating that an environmental mismatch between generations can influence offspring metabolic response. This effect did not carry over to the warm-acclimated offspring (Fig. 2.2B) as MO_2 was comparable among the parental acclimation groups.

To visually isolate the effects of maternal acclimation temperature on offspring's thermal response, we plotted the mass-adjusted MO_2 (Fig. 2.3) estimated from the GLMM to show the interaction between challenge temperature and the acclimation temperature of the mothers ($T_a \cdot T_M$; Table 2.2). For both cold- and warm-acclimated offspring (Fig. 2.3, both panels) the difference in the slope of the MO_2-temperature relationship illustrates the significant interaction between challenge temperature and acclimation temperatures of the offspring and mothers ($T_a \cdot T_O \cdot T_M$; p = 0.019; Table 2.2). Focusing on the cold-acclimated offspring (Fig. 2.3, left), at cooler challenge temperatures, the MO_2 of offspring from warm-acclimated mothers was elevated compared to offspring from cold-acclimated mothers. For warm-acclimated offspring at

challenge temperatures below approximately 19°C, individuals from warm-acclimated mothers had a higher MO_2 compared to those from cold-acclimated mothers (Fig. 2.3, left). This general trend occurred to a lesser extent in the warm-acclimated offspring (Fig. 2.3, right) with the lines of the offspring's MO_2-temperature relationship overlapping for warm- and cold-acclimated mothers.

To visually explore the paternal effect on offspring MO_2 we plotted the mass-adjusted MO_2, estimated from the GLMM (Fig. 2.4). This illustrates the significant interaction between challenge temperature and the acclimation temperatures of the offspring and fathers ($T_a \cdot T_O \cdot T_F$; $p < 0.001$; Table 2.2). For cold-acclimated offspring, the MO_2-temperature relationship of those from warm-acclimated fathers was above that of those from cold-acclimated fathers with the lines crossing at approximately 14°C (Fig. 2.4, left). A reverse trend occurred in the warm-acclimated offspring as individuals from warm-acclimated fathers had a lower MO_2 at cooler challenge temperatures compared to those from cold-acclimated fathers (Fig. 2.4, left).

Resting and peak metabolic rate

Analysis of resting MO_2 with AIC revealed six models ($\Delta AIC \leq 2$) that best predicted the trends in the data with *Mass* appearing in each of these models. The first model contained *Mass* as the only fixed variable, but this model was only 1.23 times more likely (evidence ratio, ER) than model 2, which included maternal (T_M) and paternal (T_F) acclimation temperature with their interaction, to best explain the variation in the data (Table 2.3). Interestingly, offspring acclimation temperature (T_O) appeared only twice among the six models. Maternal (T_M) and paternal (T_F) acclimation temperature

appeared most frequently among the six models, with an interaction between these terms appearing in two of these. An interaction between offspring and paternal acclimation temperature ($T_O \cdot T_F$) appeared in only one of the models (Table 2.3). Altogether this suggests that maternal and paternal environments, individually and combined, can act on the response of the offspring's resting metabolic rate to temperature acclimation.

We plotted the resting MO_2 (mass-specific values, no statistical analysis) to visually explore trends within and between the offspring and parental acclimation groups (Fig. 2.5A). The cold-acclimated offspring from warm-acclimated parents (open boxes, W♀xW♂, Fig. 2.5) had the highest mean resting MO_2 (219.2 ± 15.97 mg O_2 kg^{-1} h^{-1}), while the resting MO_2 of the other three groups ranged between 148.3 ± 11.08 and 162.1 ± 10.51 mg O_2 kg^{-1} h^{-1} (Fig. 2.5A). There was no observable trend for warm-acclimated offspring (shaded boxes, Fig. 2.5A) as resting MO_2, irrespective of parental acclimation temperature, ranged between 130.4 ± 12.56 and 154.7 ± 13.52 mg O_2 kg^{-1} h^{-1}. When comparing offspring within parental acclimation temperatures (open vs. shaded boxes; Fig. 2.5A), the mean resting MO_2 of cold-acclimated offspring from warm-acclimated parents (shaded boxes, W♀xW♂; Fig. 2.5A) was higher compared to cold-acclimated offspring (open boxes, W♀xW♂; Fig. 2.5A).

We also used an information theoretic approach to explore factors contributing to variation in peak MO_2. Of the five models that best explained the variation in peak MO_2, *mass* appeared in each model with the top model (ER = 1, W_i = 0.33) containing *mass* as the only fixed parameter (Table 2.3). Maternal (T_M) and paternal (T_F) acclimation temperature, and the interaction term between the two, occurred in the second-best model

which had a 23% (W_i) chance of being the top model (Table 2.3). The other three of the five models contained only one fixed parameter: either T_O, T_M, or T_F (Table 2.3).

Peak MO_2 (mass-specific values, no statistical analysis) was also plotted to visually explore trends within and between the offspring and parental acclimation groups (Fig. 2.5B). Overall, cold-acclimated offspring attained a higher mean peak MO_2 (mass-specific) than warm-acclimated offspring (open vs. shaded boxes, W♀xW♂, C♀xW♂, W♀xC♂, C♀xC♂; Fig. 2.5B). When comparing offspring within an acclimation temperature, peak MO_2 was comparable; cold-acclimated offspring (open boxes, Fig. 2.5B) ranged between 335.8 ± 12.97 and 386.6 ± 13.13 mg O_2 kg^{-1} h^{-1}, and warm-acclimated offspring (shaded boxes, Fig. 2.5B) ranged between 299.2 ± 18.79 and 325.5 ± 16.02 mg O_2 kg^{-1} h^{-1}.

Critical thermal maximum (CTM)

Four AIC models best explained the trends in the CTM data (ΔAIC ≤ 2). Model 1 and 2 together suggest that CTM depended on a complex interaction between offspring (T_O) and parental acclimation temperature (T_M and T_F). The top model (W_i = 0.39) was the global model containing *Mass* as a covariate with the offspring (T_O), maternal (T_M) and paternal (T_F) acclimation temperature as fixed effects, and all 2-way and 3-way interaction terms between them (Table 2.3). The second model was also the global model excluding *Mass*, however, model 1 was 1.40 (ER) times more likely to explain variation in CTM compared to model 2 (Table 2.3). The third model contained only *Mass* and offspring acclimation temperature (T_O), whereas as the fourth model contained only T_O (Table 2.3).

Critical thermal maximum within and between the groups of offspring showed subtle differences (Fig. 2.5C). The mean CTM was comparable among groups of cold-acclimated offspring (open boxes, Fig. 2.5C) with values ranging between 26.10 ± 0.2 and 26.64 ± 0.10°C. Likewise, the CTM of warm-acclimated offspring (shaded boxes, Fig. 2.5C) was similar. When comparing offspring within parental acclimation groups, warm-acclimated offspring (shaded boxes) from W♀xW♂ parents had a CTM 1.17°C higher than that of cold-acclimated offspring (open boxes) from the same parental group (W♀xW♂, Fig. 2.5C). For the rest of the parental groups (C♀xW♂, W♀xC♂, C♀xC♂;), CTM was comparable (open vs. shaded boxes, Fig. 2.5C).

Discussion

Lake trout families exhibited evidence of limited transgenerational plasticity, although the effects of TGP on MO_2 and CTM were minor compared with offspring mass and acclimation temperature. Although warm acclimation of the parents did not shift their offspring's MO_2-temperature relationship upward as predicted, we found that offspring thermal performance depended on complex interactions between parent and offspring environments. Ours is one of the few studies to investigate the relative parental contribution to TGP in a vertebrate offspring's phenotype (Shama et al. 2014; Hellmann et al. 2020A), and we demonstrate that the parents additively contribute to the limited TGP we observed (resting and peak MO_2, and CTM).

Offspring MO_2 was most strongly influenced by mass and acclimation temperature. This is not surprising given that warm-acclimated offspring were heavier than their cold-acclimated siblings, and thus had higher whole animal O_2 consumption

rates. The effect of allometric scaling was apparent when the MO_2 was expressed mass-specifically, where mean MO_2 was higher in cold-acclimated offspring overall compared with warm-acclimated offspring (Clarke and Johnston 1999). Although it is possible that partial hypoxia may have influenced offspring performance, we saw no evidence for this. The observed effect of offspring acclimation temperature on MO_2 and CTM concurs with other lake trout studies, including evidence of limited acclimation capacity (Evans 2007; McDermid et al. 2013; Kelly et al. 2014). Lastly, individual (random) effects had a significant contribution but epigenetic priming can be expected to vary within and among individuals; thus, the extent of TGP should be expected to vary among both adults and offspring.

Limited evidence for transgenerational plasticity

At lower challenge temperatures (11-20°C), the average MO_2 of cold-acclimated offspring from warm-acclimated parents was elevated compared to those from cold-acclimated parents (Fig. 2.2A), suggesting a higher cost of living (Norin and Metcalfe 2019) at these temperatures when an environmental mismatch exists between generations. At warmer acute challenge temperatures (>18ºC), cold-acclimated offspring from the different mating crosses showed similar MO_2 values, and MO_2 began to decline once temperature exceeded 24ºC (Fig. 2.2A). This disagrees with previous findings that warm-acclimated offspring from warm-acclimated parents had a lower metabolic rate (Donelson et al. 2012), although the effect of TGP can be difficult to predict and may not always be to the benefit of the offspring (Guillaume et al. 2016). In addition to the acute temperature challenge, the resting MO_2 of offspring from warm-acclimated parents (a

generational environmental mismatch) was the highest among of the cold-acclimated offspring (Fig. 2.5A), and parent acclimation temperature did not have an appreciable effect on peak MO_2 (Fig. 2.5B). While this contrasted with our predictions, standard metabolic rate is thought to be relatively plastic (Norin and Metcalfe 2019) which may explain why resting MO_2 was elevated in cold-acclimated offspring from warm-acclimated parents in our study. We interpret the high resting MO_2 in this group (11°C acclimated offspring from warm-acclimated parents) to be due to an increased effort into surviving elevated temperature. It was evident that this group was not diverting energy into increased growth considering that the mass and condition factor were no greater in this group compared to the others.

MO_2-temperature relationships were similar for the warm-acclimated offspring regardless of parental treatment (Fig. 2.2B), suggesting that parental environmental temperatures had little influence on offspring performance. This was surprising given that previous studies on fish have reported that offspring from warm-acclimated parents could tolerate warm temperatures better than offspring from cold-acclimated parents by reducing standard metabolic rate or increasing maximum metabolic rate (Donelson et al. 2012; Shama et al. 2014; Donelson et al. 2018). These earlier studies tested TGP in tropical or eurythermal species, thus it is possible that TGP is limited in stenothermal species like lake trout. Limited TGP may also be related to the limited variation in within-generation thermal plasticity in lake trout (Evans 2007; Kelly et al. 2014), and it could be that lake trout simply do not have the capacity to extend their thermal tolerance (Evans 2007; McDermid et al. 2013; Kelly et al. 2014). It is possible that multiple generations of exposure to the same stressor may be required to strengthen the effect (Burggren 2015;

Bell and Hellmann 2019; Pilakouta et al. 2020), as in the case of the polychaete, *Ophryotrocha labronica*, where the effect of multigenerational exposure to warming was strongest in the F5 and F6 generations (Gibbin et al. 2017).

Our results showed that the thermal experiences of the parents had a relatively minor role in shaping the metabolic rate of the next generation in lake trout. Although we did not explore the physiological mechanisms underlying variation in offspring metabolic rate, TGP has been shown in other species to act on physiological mechanisms that can affect metabolic rate, such as mitochondrial function and gene expression (Shama et al. 2014; Gibbin et al. 2017). For example, mitochondria from the heart tissue of warm-acclimated stickleback offspring from warm-acclimated mothers had a lower rate of oxidative phosphorylation and less proton leak at warm temperatures than those from mothers acclimated to a cooler temperature, suggesting that offspring mitochondrial function is more efficient when maternal and offspring environments match (Shama et al. 2014). TGP has also been shown to up- or down-regulate the expression of genes involved in the heat shock response, metabolism, protein catabolism, immune response and reproduction (Veilleux et al. 2015; Shama et al. 2016; Veilleux et al. 2018).

Additive parental contribution

Our results suggest that the contribution of the parents to TGP was additive. The offspring's overall MO_2 response to the acute temperature challenge was not influenced by the sole effect of either the maternal or paternal acclimation temperature, but instead on the complex interaction of maternal or paternal acclimation temperature with challenge temperature and offspring acclimation temperature (Table 2.2). The additive

effect of parental temperatures on offspring metabolic rate was confirmed when both paternal and maternal acclimation temperature appeared in the top AIC models for resting and peak MO_2 (Table 2.3). Both parents also contributed to their offspring's upper thermal tolerance (CTM; Table 2.3) even though the differences in CTM among the groups of offspring were very slight (Fig. 2.5C). Similarly, Massamba-N'Siala et al. (2014) found no effect of TGP on the upper thermal tolerance of polychaetes given an acute temperature challenge.

Transgenerational plasticity can be mediated through epigenetic modifications (summarized by Donkin and Barrès 2018) that can be transmitted to the next generation (Crean and Bonduriansky 2014; Marshall 2015), but other non-genetic effects (maternal and paternal) could have influenced the offspring's phenotype (Burgess and Marshall 2011; Shama 2015). Females, for example, can provision their eggs through changes in egg size or nutrient enrichment of the yolk which can contribute to offspring fitness (Einum and Fleming 1999; Gagliano and McCormick 2007; Jonsson and Jonsson 2016). We conducted a preliminary analysis of egg size, mass, and water and energy content but did not find evidence of maternal provisioning relating to the trends observed in MO_2 (Table A2.2). Similarly, Shama et al. (2014) found that temperature acclimation of the parents did not affect egg size. Another maternal effect includes the transfer of hormones, such as thyroid or cortisol, to the eggs which could potentially alter offspring gene expression (Sopinka et al. 2017), growth and development (Gagliano and McCormick 2009; Ruuskanen and Hsu 2018). While we did not test the hormone content of the eggs, we acknowledge that it could potentially influence metabolic rate (Burton et al. 2011) of the offspring in our study.

The paternal contribution to TGP is understudied relative to maternal effects, although there is some evidence for non-genetic paternal effects (Crean and Bonduriansky 2014; Marshall 2015; Immler 2018). The contribution of the father's thermal environment to the offspring's phenotype is variable, with effects seen in some species (e.g. marine tubeworm: Guillaume et al. 2016) but not others (e.g. stickleback: Shama et al. 2014). Further, paternal contributions to TGP can extend beyond the transmission of epigenetic machinery to their offspring, as ejaculate and sperm cytoplasmic components can also mediate paternal effects (Crean and Bonduriansky 2014; Kekäläinen et al. 2018). How or if these components could have affected the metabolic response of the offspring to temperature stress in our study was not assessed.

Perspectives and future directions

The importance of TGP may be a function of generation time and environmental fluctuation where TGP would be beneficial when the environment fluctuates predictably over multiple generations (Yin et al. 2019). Lake trout have a long generation time for a freshwater fish, reaching maturity in ~6-12 years depending on latitude and lake productivity (Martin and Olver 1980; Hansen et al. 2012), and occupy thermally stable habitats with limited seasonal variation (Wilson and Mandrak 2004; Guzzo and Blanchfield 2009). Under these circumstances, TGP is unlikely to provide an ecologically significant benefit to lake trout populations. It is also important to note that an evolutionary response would require multiple generations and be dependent on existing heritable variation within local populations, which is likely limited in lake trout (Wilson and Mandrak 2004; Perrier et al. 2017). Adaptation would require diversity at multiple

genes involved in core metabolic pathways with adaptive responses acting in concert (Willi et al. 2006). Within- and among-population phenotypic variation in upper thermal tolerance is limited in lake trout (McDermid et al. 2013; Kelly et al. 2014) suggesting that standing genetic variation for genes underlying their thermal physiology is likely very limited.

Although TGP may have an important role in adaptation (Bernatchez 2016; Smith et al. 2016), cold-adapted species with long generation times may not be able to keep up with the pace of anthropogenic climate change (Willi et al. 2006; Munday et al. 2013; Wilson et al. 2014). Based on our findings, it is unlikely that TGP effects in lake trout would be enough to sufficiently mitigate climate-related selection pressures to make much difference for population persistence under rapidly changing environmental conditions. Lake trout retreat to the cooler hypolimnion during the warmer summer months when the lake thermally stratifies (Casselman 2008; Guzzo and Blanchfield 2017), but climate change is expected to increase lake surface temperatures and prolong the duration of stratification (Lehman 2002). For this reason, lake trout may be forced to reside in the hypolimnion for an extended period, lengthening their exposure to hypoxia which could negatively impact important life history traits (Evans 2007; Guzzo and Blanchfield 2017).

From other studies, it is evident that TGP has some role to play in 'priming' offspring's response to elevated temperatures (Yin et al. 2019; but see Sánchez-Tójar et al. 2020), however, TGP had only a limited effect on lake trout thermal performance in our study. A further investigation into how TGP acts to influence physiological processes is warranted and will require examination of the mechanisms underlying thermal

tolerance, such as mitochondrial performance and gene expression in tandem with investigating which parental effects, including epigenetic inheritance (e.g. methylation, RNA interference), contribute to TGP. An understanding of how phenotypic plasticity, developmental plasticity, TGP, and genetic changes combine to influence the adaptation of populations to climate change will not only help us anticipate the effects of a changing environment but will also deepen our knowledge of the link between plasticity, acclimation and adaptation.

Tables

Table 2.1: The mass and condition factor of 11°C and 15°C acclimated lake trout offspring. Parental groups are represented as the maternal environment crossed with the paternal environment: C♀xC♂, C♀xW♂, W♀xC♂ and W♀xW♂ where C = cold and W = warm. Values are least squares means ± SEM. Statistical significance ($p < 0.05$; GLMM) between offspring acclimation temperature is indicated by an asterisk.

	11°C Acclimated offspring				15°C Acclimated offspring			
Parental group	C♀xC♂	C♀xW♂	W♀xC♂	W♀xW♂	C♀xC♂	C♀xW♂	W♀xC♂	W♀xW♂
Mass (g)	2.93 (±0.26)	2.69 (±0.23)	3.26 (±0.22)	2.14 (±0.26)	4.16* (±0.25)	4.61* (±0.28)	4.17* (±0.25)	4.19* (±0.27)
Condition factor	0.91 (±0.01)	0.88 (±0.01)	0.91 (±0.01)	0.86 (±0.01)	0.93 (±0.01)	0.94 (±0.01)	0.94 (±0.01)	0.92 (±0.01)

Table 2.2: Summary of the GLMM results to test for a transgenerational effect of acclimation temperature on lake trout offspring MO_2 during an acute temperature challenge. Offspring were from parents acclimated to either a cold or warm temperature. The offspring were also acclimated to cold or warm temperature. T_F, T_M, and T_O are the father, mother and offspring acclimation temperatures, respectively, and the acute temperature challenge is represented by T_a. Significant effects ($p<0.05$) are highlighted with bold text.

Parameter	**Coefficient**	**S. E.**	**DF**	**t-value**	**p-value**
Intercept	-0.32	0.07	18.45	-4.60	**<0.001**
Mass	0.14	0.01	155.32	10.66	**<0.001**
T_a	0.89	0.05	2212.78	17.58	**<0.001**
T_O	-0.30	0.09	1023.18	-3.40	**<0.001**
T_M	0.17	0.08	7.73	2.12	0.068
T_F	0.08	0.07	560.72	1.22	0.222
$T_O \cdot T_M$	-0.20	0.12	1158.94	-1.66	0.097
$T_O \cdot T_F$	-0.44	0.13	1247.21	-3.42	**<0.001**
$T_F \cdot T_M$	0.09	0.09	501.51	1.01	0.312
$T_a \cdot T_O$	0.24	0.09	2207.42	2.61	**0.009**
$T_a \cdot T_M$	-0.29	0.07	2209.73	-4.34	**<0.001**
$T_a \cdot T_F$	-0.23	0.07	2209.80	-3.39	**<0.001**
$T_O \cdot T_M \cdot T_F$	0.16	0.18	1233.62	0.92	0.360
$T_a \cdot T_O \cdot T_M$	0.30	0.13	2206.66	2.34	**0.019**
$T_a \cdot T_O \cdot T_F$	0.62	0.14	2210.05	4.46	**<0.001**
$T_a \cdot T_M \cdot T_F$	0.04	0.10	2207.83	0.46	0.641
$T_a \cdot T_O \cdot T_M \cdot T_F$	-0.30	0.19	2208.18	-1.60	0.109

Table 2.3: Summary of the top models determined with AIC to explain variation in resting MO_2, peak (highest achieved, thermally-induced) MO_2 and critical thermal maximum (CTM) with transgenerational acclimation of lake trout offspring. Offspring were from parents acclimated to either a cold or warm temperature and were also acclimated to cold or warm temperature. T_F, T_M, and T_O are the father, mother and offspring acclimation temperatures, respectively, and ID_M and ID_F are the mother and father individual identification (treated as random effects) which appear in each model to account for offspring relatedness.

Measure	Model #	ΔAIC	ER	W_i	R^2	Model
Resting MO_2	1	0	1.00	0.25	0.41	Mass + ID_M + ID_F
	2	0.42	1.23	0.20	0.43	Mass + T_F + T_M + (T_F · T_M) + ID_M + ID_F
	3	0.58	1.34	0.18	0.45	Mass + T_F + T_M + T_O + (T_F · T_M) + (T_F · T_O) + ID_M + ID_F
	4	1.16	1.79	0.13	0.42	Mass + T_M + ID_M + ID_F
	5	1.53	2.14	0.12	0.42	Mass + T_F + ID_M + ID_F
	6	1.55	2.17	0.11	0.42	Mass + T_O + ID_M + ID_F
Peak MO_2	1	0	1.00	0.33	0.64	Mass + ID_M + ID_F
	2	0.77	1.47	0.23	0.65	Mass + T_F + T_M + (T_F · T_M) + ID_M + ID_F
	3	1.24	1.86	0.18	0.64	Mass + T_M + ID_M + ID_F
	4	1.73	2.38	0.14	0.64	Mass + T_F + ID_M + ID_F
	5	1.97	2.68	0.12	0.64	Mass + T_O + ID_M + ID_F
CTM	1	0	1.00	0.39	0.29	Mass + T_F + T_M + T_O + (T_F · T_M) + (T_F · T_O) + (T_M · T_O) + (T_F · T_M · T_O) + ID_M + ID_F
	2	0.67	1.40	0.28	0.27	T_F + T_M + T_O + (T_F · T_M) + (T_F · T_O) + (T_M · T_O) + (T_F · T_M · T_O) + ID_M + ID_F
	3	1.65	2.28	0.17	0.27	Mass + T_O + ID_M + ID_F
	4	1.87	2.55	0.15	0.25	T_O + ID_M + ID_F

Figures

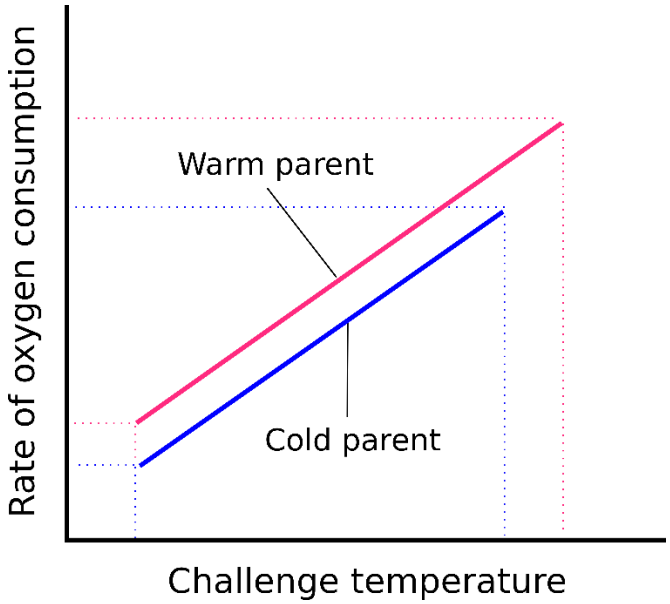

Figure 2.1: The predicted transgenerational effect of parental acclimation temperature on the rate of oxygen consumption of their offspring. The effect could be driven by either maternal or paternal acclimation temperatures, or both. Interactions would be observed as a crossing of the lines. The resting and peak (highest achieved, thermally-induced) rate of oxygen consumption are represented as the lowermost and uppermost ends of the lines.

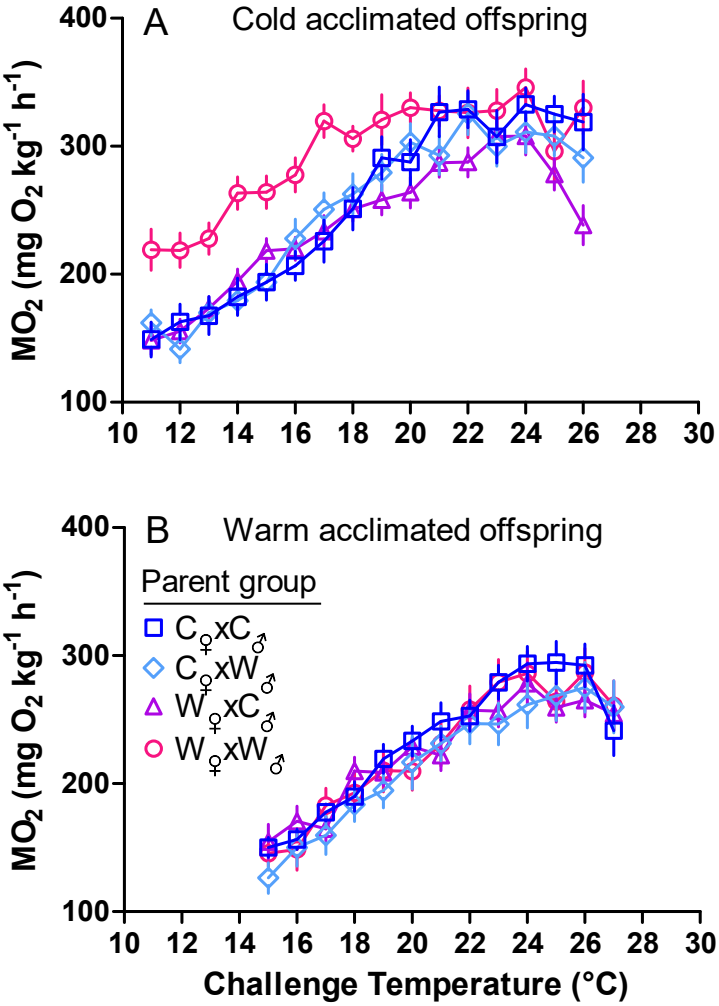

Figure 2.2: The change in the rate of oxygen consumption (MO_2) of A) cold- and B) warm-acclimated lake trout offspring given an acute temperature challenge of $+2°C \cdot h^{-1}$, showing mass-specific means ± SEM. Parental groups are represented as the maternal environment crossed with the paternal environment: C♀xC♂, C♀xW♂, W♀xC♂ and W♀xW♂ where C = cold and W = warm.

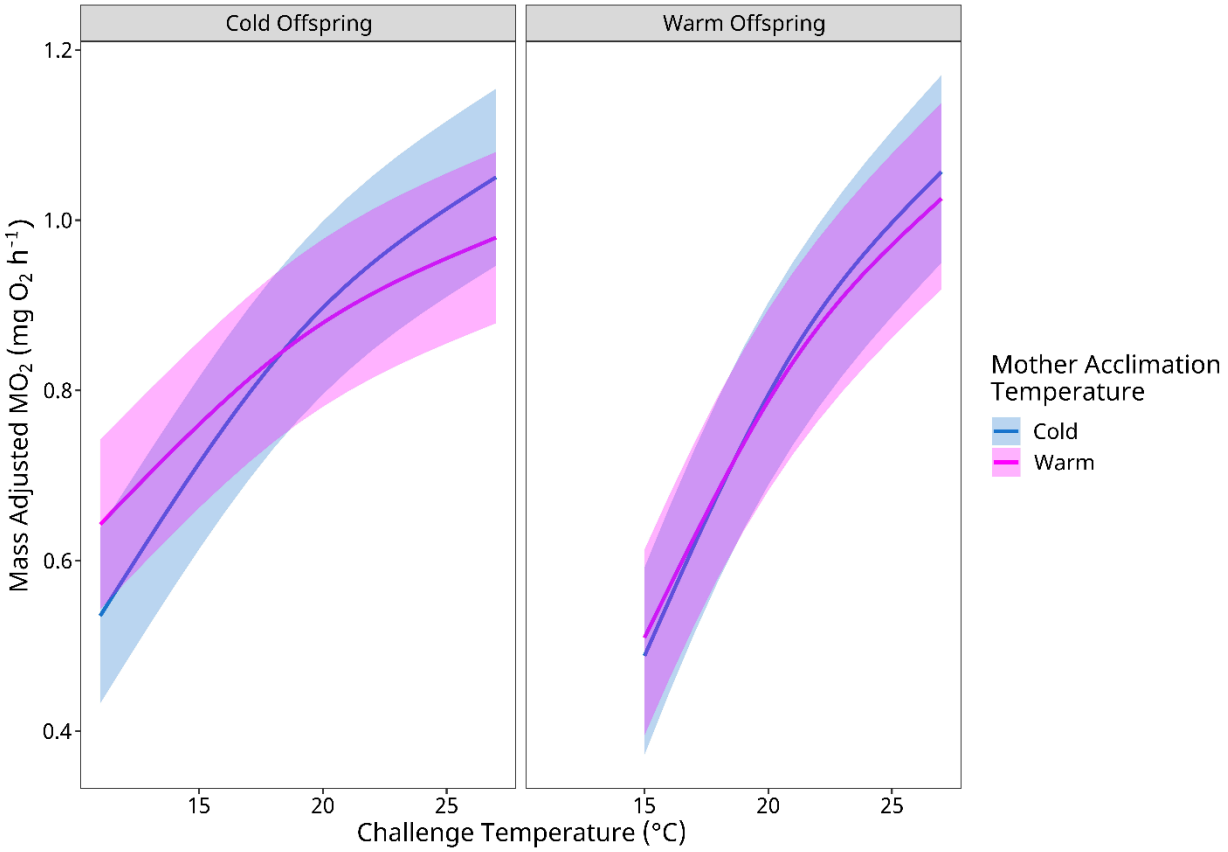

Figure 2.3: The influence of maternal acclimation temperature on the change in the rate of oxygen consumption (MO_2) of cold- and warm-acclimated lake trout offspring given an acute temperature challenge of +2°C·h^{-1}. Values are means estimated from the GLMM with 95% confidence intervals (refer to Methods).

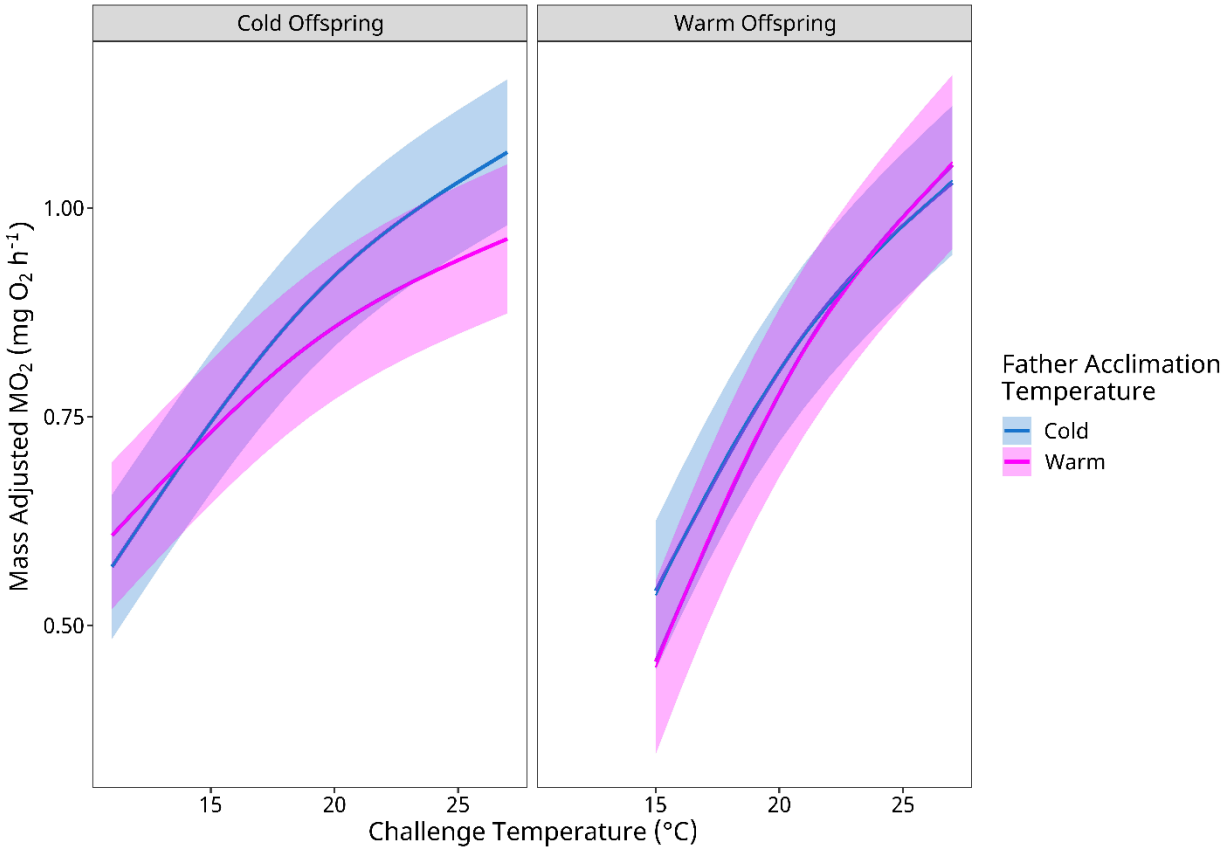

Figure 2.4: The influence of paternal acclimation temperature on the change in the rate of oxygen consumption (MO_2) of cold- and warm-acclimated lake trout offspring given an acute temperature challenge of +2°C·h^{-1}. Values are means estimated from the GLMM with 95% confidence intervals (refer to Methods).

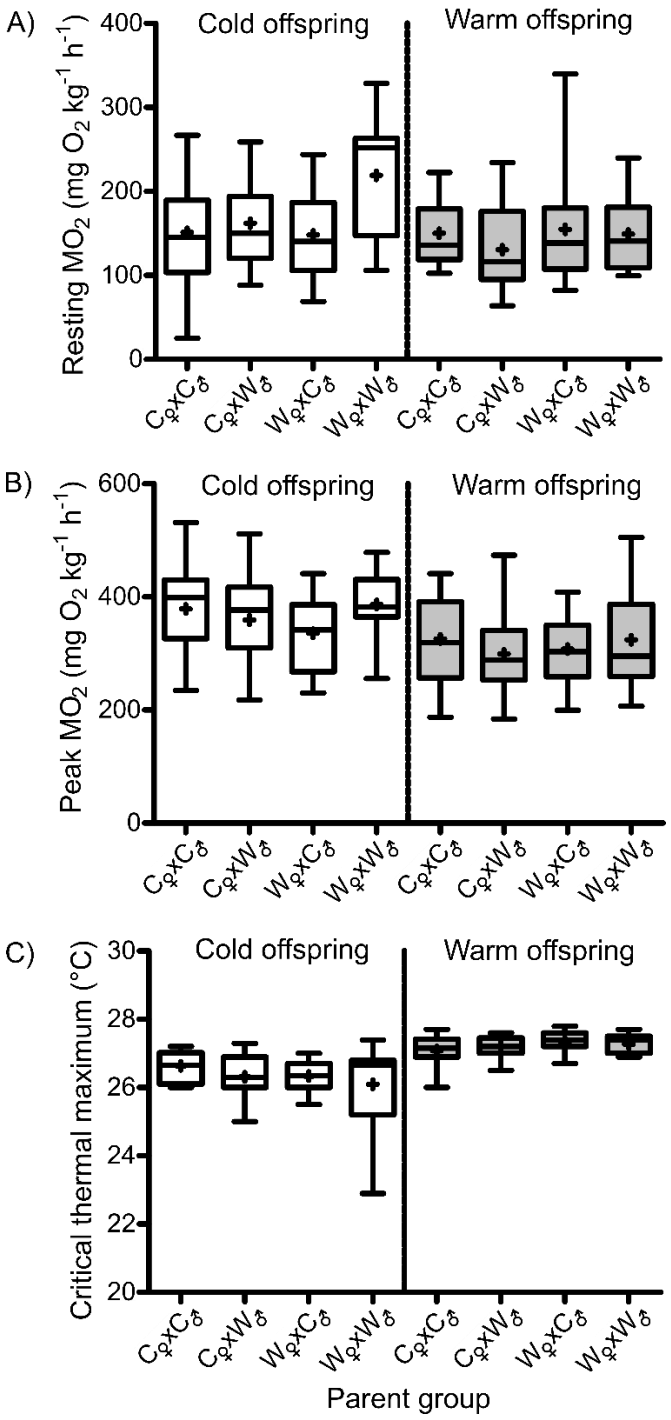

Figure 2.5: A) Resting rate of oxygen consumption (MO_2), B) peak (highest achieved, thermally-induced) MO_2, and C) critical thermal maximum (CTM) of lake trout offspring acclimated to a cold (open) or warm (shaded) temperature. Parental groups are represented as the maternal environment crossed with the paternal environment: C♀xC♂,

C♀xW♂, W♀xC♂ and W♀xW♂ where C = cold and W = warm. The plot shows the 25th and 75th quartiles with medians; means are represented as '+', and the upper and lower tails are the minimum and maximum values.

Chapter 3: Within- and transgenerational plasticity of a temperate salmonid in response to thermal acclimation and acute temperature stress

A version of this chapter has been published in Physiological and Biochemical Zoology. Penney, C. M., Tabh, J. K., Wilson, C. C., & Burness, G. (2022). Within-Generation and Transgenerational Plasticity of a Temperate Salmonid in Response to Thermal Acclimation and Acute Temperature Stress. *Physiological and Biochemical Zoology*, *95*(6), 484-499.

Abstract

The rise in temperature associated with climate change may threaten the persistence of stenothermal organisms with limited capacities for beneficial thermal acclimation. We investigated the capacity for within- and transgenerational thermal responses in brook trout (*Salvelinus fontinalis*), a cold-adapted salmonid. Adult fish were acclimated to temperatures within (10°C) and above (21°C) their thermal optimum for six months before spawning, then mated in a full factorial breeding design to produce offspring from cold- and warm-acclimated parents, and bidirectional crosses between parents from both temperature treatments. Offspring from families were subdivided and reared at two acclimation temperatures representing their current (15°C) and anticipated future (19°C) habitat temperatures. Offspring thermal physiology was measured as the rate of oxygen consumption (MO_2) during an acute change in temperature ($+2°C \cdot h^{-1}$) to observe their MO_2-temperature relationship. As performance metrics we recorded resting MO_2, the peak (highest achieved, thermally-induced) MO_2, and critical thermal maximum (CTM). Though limited, within-generation plasticity was greater than transgenerational plasticity,

with offspring warm acclimation elevating CTM by 0.5°C, but slightly lowering peak thermally-induced MO_2. Transgenerational plasticity was evident as a slightly elevated resting MO_2 and a shift of the MO_2-temperature relationship to higher rates overall in offspring from warm-acclimated parents. Further, offspring whose parents were warm-acclimated were in worse condition than those whose parents were cold-acclimated. Both parents contributed to offspring thermal responses, however, the paternal effect was stronger. Despite the existence of within- and transgenerational plasticity in brook trout, it is unlikely these will be sufficient for coping with long-term changes to environmental temperatures.

Introduction

Environmental warming due to climate change is adversely affecting the physiology and persistence of many species and populations globally (Moritz and Agudo 2013; Whitney et al. 2016). Species or populations that cannot migrate, or are restricted to localized habitats, are particularly vulnerable because they may face temperatures higher than those to which they are physiologically capable of withstanding long-term (Somero 2010). Evolutionary change may provide the best option for long-term persistence for organisms, however, the accelerated rate of climate change is likely too rapid for most organisms to respond (Comte and Olden 2017). This is especially true for those with long generation times or limited standing genetic variation (Willi et al. 2006; Munday et al. 2013; Meier et al. 2014).

Thermal acclimation (phenotypic plasticity) may help to buffer temperature effects through physiological adjustments which can occur within a single generation or over multiple generations, potentially allowing some populations to compensate for short-term (within-generation) or long-term (transgenerational) environmental change (Jablonka et al. 1992; Somero 2010; Schulte 2015; Bonduriansky et al. 2012; Norouzitallab et al. 2019). Plasticity (within- and transgenerational) is thought to have evolved in populations that experience environmental variation over time (Leimar and McNamara 2015; Beaman et al. 2016), such as for populations living in temperate regions. Here, we use the definition of transgenerational plasticity given in Bell and Hellmann (2019) and Bonduriansky (2021) which describes it as a form of plasticity where phenotypic changes occur over multiple generations through non-genetic inheritance which includes parental effects. Like within-generation plasticity,

transgenerational plasticity may not always be beneficial but if parents correctly anticipate their offspring's environment then they may pre-condition their offspring for future environmental conditions (Bonduriansky et al. 2012; Beaman et al. 2016; Norouzitallab et al. 2019). In this way, transgenerational plasticity could serve to buffer the impacts of environmental stressors and grant more time for the evolution of adaptive responses (Bernatchez 2016; Smith et al. 2016). To date, most studies of transgenerational plasticity of aquatic vertebrates have focused on temperate or tropical species (Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014), however, cold-adapted, stenothermal species are predicted to be most negatively impacted by climatic warming (Beitinger and Bennett 2000). For example, salmonid populations are currently threatened by climate change but their transgenerational responses to warming remains largely unexplored.

It is currently unclear how plasticity within- and across generations operates or interacts in organisms that experience variable habitats, or whether within-generation plasticity can override transgenerational plasticity (Shama et al. 2014; Leimar and McNamara 2015; Donelson et al. 2018). For example, lake trout (*Salvelinus namaycush*) inhabit a thermally stable environment (<10°C; Martin and Olver 1980; Wilson and Mandrak 2004) and have limited transgenerational thermal plasticity (Chapter 2; Penney et al. 2021). In contrast to lake trout, brook trout (*S. fontinalis*) occupy different thermal habitats at different life stages (Biro et al. 2008; Smith and Ridgway 2019), experience relatively high levels of environmental variation (within-lake variation: 7.2-17.7°C; Smith et al. 2020) and exhibit within-generation thermal acclimation (McCormick et al. 1972; Stitt et al. 2014; Morrison et al. 2020). It is not yet known whether organisms, like

brook trout, that display within-generation plasticity for thermal tolerance are more or less capable of transgenerational plasticity.

The brook trout is a cold-adapted salmonid native to eastern North America found in cold (10-16°C), well-oxygenated, freshwater habitats such as streams and lakes (Power 1980; Smith and Ridgway 2019). Brook trout also have a poor tolerance for warm temperatures (Beitinger and Bennett 2000) making them highly vulnerable to climate change as temperatures become warmer and suitable habitat is lost (McKenna Jr. 2019). Thermal refugia in lakes are also being reduced as epilimnetic temperatures rise and the metalimnion shrinks (King et al. 1999); in some smaller lakes brook trout populations already encounter temperatures that push them to their physiological limits (21-23°C; Smith et al. 2020) or prevent reproduction (>20°C; Warren et al. 2012).

In this study, we acclimated adult brook trout and their offspring to elevated temperatures to examine within-generation and transgenerational plasticity in offspring to a warming environment. We measured offspring thermal physiology as the rate of oxygen consumption (MO_2) and critical thermal maximum (CTM) as performance metrics. We recorded resting MO_2 at the offspring's acclimation temperature (15 or 19°C), then increased temperature at a rate of $2°C \cdot h^{-1}$ and recorded MO_2 at every +1°C during this acute temperature increase to observe the MO_2-temperature relationship. We also recorded peak (highest achieved, thermally-induced) MO_2 observed as temperature increased, and the CTM as the temperature at which the fish lost equilibrium for each acclimation group (15 or 19°C). We hypothesized that brook trout are capable of within-generation plasticity and transgenerational plasticity as responses to environmental

temperatures, and that transgenerational plasticity would enhance upper thermal tolerance (Stitt et al. 2014; Morrison et al. 2020; Chapter 2; Penney et al. 2021).

We predicted that CTM and peak MO_2 would increase with offspring warm acclimation, demonstrating within-generation plasticity (Morrison et al. 2020; Mackey et al. 2020). We also predicted that, through transgenerational plasticity, offspring with warm-acclimated parents would have a higher CTM and a higher peak MO_2 compared to offspring with cold-acclimated parents because transgenerational warm acclimation would improve thermal tolerance at elevated temperatures (Donelson et al. 2012; Shama et al. 2014; Donelson et al. 2018). The effect of transgenerational plasticity on offspring resting MO_2, and whether the effect would be beneficial or detrimental was more difficult to predict: transgenerational warm acclimation could result in a lower resting MO_2 in offspring from warm-acclimated parents, as seen in some other fish species (Donelson et al. 2012; Shama et al. 2014; Donelson et al. 2018). Alternatively, resting MO_2 could be higher, as was seen with transgenerational warming in lake trout (Chapter 2; Penney et al. 2021). To date, parental contributions to transgenerational plasticity have largely focused on the maternal environment (Shama et al. 2014; Best et al. 2018); however, paternal contributions are increasingly being reported across taxa (Hellmann et al. 2020B; Rutkowska et al. 2020). Our experimental design provided us with the opportunity to assess both maternal and paternal contributions to offspring thermal responses. We predicted that offspring and parental warm acclimation would interact (i.e., parents anticipate their offspring's environment) to strengthen the effect of transgenerational plasticity on CTM, resting and peak MO_2, and the MO_2-temperature relationship.

Methods

All experiments were approved by the Trent University Animal Care Committee (Protocol # 24794) and the Ontario Ministry Natural Resources and Forestry (OMNRF) Aquatic Animal Care Committee (Protocol FACC 136) and conducted according to the guidelines outlined by the Canadian Council on Animal Care.

The brook trout used for this study originated from wild spawn collections from a native population in Dickson Lake, Algonquin Provincial Park in south-central Ontario, Canada (45°47' N, 78°12' W). Dickson Lake has a maximum depth of 18.5 m and stratifies in the summer with temperatures ranging between approximately 8-21°C with the uppermost 10 m reaching 20-21°C, into which brook trout will venture to feed (Smith 2017; personal communication D. A. Smith 2022). Overwintering (November-May) temperatures range between 1.92-3.02°C (Cook et al. 2018B).

The captive brook trout population has been kept in the OMNRF hatchery system since 2002 under conditions to minimize hatchery selection, including equalizing family sizes, as well as rotational line crossing (Kincaid 1977) to maintain original genetic variation and minimize inbreeding (OMNRF Fish Culture Stocks Catalogue 2005; and OMNRF unpublished data). The holding facility circulated water through the tanks using a flow-through system with water from a nearby lake, and the fish experienced ambient water temperatures which was monitored daily with water temperature generally increasing from approximately 3.0 to 12°C from mid-winter to early spring.

Experimental design: Adult trout acclimation and breeding

Adult brook trout (age 5; 0.3-0.9 kg) from the Dickson Lake hatchery broodstock were transported to the OMNRF White Lake Fish Culture Station (Sharbot Lake, Ontario, Canada) in the spring of 2015 and implanted with PIT tags. A small caudal finclip (<0.25 cm^2) was taken from each individual and separately stored in 95% ethanol to enable subsequent genetic parentage analysis of offspring families (described in appendix: Supplementary methods 3.1). In May 2015, adults were divided into two groups (n = 8 and 9, 4-5 per sex), acclimated to one of two temperatures (10 ± 0.5 and 21 ± 0.5°C, respectively), and held until fall reproduction. The lower temperature was based on the temperature requirements for brook trout spawning, while the warmer temperature was selected to induce thermal stress without exceeding their physiological limits or compromising reproductive success (Hokanson et al. 1973; Blanchfield and Ridgway 1997). Each group was kept in a 6000L flow-through tank covered with opaque acrylic lids to reduce stress to the fish, with light allowed in at the inflow and outflow to provide natural photoperiod cues. The tanks received water from White Lake (44°46' N ,78°45' W), and the target temperatures (10 ± 0.5 and 21 ± 0.5°C) were achieved by mixing inflows from above and below the lake's thermocline. Fish were acclimated to these temperatures from mid-July to September, after which the temperature of each tank followed the lake's seasonal cooling beginning from September, reaching 5.2°C by mid-December for both treatment tanks. Tank water temperature and oxygen levels were checked daily, with temperature also logged every hour using two HOBO Tidbit loggers (Onset Computer Corporation, MA, USA) per tank for the duration of the adult acclimation period. The temperature data were collected from the loggers with a HOBO

USB optic reader and HOBOware Pro (v. 2.3.0; Onset Computer Corporation, MA, USA) after spawning to track acclimation temperatures throughout the duration of the experiment.

Beginning in early October, the reproductive status of the trout was checked weekly by visual inspection following mild anesthesia (0.1 g L^{-1} MS-222; Aqua Life, Syndel Laboratories Ltd., B.C., Canada), and all adults were reproductive by mid-December. As males and females came into reproductive condition, fish were dry-spawned by collecting gametes from anaesthetized fish, subdividing eggs from individual ripe females into two glass jars and fertilizing them with milt from separate males. In total, we used two males and two females from each of the two temperature treatments (8 adult fish in total) in two 2 x 2 factorial crosses (Fig. 3.1), where the offspring were from parents of matched or mismatched thermal histories: C♀xC♂, C♀xW♂, W♀xC♂ and W♀xW♂ where C = cold and W = warm. Egg numbers for all families were equalized so that 140 mL of eggs from each female were sired by each of the four males, resulting in 16 families. Fertilized egg families were transported in insulated jars packed inside a cooler with ice packs to the OMNRF Codrington Fish Research Facility (Codrington, Ontario, Canada) where they were transferred to Heath trays receiving freshwater at ambient temperature (5-6°C) under constant dim light for development.

One caveat of transgenerational studies is that parental effects cannot be accurately assessed unless a full-factorial breeding design is used (Uller et al. 2013). We used a full-factorial design, but we recognize we used only 4 males and 4 females in the crosses (technically 16 breeding pairs; 4 families in each of the C♀xC♂, C♀xW♂, W♀xC♂ and W♀xW♂ groups). One of the challenges of working with larger, non-model

organisms is providing adequate space. We opted to use fewer adults and test more offspring per family to ensure we had enough replicates from each family to test individually. A limited number of breeding adults could mean that any effects seen in offspring MO_2 may not be entirely due to parent acclimation temperature (transgenerational plasticity) but potentially due to differences in parental or family fitness. For brook trout, however, this seems somewhat unlikely based on the limited variation for standard metabolic rate observed within and among brook trout populations (Stitt et al. 2014), and the consistency of brook trout aerobic scope across independent studies that controlled for thermal acclimation (Smith and Ridgway 2019).

Experimental design: Offspring temperature acclimation

When fry reached the exogenous feeding stage, we randomly chose 20 offspring from each of the 16 families and divided them into two groups for acclimation to two different temperatures (15 and 19°C). We chose the cooler acclimation temperature based on the optimal growth temperature reported for brook trout (McCormick et al. 1972), whereas the warm temperature simulated the potential warming due to climate change in the Great Lakes region by the end of the century (Hayhoe et al. 2010). The offspring acclimation temperatures, while different than their parents, were chosen because they are ecologically relevant for the adults and juveniles (Smith et al. 2020). Further, the temperatures between the generations need not be identical for tests of transgenerational plasticity (Uller et al. 2013). Each group of 10 was moved into one of four larger (200 L) tanks: two tanks were designated for 15°C and the other two for 19°C so that each family had 10 representatives acclimated to each temperature. Each tank was separated into four

sections to keep the families separate, but due to space constraints two families were kept in each tank section where the families sharing a section had a father in common. Individuals were identified to family after measurement trials by microsatellite genotyping (described in appendix: Supplementary methods 3.1).

Temperature acclimation began after the offspring were transferred to the larger tanks. We increased the water temperature at a rate of 1°C per day using titanium heaters (500W, model TH-0500, Finnex, IL, USA) with digital temperature controllers (model 192 HC 810M, Finnex, IL, USA) until the water in each tank reached its designated temperature (15 ± 0.6 or 19 ± 0.6°C). The temperatures were checked and recorded twice daily. During this time the fish were fed 5-6 times a day at 2-3% their body weight. The experiments began after the fish had been acclimated for at least 3-4 weeks.

Respirometry set up

We explored the influence of parental thermal history on the MO_2-temperature relationship in the offspring, the resting metabolic rate, peak (highest achieved, thermally-induced) metabolic rate and upper thermal tolerance of the offspring. The metabolic rate of the offspring was measured as the rate of oxygen consumption (MO_2) using closed respirometry. We began the respirometry trial by measuring resting MO_2 at the offspring's acclimation temperature (15 or 19°C). The temperature was increased by $2°C \cdot h^{-1}$, measuring MO_2 at every 1°C increase, until the fish lost equilibrium which was recorded as the critical thermal maximum (CTM). From this dataset (MO_2 as temperature increased) we recorded the highest MO_2 achieved by each fish during the acute temperature increase. This peak (highest-achieved, thermally-induced) MO_2 may not

necessarily occur at or immediately before loss of equilibrium (CTM), as MO_2 could potentially plateau at temperatures below the CTM. Peak MO_2 also differs from maximum metabolic rate (MMR) in that peak MO_2 is the highest MO_2 observed with an acute temperature increase ($+2°C \cdot h^{-1}$) whereas MMR is usually tested with exhaustive exercise. Thus, peak MO_2 may not be the absolute maximum rate each offspring was actually capable of.

We used the same respirometry set-up and general protocol as reported previously for lake trout (Chapter 2; Penney et al. 2021). Each experimental trial used eight custom-built respirometers. Respirometers were made from a 8 cm diameter glass tube that was cut at a 4.5 cm length and sealed at one end (i.e. the floor of the chamber) for a total volume of 226 cm^3. The respirometer lids were made of acrylic. Each lid contained a fitting in the center for an O_2 probe, and valves on opposite sides of the probe fitting to allow water to circulate through the respirometer chamber. Two respirometers were placed in each of four transparent plastic tubs and the tubs were seated on top of two, side-by-side stir plates (one plate per respirometer). The plates were used to spin a magnetic stir bar in each respirometer at approximately 60 RPM to prevent the establishment O_2 gradients in the chambers and to keep water moving past an O_2 probe (Clark-type polarographic electrode, model DO-BTA, Vernier Software and Technology, OR, USA) that was inserted into the lid of each respirometer. The O_2 probes were connected to a Lab Pro interface (Vernier Software and Technology) and O_2 concentration within the respirometers was recorded every second using LoggerPro software (version 3.8.6; Vernier Software and Technology). Each respirometer also contained a perforated steel grid to separate the fish from the stir bar. Water from the tub was circulated through

the respirometer at 4.5 L per minute using a submersible pump (universal type 1005, EHEIM GmbH & Co., Deizisau, Germany), and the water in each tub was also circulated with aerated, temperature-controlled freshwater from a source tank.

Respirometry and critical thermal maximum protocol

Respirometry trials were conducted from August 9 to September 15, 2016. The night before a trial, eight fish (mass: range 1.1-5.5 g; median 3.0 g) were individually transferred into clean respirometers where they received a continuous flow of fresh water maintained at their acclimation temperature and delivered via vinyl tubing. They were left to adjust to the experimental apparatus overnight, and a thin sheet of black plastic covered each tub to minimize visual disturbance to the fish during the adjustment period and experimental trial. Fish were fasted for at least 12 hours prior to each trial to eliminate the physiological effects of digestion on the experimental results (Millidine et al. 2009).

We began measuring MO_2 in each individual fish the next morning at 7:00. To measure MO_2, the respirometer chambers were sealed by manually closing the respirometer valves and switching off the pumps that circulated water through the chambers. After a 30 second wait period, the reduction in chamber O_2 concentration was recorded for 10 minutes. Afterwards, the flow valves were reopened to restore water circulation. Water temperature was then increased at a rate of 2°C per hour and the MO_2 of each fish measured at each 1°C increase with 30 minutes between the repeated MO_2 measurements. The rate of oxygen consumption (MO_2) was calculated as,

$$MO_2 = \frac{(Rate\ of\ decline\ [O_2])(V_R - V_F)x60}{h}$$

where *(Rate of decline in [O_2])* is the decline in water oxygen concentration (mg O_2 L^{-1} min^{-1}) during the 10-minute measurement period, V_R is the volume (L) of the respirometers, V_F is the volume of the fish (L) and h is the time in hours. The background microbial respiration, measured at the end of the respirometry trials, was nil. The rate of decline was determined with LoggerPro, and we measured the linear fit of the drop in respirometer O_2 concentration over time. If the linear correlation coefficient (r) was below 0.8, the datapoint was excluded from the analysis. This resulted in the exclusion of 225 out of 2,845 total datapoints collected. Some of these excluded values were measures of resting and peak MO_2 (of 230 individuals, 43 resting MO_2 and 5 peak MO_2 were not included in the analysis).

The critical thermal maximum (CTM) for each fish was recorded as the temperature when it lost its righting response (i.e. equilibrium) and this was recognized as the point at which the fish could no longer maintain an upright position within the respirometer. All fish were closely monitored as temperature increased, and when a fish lost equilibrium it was quickly removed from the respirometer and euthanized with 0.3 g L^{-1} of tricaine methanesulfonate (MS-222; Aqua Life, Syndel Laboratories Ltd., B.C., Canada). Euthanized fish were immediately blotted dry on paper towels, measured for mass to the nearest 0.1 g and fork length (mm) using digital balance and calipers, respectively. Measurements of mass and length were used to calculate condition factor using the following formula,

$$Conditionfactor = \frac{mass}{(forklength)^3} x100$$

A tissue sample (caudal finclip) was taken from the euthanized fish and individually stored in 95% ethanol for microsatellite genotyping to identify each offspring to their

respective family (described in appendix: Supplementary methods 3.1). Twelve of the 230 fish used for this experiment died at or just prior to collecting CTM, so were not included in the analysis of CTM.

To ensure that O_2 would not become limiting at warmer temperatures, we monitored O_2 saturation of the water throughout each trial. The source tank O_2 concentration was kept at 6.0-7.0 mg L^{-1} and continuously checked with a YSI Pro probe (Hoskin Scientific, ON, Canada). If saturation levels lowered at high temperatures, O_2 was supplemented to the source tank water using airstones and a tank of compressed O_2 while ensuring that hyperoxia did not occur. Because the fish consumed O_2 with increased rates at higher temperatures, we shortened the measurement period (<10 minutes) as necessary to avoid the O_2 concentration from reaching the critical limit of 3.5 mg O_2 L^{-1} during the MO_2 measurement to avoid inducing a hypoxia response in the fish (Graham,1949; Doudoroff and Shumway 1970).

Calculations and statistical analysis

The MO_2 measured at the fish's acclimation temperature before temperature began to rise with the acute temperature challenge was considered as the fish's resting MO_2. We report peak MO_2 as the highest MO_2 achieved during the respirometry trial. We do not report aerobic scope here because our measurement of peak MO_2 may not necessarily represent the absolute maximum MO_2 achievable by the offspring; max. MO_2 is typically obtained using exhaustive exercise protocols which we did not use in this study. We analyze whole animal rates of O_2 consumption with mass as a covariate rather than perform the analysis on mass-specific values because the former is statistically more

appropriate (Hayes and Shonkwiler 1996). The mean values reported from these models are referred to as mass-adjusted MO_2, however, we also provide the data plotted as mass-specific MO_2 in the appendix (Fig. A3.1).

The effect of parent and offspring acclimation temperatures on mass and condition factor was assessed using a general linear mixed effects model (GLMM). The models for mass and condition factor included offspring acclimation temperature (T_O: cold or warm) and parental acclimation temperature (both parents combined into a single parental group: C♀xC♂, C♀xW♂, W♀xC♂ or W♀xW♂) as fixed effect predictors. An interaction term between offspring and parent acclimation temperature was also included as a fixed effect predictor to determine if parental acclimation temperature had differential effects on offspring mass and condition depending on whether the offspring were acclimated to a cold or warm temperature. Degree days was included as a random intercept to account for the potential effects of age on mass and condition factor. Degree days were calculated for each fish as the cumulative temperature experienced above 0°C (Chezik et al. 2013; Cook et al. 2018A) until the beginning of the experimental trial. A Tukey's HSD post-hoc analysis was performed if the test determined a significant effect of fixed predictors on mass or condition to uncover where differences occurred among pairwise comparisons.

To identify factors contributing to variation in resting MO_2, peak MO_2 and CTM, we evaluated competing statistical models using an Akaike Information Criterion corrected for small sample size (AICc). The possible model terms included offspring acclimation temperature (T_O), maternal acclimation temperatu™T_M) and paternal acclimation temperature (T_P) as fixed effect predictors, with interactions between all factors. Including maternal and paternal effects as separate terms (instead of as single

parental groups: C♀xC♂, C♀xW♂, W♀xC♂ or W♀xW♂) allows us to investigate the relative parental contribution to offspring resting MO_2, peak MO_2 and CTM. An additive effect of parental acclimation would be detected as both maternal and paternal acclimation temperature appearing in the models ($T_M + T_P$). Models also included offspring mass as a covariate because the warm-acclimated offspring grew heavier than cold-acclimated ones and because metabolic rate scales with mass. The effects of maternal ID (ID_M) and paternal ID (ID_P) were included as random intercepts to control for statistical non-independence of offspring relatedness as some were full- or half-siblings based on the 2 x 2 factorial mating design. From the model AIC values, we calculated the ΔAIC, evidence ratio (ER) and Akaike weight (Wi) for each model and considered the best models as those with a $\Delta AIC \leq 2$ (Burnham and Anderson 2002). All models with a $\Delta AIC \leq 2$ were therefore included in the results. We used the calculated AIC metrics to compare the models and identify common parameters among the models that explained variation in resting MO_2, peak MO_2 and CTM. We generated figures using the residuals from a model containing the natural log of mass (fixed effect), and maternal and paternal identity (random effects) to compare the direction of the effects of offspring, maternal and paternal temperature acclimation on critical thermal maximum, and resting and peak MO_2 from a presumed population mean of 0 ($y = 0$).

To detect within-generation plasticity and transgenerational plasticity in the metabolic response of the offspring to an acute temperature challenge, we tested whether offspring, maternal, and paternal acclimation temperature influenced the effect of acute temperature exposure on an offspring's MO_2. If the relationship between acute temperature exposure and an offspring's MO_2 were linear (or conformed with a low-order

polynomial function), we would achieve this end by using a GLMM with offspring MO_2 as a dependent variable, and temperature exposure, parental acclimation treatment, offspring acclimation treatment, and interactions between each parameter as independent variables. Across all offspring, however, the relationship between temperature exposure and MO_2 was not linear, nor could it be explained by a simple polynomial function. To account for this non-linearity, we first modelled the effect of acute temperature exposure alone on offspring MO_2 using a generalized additive model (GAM) with MO_2 as a dependent variable and temperature exposure as a cubic regression spline with 7 knots to avoid model overfitting. We then tested whether parental or offspring acclimation treatments (or any combination of each) could explain the remaining variation between an offspring's true MO_2 at a given temperature exposure, and that explained by temperature exposure alone (β_{Ta}; predicted by our GAM) using a GLMM (similar to Chapter 2; Penney et al. 2021). This approach is similar to using residual MO_2 as a dependent variable and allowed us to test for broad differences in the non-linear effect of acute temperature exposure on MO_2 among acclimation treatments. Unlike using residual MO_2 as a dependent variable, however, our approach allowed us to test for the influence of offspring and parental acclimation temperature on the slope of the non-linear, MO_2-temperature relationship and not just its vertical position.

Here, our GLMM included the true MO_2 of offspring as the dependant variable, with the offspring's expected MO_2 at a given temperature (β_{Ta}), offspring acclimation temperature (T_O; cold and warm), maternal acclimation temperature (T_M; cold and warm), paternal acclimation temperature (T_P; cold and warm), mass, and all interactions between β_{Ta}, T_O, T_M, and T_P as independent variables. As with our previously described models,

our GLMM also included random intercepts for maternal, paternal, and offspring identities (ID_M, ID_P, and ID_O). Finally, we included a type I autoregressive correlation structure ($\rho = 0.221$) in our model to correct for autocorrelation between MO_2 measurements as they occur at adjacent points (temperatures) during the acute temperature challenge (as per Chapter 2; Penney et al. 2021).

In our GLMM, a significant effect of β_{Ta} would indicate that changes in MO_2 across the acute temperature challenge could be reliably explained by the non-linear relationship modelled in our GAM (i.e. the expected values of offspring MO_2 correlate with their true values at each temperature). Significance of other independent factors would suggest that they shift the non-linear MO_2-temperature relationship up or down, while significant interactions between β_{Ta} and the other factors mean that they tilt the MO_2-temperature relationship.

All statistical analyses were conducted in JMP 13 (v. 18.1) or R (v. 3.5.2) with the level of significance set to 0.05. Linearity, homogeneity of variance, sample independence and residual normality were confirmed visually, and with the Shapiro-Wilk W, Levene's and Brown-Forsythe tests. The factors that contributed to variation in body mass and condition factor were investigated using JMP 13. Statistical analyses of the resting and peak MO_2, CTM, and MO_2 during the temperature challenge were conducted using R with the 'MuMIn' (version 1.43.15; Barton 2019), 'lme4' (Bates et al. 2015), 'nlme' (version 3.1-143; Pinheiro et al. 2019) and 'mgcv' (Wood 2011) packages. We discovered that one of the peak MO_2 datapoints was five standard deviations below the mean, therefore, this datapoint was not included in the final analysis.

Results

Mass and condition factor

Warm-acclimated offspring were heavier overall compared with the cold-acclimated offspring (19°C offspring: 3.31 ± 0.08 g; 15°C offspring: 2.73 ± 0.08 g, GLMM: $F_{1,24.60} = 25.22$, $p < 0.01$). Parental acclimation temperature had a significant effect on offspring mass (GLMM: $F_{3,52.71} = 13.78$, $p < 0.01$) where offspring from parental groups C♀xC♂ and W♀xC♂ (3.50 ± 0.11 vs. 3.22 ± 0.11 g) were significantly heavier ($p < 0.05$) than those from the C♀xW♂ and W♀xW♂ parental groups (2.66 ± 0.12 vs. 2.69 ± 0.11 g), indicating that offspring with cold-acclimated fathers (C♀xC♂ and W♀xC♂) were heavier than those with warm-acclimated fathers. No other parental group comparisons were significantly different. There was no interaction between offspring acclimation and parental acclimation group (GLMM: $F_{3,52.71} = 0.34$, $p = 0.80$).

Warm-acclimated offspring had higher condition factor than cold-acclimated offspring (1.0 ± 0.01 vs. 0.96 ± 0.01; GLMM: $F_{1,23.25} = 27.16$, $p < 0.01$) and the condition factor of offspring was significantly affected by parental acclimation temperature (GLMM: $F_{3,53.89} = 6.10$, $p < 0.01$). There was a transgenerational effect of parental temperature acclimation on offspring condition factor: offspring from parents that were both cold-acclimated (C♀xC♂) were in significantly better condition than offspring from parents that were both warm-acclimated (W♀xW♂; 1.0 ± 0.01 vs. 0.95 ± 0.01, respectively; $p < 0.05$). No other parental groups differed significantly from each other. There was no significant interaction between offspring acclimation temperature and parental acclimation group (GLMM: $F_{3,53.89} = 0.13$, $p = 0.94$).

Critical thermal maximum

Critical thermal maximum was influenced by offspring acclimation temperature (within-generation plasticity) but not transgenerational (i.e. parental) acclimation. Offspring acclimation temperature (T_O) along with maternal and paternal ID (random effects: ID_M and ID_P) best explained the variation in offspring CTM ($\Delta AIC \leq 2$, Table 3.1), no other model was within a $\Delta AIC \leq 2$. The effect of offspring acclimation temperature resulted in an approximately 0.5°C higher average CTM in warm-acclimated offspring versus cold-acclimated offspring (28.6 ± 0.03 vs. 29.1 ± 0.02; Fig. 3.2A and B).

Resting and peak metabolic rate

The brook trout MO_2 values were within the expected range reported for similar-sized trout (Myrick 2003). Resting MO_2 was affected by offspring and parental acclimation temperature. The AIC revealed four models that best explained variation in resting MO_2, and each included *Mass* and maternal acclimation temperature (T_M), with maternal and paternal ID (ID_M and ID_P) as random effects (Table 3.1). The first model contained only these factors, while offspring acclimation temperature (T_O) appeared in models 2 and 3 with an interaction occurring between offspring and maternal acclimation temperature in model 3 suggesting that resting MO_2 depends on whether or not offspring and maternal environment are consistent with each other (Table 3.1). Paternal acclimation temperature (T_P) appeared only once in the top four models and occurred in model 4, which was 2.11 (ER) less likely to best explain variation in the data when compared with model 1 (Table 3.1). We plotted the resting MO_2 residuals to observe the direction of the effects and saw that warm-acclimated offspring tended to have residual resting MO_2

values slightly below the population mean (Fig. 3.3A & B). With regard to parental acclimation temperature, the residual resting MO_2 of cold-acclimated offspring from warm-acclimated mothers and fathers (Fig. 3.3A & B) was higher than the population mean. In contrast, resting MO_2 was lower than the population mean for warm-acclimated offspring from warm-acclimated mothers (Fig. 3.3A) and warm-acclimated fathers (Fig. 3.3B). Together this suggests a transgenerational effect of lowering resting MO_2 when parents and offspring each experience warming. The interaction between maternal and offspring acclimation temperature ($T_O \cdot T_M$) was evident in the residual plot (Fig. 3.3A): when mothers were cold-acclimated the residual resting MO_2 of their cold-acclimated offspring was slightly lower than that of their warm-acclimated offspring, this trend reversed when the mothers were warm-acclimated. Although Fig. 3.3 shows the direction of the effects detected by the AIC, it is important to note that in each case the confidence intervals overlap zero suggesting the effect size is small.

Peak MO_2 was also affected by offspring and parental acclimation temperature. Three models best explained variation in peak MO_2 ($\Delta AIC \leq 2$), each with the random effects of maternal and paternal ID (ID_M and ID_P) (Table 3.1). *Mass* and offspring acclimation temperature (T_O) were the best predictors of peak MO_2 as these factors occurred in all three models and were the only factors in the first model. Paternal (T_P, model 2) and maternal (T_M, model 3) acclimation temperature each appeared only once among the three models. Plots of the residual peak MO_2 showed only a slight effect of offspring acclimation temperature, being marginally lower in warm-acclimated offspring (Fig. 3.3C & D). Warm-acclimated mothers and warm-acclimated fathers slightly elevated the peak MO_2 in their offspring (Fig. 3.3C & D). As with resting MO_2, the

confidence intervals around peak MO_2 in Fig. 3.3 overlap zero suggesting a small effect size.

Metabolic response of offspring to an acute temperature challenge

Offspring MO_2 increased with challenge temperature and the correlation between acute challenge temperature and MO_2 (GAM) was supported (β_{Ta}: $p < 0.001$; Table 3.2; Fig. 3.4). Offspring mass had a significant effect on oxygen consumption, as rates were higher in heavier fish (*Mass*: $p < 0.001$; Table 3.2), but the MO_2-temperature relationship was similar between offspring of cold and warm acclimation temperatures (T_O: $p = 0.880$; Table 3.2).

There was a transgenerational effect of parental acclimation temperature on offspring MO_2 responses to an acute temperature challenge. The acclimation temperature of the mothers and fathers (Fig. 3.4A & B, respectively) each significantly affected the offspring's metabolic response to the challenge temperature. That is, the MO_2 of the offspring from warm-acclimated parents was elevated compared with offspring from cold-acclimated parents (Fig. 3.4A & B). While the effect was significant for both parents, a stronger effect occurred on the paternal side (T_M: $p = 0.042$; T_P: $p = 0.010$; Table 3.2).

There was a significant statistical interaction between β_{Ta} and paternal acclimation temperature ($\beta_{Ta} \cdot T_P$: $p = 0.007$; Table 3.2) indicating a tilt in the offspring's MO_2-temperature curve depending on the acclimation temperature of the father. Offspring mass-adjusted MO_2 was lower in offspring from cold-acclimated fathers compared to those from warm-acclimated fathers when challenge temperatures were below 25°C, but

the groups tended to converge at temperatures above 25°C (Fig. 3.4B). No other interaction terms were significant.

Discussion

We found evidence of within- and transgenerational plasticity in brook trout though both forms of plasticity were limited. Thermal acclimation had overall greater effects on within-generation plasticity than on transgenerational plasticity. Offspring warm acclimation resulted in a small (0.5°C) increase in critical thermal maximum as predicted, but it unexpectedly lowered resting MO_2 and peak MO_2 slightly. Limited within-generation plasticity was also seen with the acute temperature challenge, where the MO_2-temperature relationship did not differ between the two offspring acclimation temperatures (T_O).

Our results partially supported the prediction that transgenerational plasticity would improve thermal tolerance at elevated temperatures (Table 3.1). Parental warm acclimation had no effect on offspring critical thermal maximum, but both maternal and paternal acclimation temperature influenced offspring resting MO_2, and to a lesser extent peak MO_2. Maternal and paternal warm acclimation each contributed to an overall upward shift in the offspring's MO_2-temperature relationship. Surprisingly, the paternal contribution to offspring thermal performance (MO_2-temperature relationship) was larger than the maternal contribution.

Within-generation plasticity

Within-generation plasticity was observed in the critical thermal maximum, in that it increased with offspring acclimation temperature. Critical thermal maximum was in the expected range for brook trout acclimated to our temperatures (Wehrly et al. 2007; O'Donnell et al. 2020), however, it only increased by ~0.5°C in warm-acclimated offspring despite the 4°C difference in acclimation temperature between the two groups of offspring. We cannot be certain whether this modest increase in CTM was genuine within-generation plasticity or an artefact of experimental starting temperature. Though the rate of heating used in our experiment ($+2°C \cdot h^{-1}$) has been deemed appropriate rate for measuring critical thermal maximum in brook trout (Galbreath et al. 2004), it is unclear whether significant differences in critical thermal maximum would have occurred with a slower rate of heating. For example, Morrison et al. (2020) found that the critical thermal maximum of 20°C acclimated brook trout was significantly higher than that of 15°C acclimated trout (approximately 31.7 vs. 30.5°C) using a heating rate of 0.3°C per minute (i.e. $+18°C \cdot h^{-1}$).

We detected an effect of thermal acclimation on offspring resting MO_2, however, the effect was small (Table 3.1). Resting metabolic rate typically increases with acclimation temperatures until the individual reaches its *pejus* temperature (approximately 20°C for brook trout: Hartman and Cox 2008). Thus, we anticipated that resting MO_2 would be higher in warm-acclimated offspring compared to cold-acclimated offspring, largely because they were being measured at a warmer temperature, but we did not see this trend. Further analysis revealed that resting MO_2 also did not differ at common temperatures (19°C-acclimated at 19°C vs. 15°C-acclimated at 19°C). A

previous study on variation in upper thermal tolerance and metabolic rate of brook trout found that individuals originating from Dickson Lake (the same lake from which our brook trout originated) had a higher standard metabolic rate following acclimation to 16°C compared to 20°C, though the authors did not suggest the lower standard metabolic rate at 20°C was due to the fish reaching their *pejus* temperature (Stitt et al. 2014). Stitt et al.'s (2014) experimental temperatures were comparable to ours (15-16°C and 19-20°C), however, the fish tested were of different life stages (yearling vs. adult).

Offspring acclimation temperature had a modest effect on peak MO_2, demonstrating some within-generation plasticity in this parameter. Some fish species are capable of extending the upper limit of MO_2 (i.e. peak MO_2) when acclimated to warmer temperatures (reviewed by Schulte 2015). For example, exercise-induced maximum metabolic rate (MMR) increased by 20-30% in lake trout acclimated from 8 to 15°C (Kelly et al. 2014). It is important to note that peak MO_2 and MMR differ in that peak MO_2 is the highest MO_2 observed with an acute temperature increase ($+2°C \cdot h^{-1}$) whereas MMR is usually tested with exhaustive exercise. Though related to MMR, peak MO_2 may not be the absolute maximum rate each offspring was actually capable of achieving. The small effect of offspring acclimation temperature could suggest that brook trout peak MO_2 is not capable of further increases. This generally agrees with the idea that metabolic ceilings, like peak MO_2 or critical thermal maximum, are relatively thermally (acclimation) insensitive (Sandblom et al. 2016; Norin and Metcalfe 2019; Morrison et al. 2020).

Offspring acclimation temperature did not influence the offspring's MO_2 response to an acute temperature challenge ($+2°C \cdot h^{-1}$). Interestingly, offspring MO_2 did not begin

to rise until the challenge temperature exceeded 23°C. Although unusual, we are confident this result was not an experimental artefact. We used the same respirometry set up and experimental protocol to address parallel questions in lake trout, which displayed an increase in MO_2 with increasing temperature, as would be predicted (Chapter 2; Penney et al. 2021). For this same reason, we do not suspect an effect of thermal inertia in brook trout in our study. The sudden increase in MO_2 at ~23°C in brook trout could be due to a physiological stress response(s) being initiated at this temperature, especially considering that 23°C is near the upper incipient lethal temperature recorded for these fish (24°C; Fry et al. 1946; Wehrly et al. 2007). Such a rapid increase could also occur with hypoxia stress. Although we monitored O_2 concentration throughout each respirometry trial to ensure levels did not reach the limit that would induce a hypoxia stress response (3.5 mg O_2 L^{-1}; Graham 1949; Doudoroff and Shumway 1970) we acknowledge that hypoxemia could have occurred. Identifying the physiological processes that result in the increased MO_2 at ~23°C in brook trout would require further study. Chadwick et al. (2015) saw that levels of HSP70 and glucose increased in juvenile brook trout when challenge temperatures reached approximately 21°C. It is possible that stress responses, such as induction of molecular chaperones or mobilization of energetic resources were initiated at 23°C in the juvenile brook trout in our study, thus increasing metabolic rate at this temperature. It is also unclear whether metabolic compensation may have been occurring in our brook trout to keep O_2 consumption at a steady rate up to the point of 23°C, however, further experimentation would be required to confirm this.

Transgenerational plasticity

Offspring condition factor was reduced overall with transgenerational warm acclimation. We cannot confirm with certainty that this is a condition transfer effect (Bonduriansky and Crean 2018). However, a reduced condition factor could potentially have negative downstream effects on fecundity though it is unclear whether a low condition factor would persist into adulthood.

Both maternal and paternal acclimation temperature affected the offspring's MO_2-temperature relationship, with an overall upward shift for offspring from warm-acclimated parents. This was also reflected in offspring resting MO_2. A higher resting metabolic rate could indicate faster growth (especially if food is plentiful), meaning that fish may mature faster. Similar to our findings for lake trout (Chapter 2; Penney et al. 2021), however, parental warm acclimation did not contribute to faster growth in brook trout when fed in amounts of 2-3% their body weight; offspring from warm-acclimated parents were not larger than those from cold-acclimated parents. A higher MO_2 could also mean that physiological systems are upregulated to respond to stressors, keeping the fish alive until the stressors subside (Norin and Metcalfe 2019; Rosenfeld et al. 2020). While this may benefit short-term survival, prolonged elevated resting MO_2 due to environmental stressors could reduce the energetic resources necessary for growth and, later in life, reproduction (Somero 2010; Rosenfeld et al. 2020). Our results suggest that brook trout offspring will incur a higher cost of living (Norin and Metcalfe 2019) when their parents experience warmer summers.

It is thought that transgenerational plasticity is adaptive when the environment varies across generations and parents can correctly anticipate their offspring's

environment (Jablonka et al. 1992; Bonduriansky et al. 2012; Norouzitallab et al. 2019). Based on this idea, transgenerational plasticity would be predicted to be weak in stenothermal organisms that have adapted to habitats that are thermally stable across generations. The limited available evidence supports this: in lake trout (*S. namaycush*), a cold-adapted stenothermal congener of brook trout, transgenerational plasticity was limited, and most evident as elevated MO_2 in warm-acclimated offspring from warm-acclimated parents (Chapter 2; Penney et al. 2021). In contrast, in eurythermal or warm-adapted fish species, metabolic rates are *reduced* in warm-acclimated offspring from warm-acclimated parents compared to cold-acclimated parents (Donelson et al. 2012; Shama et al. 2014; Donelson et al. 2018). How changes in MO_2 through transgenerational plasticity influences fitness in future generations is not immediately clear. An increase in MO_2 with warming could indicate an increase in the use of energy for certain physiological processes like protein synthesis for growth or repair. This may be sustainable if food is plentiful and there is sufficient metabolic scope remaining for reproduction (Schulte 2015; White and Whal 2020). Conversely, energy reallocation is also possible where more energetic resources are diverted to thermal responses (i.e. survival), potentially reducing growth or reproduction. In this case, a change in MO_2 may not be observed, but its effects on body size and fecundity could be apparent later in life.

In our study, peak MO_2 varied only slightly with maternal or paternal acclimation temperature, and no transgenerational effect on offspring critical thermal maximum was detected. Our results agree with the limited number of studies on the transgenerational effects in temperate fish (Sandblom et al. 2016; White and Wahl 2020) and other cold-adapted fish (Chapter 2; Penney et al. 2021). Together, these studies suggest

transgenerational plasticity is unlikely to significantly alter critical thermal maximum or peak MO_2 in response to increased environmental temperatures over relatively short multi-generation timespans, reinforcing evidence that these metabolic ceilings are likely to be exceeded in ecological timeframes (Sandblom et al. 2016; Norin and Metcalfe 2019; Morrison et al. 2020).

Relative parental contributions

Although both maternal and paternal thermal history (temperature acclimation) each contributed to offspring thermal physiology by elevating the MO_2 of their warm-acclimated offspring, we did not find strong evidence that transgenerational effects were additive (i.e., stronger when the offspring had a warm mother *and* a warm father; $T_M + T_P$). Maternal and paternal acclimation temperature appeared in the same model only once for resting MO_2 (model 4, Table 3.1), but not for peak MO_2 or critical thermal maximum. Similarly, each parent contributed to their offspring's MO_2 response to an acute temperature increase.

Paternal effects have received less attention relative to maternal effects (Rutkowska et al. 2020) and the size of the epigenetic paternal contribution to such changes relative to the maternal contribution is still debated (reviewed by Best et al. 2018). In the few studies that have tested relative parental contributions to transgenerational plasticity in metabolic traits in fish, the paternal contribution is either less than (Shama et al. 2014) or comparable to the maternal contribution (Chapter 2; Penney et al. 2021). In this study, fathers surprisingly appeared to have greater contributions to transgenerational plasticity than did mothers. Paternal effects are complex, can depend on the sex of the offspring, and can vary depending on the

environment experienced by paternal grandparents (Crean and Bonduriansky 2014; Hellmann et al. 2020B). Environmentally-mediated epigenetic changes do occur in sperm (Immler 2020; Ord et al. 2020) and these along with cytoplasmic components can influence offspring phenotypes (summarized by Donkin and Barrès 2018). Parents can also have opposing effects on gene expression in their offspring despite both parents having received the same treatment, in that a gene may be maternally downregulated but paternally upregulated in the offspring (Bautista et al. 2020). While epigenetic regulation of gene expression may be an underlying factor in the paternal contribution we observed in our study, we are not aware of another study showing such a large paternally-mediated transgenerational plasticity contribution to thermal responses relative to the maternal contribution.

Summary and perspectives

While within-generation plasticity was evident in peak MO_2 and critical thermal maximum, it was through transgenerational plasticity that warm-acclimated parents elevated resting MO_2 and affected the MO_2-temperature relationship in offspring. The importance of transgenerational plasticity relative to within-generation plasticity may depend on life stage and variation in the habitat experienced at each life stage. It is possible that transgenerational effects are strongest in early-juvenile life stages (Yin et al. 2019), but only when the environment is stable. In contrast, within-generation plasticity may be favoured when temperatures are more variable (Leimar and McNamara 2015). In fact, in situations where environmental temperature variation exists, transgenerational plasticity effects may be overridden by within-generation plasticity, as found in

stickleback (Shama 2017). Our study examined juvenile brook trout 5-6 months after hatching, at a time when they would be feeding in shallow depths near shore and near the surface in warmer water (Biro et al. 2008). Experiments examining within- and transgenerational plasticity at multiple life stages could be very informative, though we are not aware of any such studies to date.

Brook trout exhibited less of a response to within- and transgenerational acclimation than expected. It is thought that plasticity occurs in populations experiencing predictable environmental variation over time (Bonduriansky et al. 2012; Beaman et al. 2016; Norouzitallab et al. 2019). For example, compared to brook trout, lake trout live in a more thermally stable habitat and have little variation in within-generation thermal plasticity (Kelly et al. 2014) and limited transgenerational plasticity (Chapter 2; Penney et al. 2021). As such, we had expected to detect greater plasticity in brook trout given the greater degree of thermal variation experienced by brook trout over their lifetime (McCormick et al. 1972; Stitt et al. 2014; Morrison et al. 2020). Although our results represent the response of brook trout to anticipated warming due to climate change (+4°C; Hayhoe et al. 2010), it is possible that a within-generation plastic response may have been stronger with acclimation temperatures that differ by more than 4°C.

Transgenerational effects on offspring phenotypes depend on genotype or ecotype (Verhoeven and van Gurp 2012; Vayda et al. 2018), and transgenerational plasticity is predicted to arise in populations that experience variation in temperature over multiple generations (Beaman et al. 2016; Yin et al. 2019). Given that different populations of brook trout display variation in thermal tolerance and capacity for acclimation across populations (McDermid et al. 2012; Stitt et al. 2014) it would be informative to assess

whether transgenerational responses to warming vary among stream and lake populations of brook trout, and across the species' range. One might predict, for example, that daily as well as seasonal thermal variation in stream environments (Chadwick and McCormick 2017) would select for increased transgenerational plasticity compared with lake habitats. Family can also be an important contributor to variation in the metabolic response to temperature (Cook et al. 2018A). We simply accounted for this variation by including family as a random effect in our analyses, but we acknowledge that this existing variation among families could serve as potential substrate for selection.

Transgenerational plasticity may be adaptive for some species of tropical or eurythermal fish (Donelson et al. 2012; Shama et al. 2014; Donelson et al. 2018), buffering the impact of environmental stressors associated with climate change (Bonduriansky et al. 2012; Bernatchez 2016; Smith et al. 2016), but this may not be true for some temperate or stenothermal fish species (*Salvelinus namaycush*: Chapter 2; Penney et al. 2021; brook trout: this study; but see Houle et al. 2023). Our results underscore the importance of conservation programs and environmental monitoring to protect species that are threatened by climate change and have no opportunity for migration, have long generation times or limited standing genetic variation, and limited plasticity (within and across generations).

Tables

Table 3.1: Summary of the top models determined with AIC_C to explain variation in brook trout offspring resting rate of oxygen consumption (MO_2), peak MO_2 and critical thermal maximum (CTM) with transgenerational acclimation.

Measure	Model	ΔAIC	ER	W_i	R^2	Model
CTM	1	0	1	1	0.51	$T_O + ID_M + ID_p$
Resting MO_2	1	0	1	0.39	0.19	$Mass + T_M + ID_M + ID_P$
	2	1.10	1.73	0.22	0.21	$Mass + T_O + T_M + ID_M + ID_P$
	3	1.26	1.88	0.21	0.22	$Mass + T_O + T_M + (T_O \cdot T_M) + ID_M + ID_P$
	4	1.49	2.11	0.18	0.17	$Mass + T_M + T_P + ID_M + ID_P$
Peak MO_2	1	0	1	0.48	0.52	$Mass + T_O + ID_M + ID_P$
	2	1.02	1.67	0.29	0.51	$Mass + T_O + T_P + ID_M + ID_P$
	3	1.53	2.15	0.23	0.53	$Mass + T_O + T_M + ID_M + ID_P$

Offspring (age: 5 months) were from parents acclimated to either a cold or warm temperature and were similarly acclimated to cold or warm temperature. T_P, T_M, T_O are the paternal, maternal and offspring acclimation temperatures, respectively, and ID_M and ID_P are the maternal and paternal individual identification (random effects). All models with a ΔAIC value ≤ 2 were included.

Table 3.2: Factors contributing to variation in brook trout rate of oxygen consumption (MO_2).

Parameter	Coefficient	S. E.	DF	t-value	p-value
Intercept	-0.84	0.11	17.87	7.48	**<0.001**
Mass	0.20	0.02	182.14	12.27	**<0.001**
β_{Ta}	1.19	0.09	2338.40	13.03	**<0.001**
Offspring acclimation temperature, T_O	0.02	0.12	483.13	0.15	0.880
Maternal acclimation temperature, T_M	0.22	0.11	180.89	2.05	**0.042**
Paternal acclimation temperature, T_P	0.44	0.14	11.08	3.08	**0.010**
$T_O \cdot T_M$	0.011	0.15	545.40	0.07	0.941
$T_O \cdot T_P$	-0.05	0.16	869.28	0.30	0.766
$T_M \cdot T_P$	-0.22	0.16	943.48	1.38	0.168
$\beta_{Ta} \cdot T_O$	-0.003	0.12	2339.95	0.02	0.981
$\beta_{Ta} \cdot T_M$	-0.17	0.12	2337.07	1.43	0.152
$\beta_{Ta} \cdot T_P$	-0.36	0.13	2338.77	2.70	**0.007**
$T_O \cdot T_M \cdot T_P$	-0.15	0.22	646.15	0.70	0.487
$\beta_{Ta} \cdot T_O \cdot T_M$	-0.13	0.17	2345.00	0.78	0.435
$\beta_{Ta} \cdot T_O \cdot T_P$	-0.05	0.18	2343.07	0.27	0.784
$\beta_{Ta} \cdot T_M \cdot T_P$	0.17	0.18	2338.35	0.94	0.348
$\beta_{Ta} \cdot T_O \cdot T_M \cdot T_P$	0.30	0.24	2344.12	1.27	0.205

Offspring (age: 5 months) from parents acclimated to either a cold or warm temperature were similarly acclimated to cold or warm temperature. β_{Ta} represents the predicted MO_2 derived from a GAM (see methods). Significant effects are highlighted in bold text.

Figures

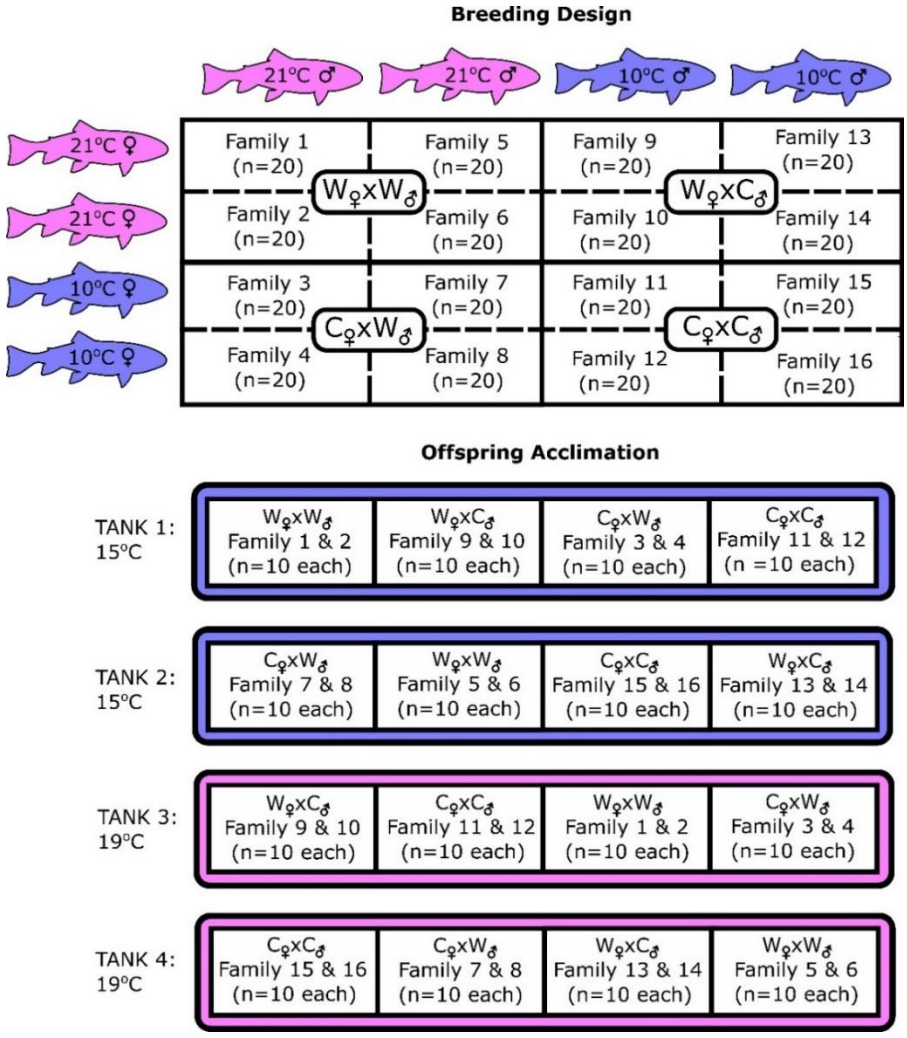

Figure 3.1: Graphic representation of the experimental design. Adult brook trout were acclimated to either a warm (21°C) or cold (10°C) temperature, then mated using a full factorial design to generate offspring from pure ($W_♀xW_♂$, $C_♀xC_♂$; W = warm, C = cold) or mixed ($W_♀xC_♂$, $C_♀xW_♂$) thermal histories. The full factorial mating design generates both maternal and paternal half-sibling families: each row in the mating design shows families generated from a single female, and each column shows families from a single male. Offspring from each family were divided and separately acclimated to either a warm (19°C) or cold (15°C) temperature.

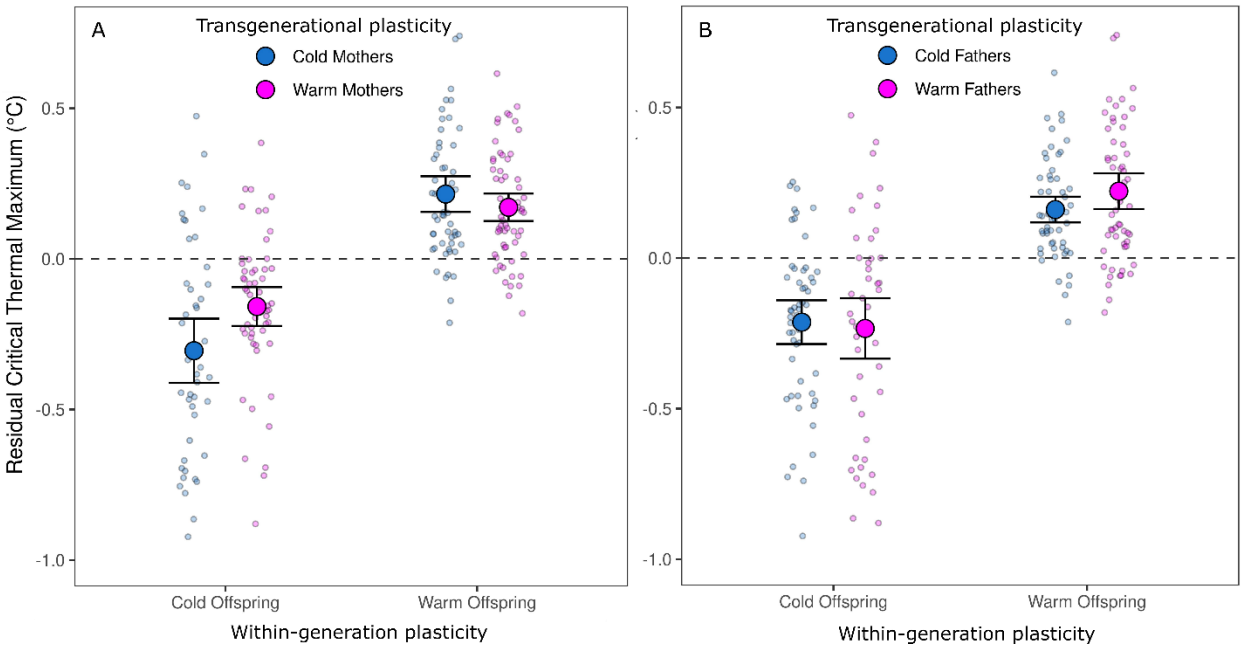

Figure 3.2: The effect maternal (A) and paternal (B) acclimation temperature on the critical thermal maximum (CTM) of brook trout offspring (age: 5 months) acclimated to a cold (all 15°C acclimated offspring, n = 100) or warm (all 19°C acclimated offspring, n = 116) temperature. On average, CTM was approximately 0.5°C higher in warm-acclimated offspring versus cold-acclimated offspring (28.6 ± 0.03 vs. 29.1 ± 0.02°C). Values represent the residuals (± confidence intervals) from a model containing the natural log of mass (fixed effect), and maternal and paternal identity (random effects).

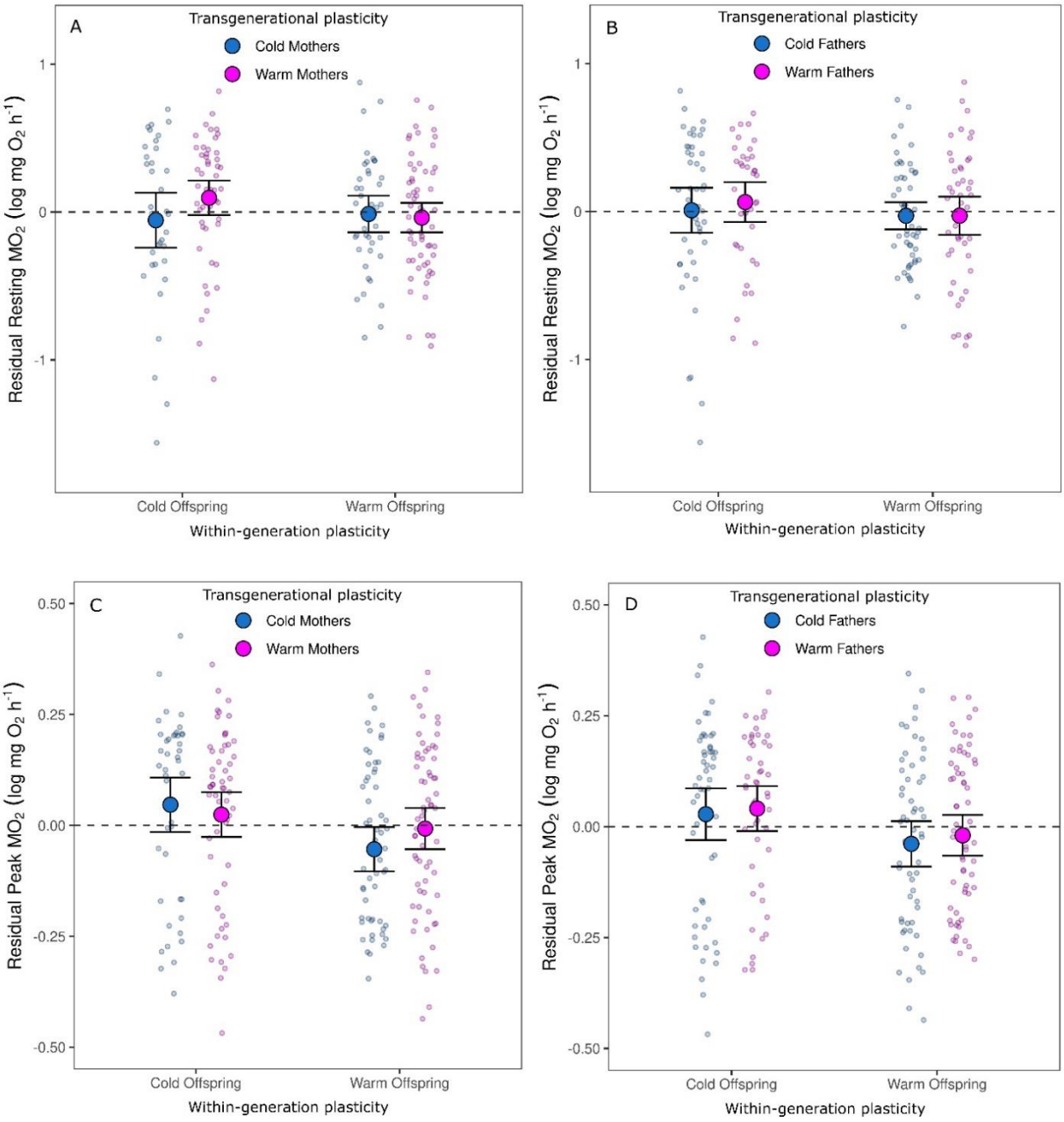

Figure 3.3: The effect of maternal (A and C) and paternal (B and D) acclimation temperature on the resting rate of oxygen consumption (MO_2), and peak MO_2 of brook trout offspring (age: 5 months) acclimated to a cold (all 15°C acclimated offspring) or warm (all 19°C acclimated offspring) temperature (n = 85-122). Values represent the residuals (± confidence intervals) from a model containing the natural log of mass (fixed effect), and maternal and paternal identity (random effects).

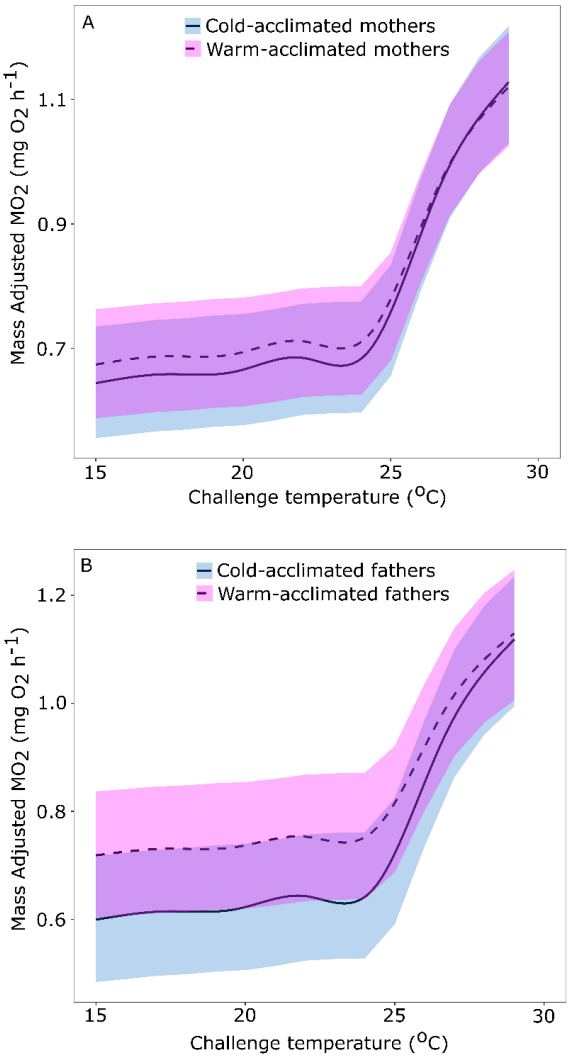

Figure 3.4: The influence of A) maternal and B) paternal acclimation temperature on the change in the rate of oxygen consumption (MO_2) of cold- (15°C, n = 105) and warm- (19°C, n = 125) acclimated brook trout offspring (age: 5 months) given an acute temperature challenge of +2°C·h^{-1}. Plots show means and 95% confidence intervals for cold- and warm-acclimated parents shown in blue and red respectively as estimated from the GLMM where challenge temperature corresponds to a spline. Rates of oxygen consumption (MO_2) were statistically adjusted for effects of body mass (see text).

Chapter 4: Transcriptomic responses to multigenerational environmental warming in a cold-adapted salmonid

Abstract

Cold-adapted species are particularly threatened by climate change as rates of environmental warming outpace the ability of many populations to adapt. Recent evidence suggest that transgenerational thermal plasticity may play a role in the response of cold-adapted organisms to long-term changes in temperature. Using RNA sequencing, we explored differential gene expression in the liver of lake trout (*Salvelinus namaycush*), a stenothermal cold-adapted species, to examine the molecular processes that respond to elevated temperatures under conditions of within-generation (offspring) and transgenerational (parental) warm acclimation. We hypothesized that genes associated with liver metabolism, growth and thermal stress/tolerance would be differentially expressed in juvenile lake trout offspring depending on their own acclimation temperature and that of their parents. We found enriched pathways for thermal stress, signaling processes, immune function, and transcription regulation, although the specific genes and direction of differential expression (up- or downregulation) depended on the combination of offspring and parental thermal rearing conditions. While parental warm acclimation did have a transgenerational effect on gene expression in their offspring, within-generation warm acclimation had a larger effect on differential expression, enriching more pathways. We provide evidence of the transgenerational response to warming at the transcriptional level in lake trout, which should be useful for future studies of transcriptomics and plasticity in this and other cold-adapted species.

Introduction

Temperate and Arctic regions are warming rapidly, particularly in North America; surface temperatures in Canada have increased by ~1.7℃ in under a century with even greater warming happening in the Arctic (~2.3℃; Zhang et al. 2019). As a result, high-latitude, cold-adapted organisms are experiencing, and will continue to experience, rapid temperature increases that may threaten their survival (Beitinger and Bennett 2000; Burkhead 2012; IPCC 2021). Range alteration to track suitable conditions is particularly challenging for species and populations with limited dispersal options, such as many freshwater fishes (Guzzo and Blanchfield 2017; Smith et al. 2020). Warming rates may outpace adaptive responses (Visser 2008; Crozier and Hutchings 2014; Comte and Olden 2017), particularly for those with low standing genetic variation (Willi et al. 2006; Wilson 2017). In the absence of standing genetic variation for coordinated adaptive responses throughout core metabolic pathways, population responses would be limited to thermal acclimation (Hendry 2016; Fox et al. 2019; Burton et al. 2022), but the low physiological plasticity typical of many cold-adapted species means they likely will be particularly vulnerable to rapidly changing environments (Somero 2010; Kelly et al. 2014). Individuals that can withstand warming may have to rely heavily on energetic resources that would normally support growth or reproduction, thus having potentially negative downstream effects on population viability (Somero 2010; Rosenfeld et al. 2020). A lack of adaptive genetic variation, low capacity for temperature acclimation, and rapid environmental change are predicted to result in widespread population losses and regional species extirpations (Bennett et al. 2019; Morash et al. 2021).

Some species may be able to buffer the negative impact of environmental warming through transgenerational acclimation, also known as transgenerational plasticity (TGP). As the name suggests, TGP refers to the phenotypic changes that occur over the course of multiple generations (Jablonka et al. 1992; Bell and Hellmann 2019; Bonduriansky 2021). This involves non-genetic inheritance of environmentally-driven changes, including parental effects, where the experiences of one generation are reflected in the morphology, physiology or behaviour of subsequent generations (Hellmann et al. 2020B; Lee et al. 2020; Bonduriansky 2021).

TGP occurs through modulation of gene expression through non-genetic inheritance, which includes parental effects and the transfer of epigenetic factors (Dai et al. 2020; Spadafora 2020; Venney et al. 2020). TGP can potentially be beneficial when parent and offspring environmental experiences coincide (Bernatchez 2016; Ashe et al. 2021). For example, TGP could be beneficial in a population that experiences environmental warming over multiple generations whereby the offspring are phenotypically "primed" for a warmer environment (Donelan et al. 2020; McCaw et al. 2020; Venney et al. 2022). In the context of climate change, this phenotypic priming could buffer the effects of rapid warming, allowing additional time for evolutionary processes to occur (Bernatchez 2016; Ashe et al. 2021), although the extent to which TGP can be considered adaptive is open to debate (Uller et al. 2013; Sánchez-Tójar et al. 2020). Nevertheless, temperature-related TGP has been identified in a number of species (Greenspoon and Spencer 2018; Yin et al. 2019). For example, the coral reef fish *Acanthochromis polyacanthus* exhibits TGP for increased temperatures by widening its aerobic scope in a warm environment by increasing maximum metabolic rate or

decreasing resting metabolic rate (Donelson et al. 2012; Bernal et al. 2018). Many of the studies on thermal TGP in aquatic organisms have used eurythermal, temperate or tropical model species; by contrast, cold-adapted aquatic vertebrates are relatively understudied but could stand to benefit from TGP given their vulnerability to environmental warming due to climate change.

Acute and chronic temperature acclimation has obvious impacts on the metabolism of ectotherms, at both the whole organism and molecular level (Pérez-Ruzafa et al. 2018; Petitjean et al. 2019; Morash et al. 2021). Relatively few studies to date report the effect of transgenerational warming on phenotypic variation in tandem with gene expression, or with follow-up experiments testing the differential gene expression underlying transgenerational effects (Shama et al. 2016; Bernal et al. 2018; Veilleux et al. 2018). Examining differential gene expression associated with changes in metabolism with thermal experiences can be informative (Oomen and Hutchings 2017). For example, Shama et al. (2016) found that genes involved in metabolism, mitochondrial protein synthesis, hemostasis and apoptosis were differentially expressed in the muscle of offspring depending on the multigenerational thermal experiences down the maternal line. These findings underscore the relationship between the molecular mechanisms underlying physiological plasticity and parental thermal experiences.

We sought to explore differential gene expression in lake trout (*Salvelinus namaycush*) to examine the molecular processes potentially underlying transgenerational responses to elevated temperatures. The lake trout is a long-lived, cold-adapted salmonid that is largely limited to cold, oligotrophic lakes in formerly glaciated regions of North America (Riley et al. 2021; Wilson and Mandrak 2021) and is under significant threat

from climate change (Casselman 2008; Guzzo and Blanchfield 2017). Being stenothermal, lake trout behaviourally thermoregulate by migrating to deeper, cooler water when lakes thermally stratify during the summer (Martin and Olver 1980; Guzzo and Blanchfield 2017). Low genetic variation for some lake trout populations (Perrier et al. 2017), and little within- and among-population variation for thermal acclimation capacity (McDermid et al. 2013; Kelly et al. 2014) may limit the adaptive potential of lake trout populations in response to warming temperate and arctic habitats. We investigated the capacity for transgenerational thermal plasticity in lake trout as a means of coping with warming associated with climate change and found that they exhibit only limited transgenerational plasticity at the whole organism (phenotypic) level (Chapter 2; Penney et al. 2021). Unexpectedly, cold-acclimated offspring of parents from elevated temperatures exhibited higher resting metabolic rate than those with one or both cold-acclimated parents (Chapter 2; Penney et al. 2021). This study suggests that parental environments can influence offspring phenotypic expression but did not investigate the molecular mechanisms underlying this transgenerational response.

For this study, we assessed gene expression in previously studied offspring from factorial mating crosses between cold- and warm-acclimated lake trout adults (Chapter 2; Penney et al. 2021) to assess transcriptional responses to within- and transgenerational thermal acclimation. Acclimation of the parents (transgenerational) to one of two environmental temperatures (10 or 17°C) was combined with subsequent acclimation of the offspring (within-generation) to cold (11°C) or warm (15°C) temperatures (Fig. 4.1). Evidence exists to support both maternal and paternal contributions to transgenerational effects (Marshall 2015; Shama et al. 2016; Chapter 2; Penney et al. 2021). Our

experimental design allowed us to examine the transgenerational effect of temperature acclimation of each parent, and both parents together, in combination with the offspring's (within-generation) temperature acclimation on gene expression in juvenile lake trout.

We hypothesized that genes associated with metabolism, growth and thermal stress/tolerance would be differentially expressed in the liver of juvenile lake trout depending on both the acclimation temperature of the offspring and the acclimation temperature of their parents. Based on studies of within-generation and transgenerational warming on differential gene expression in fish (Quinn et al. 2011; Veilleux et al. 2015; Akbarzadeh and Leder 2016; Shama et al. 2016), we predicted that 1) genes involved in growth, heat shock and hypoxia responses, and metabolic pathways would be upregulated in warm-acclimated offspring, and 2) differential expression of these genes would vary depending on whether one or both parents were warm-acclimated, although the size and direction of the effect is difficult to predict given that parental influence on offspring gene expression has been shown to be sex-specific; depending on the sex of the offspring and that of the parent (Best et al. 2018; Bautista et al. 2020).

Methods

Hatchery-raised adult lake trout from the Ontario provincial Seneca Lake hatchery broodstock were used as parents for the experiment. This hatchery strain originated from Seneca Lake, one of the Finger Lakes in central New York State (42°41' N, 76°54' W). The Ontario Ministry of Natural Resources and Forestry (OMNRF) hatchery system has maintained this lake trout strain for over five generations, using rotational line crossing

(Kincaid 1977) to minimize inbreeding and maintain the founding genetic diversity of the strain (OMNRF Fish Culture Stocks Catalogue 2005).

The liver tissue samples used for these experiments were collected from juvenile (young of year) lake trout that were previously used in a separate study on metabolic rate with transgenerational warming described in Chapter 2 and Penney et al. (2021). We chose to study gene expression in the liver because it is a metabolically active tissue involved in a number of physiological processes that respond to warming (Quinn et al. 2011; Akbarzadeh and Leder, 2016; Dammark et al. 2018). Additionally, transcriptional responses to multigenerational warming have been observed in the livers of zebrafish (Luu et al. 2021). Therefore, the liver ought to provide insight into the metabolic pathways that are affected by transgenerational temperature acclimation in lake trout. In the previous study (Chapter 2; Penney et al. 2021), adult and juvenile offspring lake trout were acclimated to two different temperatures and the offspring were subjected to an acute thermal challenge of $+2°C \cdot h^{-1}$ to determine the effect of within- and transgenerational temperature acclimation on upper thermal tolerance and metabolic rate. Details of the prior experiment (Chapter 2; Penney et al. 2021) that are relevant to the current study are summarized below.

All experiments adhered to the Canadian Council on Animal Care guidelines and were approved by the Trent University Animal Care Committee (Protocol # 24794) and the OMNRF Aquatic Animal Care Committee (Protocol # FACC 136).

Experimental design and rearing conditions

Two groups (n = 8 and 9) of mature lake trout (age 8; 2.3 - 4.2 kg) were each held in 6,000 L tanks (1 x 1 x 6 m) at the OMNRF White Lake Fish Culture Station (Sharbot Lake, Ontario, Canada) with water being supplied from a nearby lake. Males and females were kept together in each tank. As the source waterbody (White Lake) began to stratify in early June, 2015, we began to increase tank water temperature by 1°C per day starting from approximately 9°C until one tank reached 10 ± 0.5°C and the other 17 ± 0.5°C. These temperatures were chosen because 10°C represents the thermal requirements for lake trout spawning while 17°C was intended to induce physiological thermal stress without preventing reproduction at the higher temperature (Casselman 2008; Chapter 2; Penney et al. 2021). Temperatures were controlled and maintained by drawing in and mixing water from above and below the lake's thermocline as it flowed into the holding tanks. Fish were acclimated to these temperatures for approximately 3 months, mirroring thermal stratification in the source waterbody. Starting in mid-September, the temperature in the warm-water treatment was allowed to gradually cool by holding the proportional inflows from above and below the thermocline constant as the lake's surface water cooled. Temperatures in the two tanks converged at 10°C after fall turnover (late September) in the lake, and gradually cooled to 7.8°C by the end of the breeding interval (mid-November).

Adult lake trout were reproductive by the end of October with the warm-acclimated fish first spawning on October 30th. The cold-acclimated adults were first ready to spawn on November 5th and all mating crosses were completed by November 19th. Experimental offspring families were produced by a full factorial mating cross

using two males and two females from each of the two temperature treatments (4 x 4, 8 adults in total) resulting in a total of 16 offspring families; four families from each of four parental treatment groups (W♀xW♂, W♀xC♂, C♀xW♂ and C♀xC♂, where W = warm-acclimated, and C = cold-acclimated; Figure 1). Fertilized eggs were transported to the OMNRF Codrington Fish Research Facility (Codrington, Ontario, Canada) where they were transferred into 200 L tanks receiving freshwater at ambient temperature (5-6 ℃) and natural photoperiod under dim light with each family separately contained in fine-mesh, flow-through stainless steel boxes (9 x 9 x 7.5 cm, one family per box).

In March 2016, when hatched offspring were ready to begin exogenous feeding, we randomly selected 14 individuals from each of the 16 families to transfer to 200 L tanks for temperature acclimation. Seven fry from each family were acclimated to a cold temperature (11°C) and the other 7 from each family were acclimated to a warm temperature (15°C). The offspring's lower acclimation temperature differed from that of their parents because of reported differences in optimum temperature for both life stages (11 vs 10°C; Edsall 2000, Casselman 2008). The upper acclimation temperature for the offspring represents the anticipated increase in temperature associated with climate change by year 2100 (Hayhoe et al. 2010; IPCC 2021), however, for the parents the upper acclimation temperature was two degrees higher to ensure that temperature was high enough to elicit a physiological thermal response without compromising reproduction. To begin acclimating the offspring, water temperature was increased at a rate of 1℃ per day until target temperatures were reached (11 and 15°C). Due to space constraints, tanks were subdivided by adding steel inserts with perforated bottoms. Each insert was divided into 4 sections (24 x 25 x 28 cm per section) with two paternal half-sibling families (n = 7

each; shared male parent) housed per section. Offspring from pooled families were later individually identified to family using microsatellite genotyping (Chapter 2; Penney et al. 2021).

After the 3-4 week acclimation period, offspring were subjected to an acute temperature increase of $+2℃·h^{-1}$ from their acclimation temperature until loss of equilibrium was observed. Eight fish from the same acclimation temperature (11°C or 15°C) were simultaneously tested per day, with each fish held in its own experimental chamber (Chapter 2; Penney et al. 2021). Following loss of equilibrium, each fish was quickly transferred to a bath of 0.3 g l−1 of buffered tricaine methanesulfonate (MS-222; Aqua Life, Syndel Laboratories Ltd, BC, Canada) for euthanasia. Whole liver was rapidly dissected from each fish, blotted on a lab wipe and preserved in RNAlater (Invitrogen, Thermo Fisher Scientific) as per manufacturer's instructions. After 24-48 hours in a 4°C refrigerator, the RNAlater was pipetted from the tissues and the samples were stored at -80°C until RNA extraction. Liver tissue was collected between June 28 and August 9 in 2016. Euthanized individuals were genetically identified to family using microsatellite genotyping (Chapter 2; Penney et al. 2021).

RNA isolation and sequencing

RNA was extracted from the preserved liver tissues using a phenol-chloroform extraction method (Chomczynski and Sacchi 2006) after the tissues had been individually homogenized via a FastPrep-24 BeadBeater (MP Biomedicals) with 2 mL Lysing Matrix D tubes (MP Biomedicals) and 1 mL of Trizol reagent (Invitrogen, Thermo Fisher Scientific). RNA was precipitated with RNA precipitation solution (Sambrook and

Russel 2001) and isopropanol, and washed with 75% ethanol. RNA samples were resuspended in nuclease-free water (Thermo Fisher Scientific). The purity and concentration of the RNA was quantified using a NanoDrop-8000 spectrophotometer, and RNA quality was assessed by gel electrophoresis of glyoxylated RNA on a 1.5% agarose gel (Sambrook and Russel 2001).

Two lots of RNA samples were sent for high-throughput sequencing over a two-year span. In 2018, liver RNA samples from 24 individuals were sent to The Centre for Applied Genomics (Sick Kids Hospital, Toronto, Ontario, Canada), and in 2020 another 30 liver samples (individuals) were sent to Genome Quebec (Montreal, Quebec, Canada). Altogether, the 54 samples included tissue from six individuals from each of the seven experimental treatment groups, however, the control group (11°C-acclimated offspring from cold-acclimated (CxC) parents) had a total of 12 individuals sequenced: 6 individuals in 2018 and an additional 6 in 2020, so that each treatment group was compared against the control group sequenced in the same year to eliminate batch effects in the analysis of differential expression (Table 4.1). Individuals from each family were represented in their respective treatment group. There were 4 families per offspring-parent treatment group; because each sequenced group was comprised of 6 individuals, each group had 2 representatives from 2 of the 4 families. Overall, this did not lead to a bias toward any one family being overrepresented in the samples, except for the 2020 control group which had 3 individuals from one family, but this family was not overrepresented in any of the other groups. Subsequent genetic testing for males and females (Yano et al. 2013) confirmed that each group also contained members of both sexes (Table 4.1). Both facilities assessed the RNA quality via a Bioanalyzer (Agilent

Technologies) and all samples passed quality control (RNA integrity number: ≥ 7.5). cDNA libraries were constructed by enriching the poly(A) tails of mRNA with oligo dT-beads using NEBNext Ultra II Directional polyA mRNA Library Prep. In 2018, barcoded libraries were distributed among two and a half lanes and were sequenced on the Illumina HiSeq2500 instrument producing an average of 28 million reads per sample (n = 24, paired-end, 2x126 bp). In 2020, libraries were sequenced on the Illumina HiSeq4000 with barcoded libraries distributed among three lanes producing 36 million reads per sample (n = 30, paired-end, 2x126 bp).

De novo assembly, annotation and analysis

For our study, we opted to use a *de novo* transcriptome assembly for the reads rather than a genome-guided approach. For non-model species with complex genomes, a *de novo* approach can often produce better assemblies than a genome-guided approach (DeWoody et al. 2013), and the *de novo* approach using Trinity has been successfully used for transcriptome assembly for salmonids (Carruthers et al. 2018; Narum and Campbell 2015; Nazari et al. 2021). We did not use the recently published lake trout genome as a reference because the reads generated from this experiment were also used to develop the lake trout genome (Smith et al. 2022).

RNA reads from both years were assembled and annotated by the Canadian Centre for Computational Genomics (C3G, Quebec) in 2020. Adapters and reads with a Phred quality score <30 were trimmed using Trimmomatic (v.0.36), and normalization and *de novo* assembly was performed using Trinity assembly software (v.2.0.4) following Haas et al. (2013) and Grabherr et al. (2011). Pre- and post-trimming read counts and

Trinity statistics can be found in the appendix (Tables A4.1 and A4.2). Functional annotation was performed with the Trinotate tool (v.2.0.2) and using the TransDecoder pipeline (v.2.0.1) to identify candidate coding regions and open reading frames. Candidate peptides were searched against known proteins in the Swiss-Prot (UniProtKB/Swiss-Prot) database via BLASTp (v.2.3.0) and the pfam database. The pfam database was also used with HMMER (v.3.1b2) to identify protein domains. Signal peptides, transmembrane regions and ribosomal RNA genes were predicted using the programs signalP (v.4.1), tmHMM (v.2.0c) and RNAMMER (v.1.2), respectively.

Seven comparisons were made where offspring treatment groups were each compared to the control group (cold-acclimated offspring with cold-acclimated parents) to observe the effect that within- and transgenerational plasticity (acclimation) had on gene expression in juvenile lake trout (Table 4.1). Transcript abundance was estimated using 'RNA-Seq by Estimation Maximization' (RSEM; Li and Dewey, 2011) in Trinity with the 'align_and_estimate_abundance.pl' utility. Differential gene expression was analyzed between the groups of lake trout offspring in R using the limma package (v.3.40.6; Ritchie et al., 2015). The log2fold change (LFC) was calculated for each gene, representing the magnitude of up- or downregulation; negative LFC are downregulated genes. A principal component analysis (PCA) was performed to explore the DEGs for possible batch effects between the years given the differences in sequencing depth (28 vs. 36 million reads per sample), and we tested the correlation between DEGs for each of the pairwise comparisons to check whether application of a batch correction, using the twelve control group individuals as a shared baseline, changed the level of expression of DEGs.

From the list of annotated genes, we applied a LFC cutoff of $\leq$ -1 to $\geq$ 1 and used this limited list of DEGs for an enrichment analysis performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool (v.6.8) (Huang et al. 2009A; Huang et al. 2009B). The DAVID tool required that a model species be specified for our gene list: we opted to use *Salmo salar* as DAVID does not yet include *S. namaycush* despite the recent publication of the *S. namaycush* reference genome (Smith et al. 2022), and *S. salar* had the best coverage for annotated proteins among orthologous species available in the Swiss-Prot database (determined via NCBI Eukaryotic genome annotation pipeline, v.8.5). The output from the DAVID tool identified genes in functional categories (GO) and metabolic pathways (Kyoto Encyclopedia of Genes and Genomes: KEGG); we ran our list of DEGs against backgrounds for fish species in the database (*Oncorhynchus mykiss*, *S. salar*, *Danio rerio*, *Oryzias latipes*), using the DAVID tool default criterion of reporting functions that are enriched by a minimum count of 2 genes and $p<0.1$. In our study, we only discuss DEGs that were recognized in GO or KEGG via DAVID tool because the function of these DEGs have been confirmed in fish species. Tables outlining the enriched functions are included in the appendix. DEGs that were not included in the enrichment analysis are only discussed in the terms of the overall size of the effect (i.e., total number of DEGs, annotated and not annotated) on gene expression under conditions of within- and or transgenerational warm acclimation.

Results

Transcriptome sequencing yielded 52-57 million reads per sample sequenced in 2018, and 55-104 million reads per sample sequenced in 2020 (Table A4.1). Trinity

assembled 524,988 transcripts with 261,620 genes for all groups sequenced in 2018 and 413,029 transcripts with 269,149 genes for all groups sequenced in 2020 (Table A4.2). The transcriptome data (Table A4.2) showed both transgenerational and within-generation environmental influences on gene expression. Offspring within-generation warm acclimation resulted in 3,853 DEGs with a LFC of $\leq$ -1 to $\geq$ 1 (Fig. 4.2). By comparison, transgenerational warm acclimation resulted in fewer differentially expressed genes (maternal: 1,958; paternal: 1,777; both parents: 2,244; Fig. 4.3). The combined effect of offspring warm acclimation with that of either or both parents often resulted in more DEGs than offspring within- or transgenerational warm acclimation in isolation (offspring and maternal: 4,478; offspring and paternal: 3,638; offspring and both parents: 4,712; Fig. 4.4). Among all groups, 1,005 of these DEGs were recognized by the DAVID tool and used for enrichment analysis. The level of differential expression of these genes are displayed in a heatmap (Fig. 4.2) and sorted by LFC according to gene expression level in warm-acclimated offspring. The list of genes along with their LFC values and adjusted *p*-value can be found in the appendix (Table A4.17).

Principal component analysis revealed a potential batch effect between the two years of sequence data, with 11% of the variation associated with offspring acclimation temperature and 4-6% of the variation associated with different sequencing years (Fig. A4.1). An analysis of the log2 fold-changes before and after correction for year showed that there was a strong correlation across all comparisons ($r \geq 0.91$), indicating that the batch correction provided essentially the same results between the two years (Fig. A4.2). Although there is still a potential for batch effects, the trends observed in both years reflect the effects of the experimental temperature treatments. For example, the within-

generation (offspring) warm acclimation group had a higher number of DEGs than groups receiving either maternal or paternal transgenerational warm acclimation. As these latter two (parent/transgenerational) groups were sequenced in 2020, they had greater sequencing depth yet still had fewer differentially expressed genes compared to the within-generation group which was sequenced at a lower depth in 2018.

Within-generation acclimation: offspring warm acclimation

Warm acclimation of lake trout offspring resulted in differential gene expression compared to cold-acclimated offspring, upregulating genes associated with metabolism, growth and thermal tolerance. Our enrichment analysis determined that 29 of these upregulated DEGs were involved in signalling processes, 14 in cytoplasm functions and 9 were immunoglobulins (Table A4.3). Upregulated DEGs that associated with metabolic (KEGG) pathways included those involved in degradation (3-hydroxymethyl-3-methylglutaryl-CoA lyase like 1: *HMGCLL1*, CCR4-NOT transcription complex subunit 1: *CNOT1*) such as peroxisome activity and RNA and ketone degradation (Table A4.4). Genes involved in the heat shock response were also upregulated, specifically heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 1 (*hsp90aa1.1*; Table 4.4). Others included cell adhesion (CD99 molecule like 2; *CD99L2*, catenin alpha 2: *CTNNA2*) and signalling genes (Janus kinase 1: *JAK1*, PYD and CARD domain containing protein: *PYCARD*, chromodomain helicase DNA binding protein 8: *CHD8*; Table A4.4). Offspring warm acclimation downregulated 19 DEGs related to metal- and heme-binding and 3 DEGs associated with cyclin activity (Table A4.3). Contrary to our predictions, downregulated DEGs involved in KEGG pathways included

glycolysis/gluconeogenesis (glyceraldehyde-3-phosphate dehydrogenase: *GAPDH*) and metabolism of fructose and mannose (aldolase: *ALDOB*; Table A4.4). Other DEGs involved in peroxidase activity were also downregulated with offspring warm acclimation (MPV17 mitochondrial inner membrane protein like 2: *MPV17L2*; Table A4.4).

Transgenerational acclimation: parental warm acclimation

Parental warm acclimation influenced gene expression in cold-acclimated offspring, however, transgenerational warm acclimation (either or both parents) affected fewer genes than did within-generation warm acclimation (Fig. 4.3). The direction of differential expression (up- or down-regulation) in the offspring depended on which parent was warm-acclimated. Sometimes expression of a gene due to maternal or paternal warm acclimation contrasted with the expression level change associated with offspring warm acclimation (positive vs. negative LFC; Fig. 4.2). Nevertheless, parental warm acclimation influenced differential gene expression in liver of the lake trout offspring.

Maternal warm acclimation had the effect of upregulating processes related to muscle function in their offspring, specifically 2 DEGs involved in calcium-binding and 3 DEGs in troponin synthesis (Table A4.5). There were 13 warm-acclimation upregulated DEGs associated with transferases and 3 DEGs related to glycolysis (Table A4.5). Upregulated genes involved in KEGG pathways included DNA damage/repair and fatty acid metabolism (DNA damage inducible transcript 4: *DDIT4*, hydroxysteroid (17-beta) dehydrogenase 12a: *hsd17b12a*; Table A4.6). There were 14 warm-acclimation downregulated DEGs involved in cell signalling and 10 involved in hydrolase/protease activity (Table A4.5). The KEGG pathway analysis revealed that maternal warm

acclimation downregulated *GAPDH* involved in glycolysis/gluconeogenesis, and functions related to RNA transport and degradation (*CNOT1* and eukaryotic translation initiation factor 3 subunit C: *EIF3C*; Table A4.6).

Paternal warm acclimation also affected offspring gene expression in liver, although fewer genes were differentially expressed when compared to maternal warming effects (Fig. A4.3). There were 10 upregulated DEGs related to transferase activity along with 3 upregulated DEGs involved in glycolytic processes (Table A4.7). Upregulated KEGG pathways included the mTOR signalling pathway (*DDIT4*) and protein processing (endoplasmic reticulum oxidoreductase 1 alpha: *ERO1A*; Table A4.8). Downregulated DEGs included 16 DEGs involved in cell-signalling and 7 DEGs associated with protease activity (Table A4.7). The KEGG pathway analysis revealed there were two transcripts (one upregulated and the other downregulated), that were identified as *GAPDH* and associated with glycolysis (Table A4.8).

Transgenerational effects were also observed in offspring gene expression when both mothers and fathers were warm-acclimated. The combined effect of both parents resulted in more differentially expressed genes in the offspring compared to the transgenerational effect of warm acclimation of either parent in isolation (Fig. A4.3). We observed 16 upregulated DEGs that were associated with cell signalling processes, 9 DEGs related to hydrolase/proteases/peptidases function and 2 DEGs associated with peroxisome activity (Table A4.9). Upregulated KEGG pathways included the mTOR pathway (*DDIT4*), RNA degradation (*CNOT1*) and amino acid transferase activity (betaine-homocysteine S-methyltransferase: *BHMT*, glycine amidinotransferase: *GATM*, guanidinoacetate N-methyltransferase: *GAMT*; Table A4.10). Downregulated functions

involved 4 DEGs included in glycosyltransferase activity, 3 DEGs related to lectin and 2 DEGs associated with tRNA function (Table A4.9). Downregulated KEGG pathways included peroxisome activity (*HMGCLL1*) and apoptotic signaling (*pycard*) and Notch signaling pathways (aph-1 homolog B, gamma-secretase subunit: *APH1B*; Table A4.10). Again, *GAPDH* appeared as both up- and downregulated in the KEGG analysis.

Multigenerational acclimation: offspring and parental warm acclimation

Genes associated with metabolism, growth and thermal stress/tolerance were differentially expressed with the combined effect of offspring and parent warm acclimation. The combination of offspring and parent (both together) warm acclimation resulted in more DEGs (e.g. >200) being up- or downregulated relative to the offspring group that experienced only within-generation warm acclimation (Fig. 4.4). We must acknowledge here that the groups that represent the combined effect of offspring and maternal warm acclimation, and the combined effect of offspring and paternal warm acclimation were sequenced at a higher read depth than the within-generation only group (36 vs 28 million reads, respectively), meaning that the differences in differential expression here may be partly due to an experimental artefact of sequencing depth. For example, a total of 4,478 transcripts were differentially expressed in the offspring that received the combined treatment of within- and maternal transgenerational warm acclimation compared to the 3,853 transcripts of the group that received the within-generation warm acclimation only (Fig. 4.4).

The combined effect of offspring and maternal warm acclimation resulted in the upregulation of 20 DEGs related transferase function, 4 DEGs involved in

metalloprotease/endopeptidase activity and 4 associated with the PDZ domain (Table A4.11). There were also 3 DEGs associated with protein folding, 3 DEGs associated with DNA repair, and 3 associated with muscle contraction/ZASP (Table A4.11). Genes that encode heat shock proteins (heat shock protein 70: *HSP70*, heat shock protein, alpha-crystallin-related, 1: *HSPB1*, heat shock cognate 71 kDa protein: *HSP7C*, heat shock protein 30: *HSP30*) and proteins involved in the respiratory chain (cytochrome c oxidase subunit 1: *COX1*, cytochrome c oxidase subunit 2: *COX2*) were also upregulated, though these functions were not significantly enriched (p=0.07; Table A4.11). Similarly, upregulated KEGG pathways included heat shock responses and RNA degradation (Table A4.12). Downregulated functions included 21 DEGs with transferase functions, 20 with function related to the cell nucleus and 19 DEGs involved in ATP binding (Table A4.11). Additionally, there were 12 DEGs associated with kinases, 5 that had cyclin functions and 5 associated with DNA replication (Table A4.11). Downregulated KEGG pathways associated with combined maternal and offspring warm acclimation included glycolysis/gluconeogenesis (*ALDOB*, *GAPDH*; Table A4.12).

Offspring and paternal warm acclimation combined to influence the differential expression of similar numbers and sorts of pathways as did maternal warm acclimation, again supporting our hypothesis. This combination of offspring and paternal warm acclimation had the effect of upregulating 4 DEGs that have proteases/peptidases functions and 2 DEGs involved in glycolysis (Table A4.13). There were 2 upregulated DEGs involved in complex IV of the respiratory chain, however, this function was not significantly enriched (p=0.07; Table 4.13). Similar to the combined effect of offspring and maternal warm acclimation, offspring and paternal warm acclimation upregulated

KEGG pathways involved in cell signaling/inflammation (*mapk14a*), but offspring and paternal warm acclimation uniquely upregulated KEGG pathways associated with protein folding (hypoxia up-regulated 1: *HYOU1*) and insulin regulation (forkhead box O1 a: *foxo1a*; Table A4.14). Enriched downregulated functions included ATP binding (14 DEGs), hydrolase/protease activity (13 DEGs), DNA replication (4 DEGs) and cyclin function (4 DEGs; Table A4.13). There were also 2 DEGs associated with tRNA ligase activity and 2 DEGs related to peroxisome function (Table A4.13). Downregulated KEGG pathways included glycolysis (*ALDOB*, *GAPDH*), amino acid metabolism (4-hydroxyphenylpyruvate dioxygenase a: *hpda*, aldehyde dehydrogenase 4 family member A1: *ALDH4A1*, ribonucleotide reductase M1 polypeptide: *RRM1*, ribonucleotide reductase M2 polypeptide: *RRM2*, tryptophan 2,3-dioxygenase a: *tdo2a*), fatty acid metabolism (ELOVL fatty acid elongase 6: *ELOVL6*; Table A4.14).

Of all groups compared so far, the combined multigenerational effect of offspring, maternal and paternal warm acclimation had the greatest effect on differential gene expression in the offspring (Fig. 4.4). There were 41 upregulated DEGs associated with signalling function (Table A4.15). Immunoglobulin and protease/peptidase activity were also enriched (9 and 5 DEGs, respectively; Table A4.15). There were 2 DEGs involved in the respiratory chain complex IV but complex IV was not significantly enriched (p=0.07; Table A4.15). Some of the affected KEGG pathways included the heat shock response (*hsp90aa1.1*), signalling pathways associated with inflammation (mitogen-activated protein kinase 14a: *mapk14a*) and insulin regulation (*foxo1a*; Table A4.16). Downregulated functions included transferase activity (24 DEGs), DNA replication (6 DEGs) and cyclin functions (4 DEGs; Table A4.15). Binding functions were

downregulated, such as those related to magnesium, nucleic acid and iron binding (5 DEGs each; Table A4.15). Downregulated DEGs involved in KEGG pathways included peroxisome activity (*MPV17L2*), glycolysis/gluconeogenesis (*ALDOB*), and response to DNA damage (*DDIT4*; Table A4.16).

Discussion

Our hypothesis that genes associated with metabolism, growth and thermal stress/tolerance would be differentially expressed in juvenile lake trout depending on the acclimation temperatures of the offspring as well as their parents was supported by the sequence data. Genes involved in growth, heat shock and hypoxia responses, and metabolic pathways were differentially expressed in liver with within-generation, transgenerational and multigenerational warm acclimation. The degree to which each gene was affected depended on whether it was the offspring or parent that had been warm-acclimated, with the offspring thermal experience often having a stronger effect compared to the parental thermal experience. Maternal and paternal warm acclimation sometimes had opposing effects on the direction of differential expression of certain genes in their offspring with one parent stimulating gene upregulation and the other parent stimulating downregulation.

We identified five noteworthy trends: 1) Within-generation (offspring) warm acclimation had a larger effect on the overall number of DEGs compared to transgenerational contributions from warm-acclimated parents (mother, father or both). The combination of offspring, maternal and paternal warm acclimation had the greatest effect on the level of differential expression in the offspring with 471 DEGs. 2) Cell

signalling processes were upregulated with offspring (within-generation) warm acclimation (29 DEGs) and when both parents (transgenerational) were warm acclimated (16 DEGs), but maternal or paternal (transgenerational) warm acclimation alone had the opposite effect (14-16 downregulated DEGs). Cell signalling was most enriched (41 upregulated DEGs) with multigenerational (offspring, maternal and paternal) warm acclimation. 3) Transferase functions appeared to be regulated mainly by transgenerational (parental) warm acclimation rather than with within-generation (offspring) warm acclimation, however, the degree of enrichment (i.e. gene count) and the direction of the effect (up- or downregulation) depended on whether one or both parents were warm-acclimated. 4) Regulation of cyclin functions appeared to be primarily under the control of the offspring because this process was only downregulated when offspring were warm-acclimated, thus not influenced by transgenerational (either parent or both) warm acclimation. 5) Mothers seemed to have an influence over muscle functions (calcium binding, troponin synthesis, ZASP) in their offspring, upregulating these functions when mothers experienced a warmer environment, whereas there was no evidence of a paternal influence. It is therefore possible that the liver samples may have been contaminated by muscle tissue during the dissection in our experiment, as genes related to muscle function would not be expressed in liver tissue. As patterns of gene expression are tissue-specific (Sonawane et al. 2017), we attempted to primarily compare our findings to other studies on thermal stress and temperature acclimation in fishes that measured gene expression in the liver. In some cases, however, we compared our liver tissue gene expression results to other findings that used different tissue types (muscle,

gill, gonad), so it is possible that discrepancies may exist where differences in gene expression due to tissue type occur between our findings and those of other studies.

General effect of warming (parental or offspring) on differential gene expression

Several DEGs that were associated with thermal stress were apparent across all comparisons. Among these were upregulated DEGs associated with molecular chaperone and co-chaperone functions (*hsp90aa1.1, hsp70, hsp30, hsp7c, hspB1,* heat shock protein family member 7: *hspB7*, *HYOU1*, FKBP prolyl isomerase 5: *FKBP5* and FKBP prolyl isomerase 8: *FKBP8*). Upregulation of heat shock proteins and FK506-binding proteins (heat shock protein 90 alpha: *HSP90a*, *HSP70*, FKBP prolyl isomerase 10: *FKBP10*) has similarly been observed in other salmonids experiencing warm acclimation and with an acute temperature increase (liver: Akbarzadeh et al. 2018; gill; Houde et al. 2019; liver: Shi et al. 2019). Another common indicator of heat stress in salmonids is heat shock protein 47 (*hsp47*), also known as *serpinh1* (gill: Tomalty et al. 2015; liver: Akbarzadeh et al. 2018). We did not see differential expression of *serpinh1* in our study, however, upregulation of this gene does not always occur with heat stress (liver: Mackey et al. 2021). *HYOU1* in particular was upregulated with within- or multigenerational warm acclimation, but not with transgenerational warm acclimation in isolation (Table A4.17). *HYOU1* (also known as *GRP170* and *HSP12A*) is part of the heat shock protein 70 family and its expression is primarily induced with hypoxia. Although, upregulation of this gene has previously been observed in response to heat stress in fish in other tissues (kidney: Huang et al. 2018; gill: Bilyk et al. 2021), its expression may be linked to a hypoxia response or may potentially reflect the increased demands on aerobic

metabolism/oxidative stress (Olsvik et al. 2013; Huang et al. 2018). The temperature-controlled water in the source tank supplying the respirometer setup was supplemented with compressed oxygen at higher temperatures in an effort to maintain saturation levels and minimize exposure to hypoxia. However, we must acknowledge the possibility that some hypoxic responses may still have occurred.

In general, within- and transgenerational warm acclimation resulted in upregulated mitogen-activated protein kinases (MAPK): *MAPK3*, *MAPK14a* and *MAPK14b* (also known as *ERK1*, *p38a* and *p38b*, respectively). MAPKs are important in the regulation of many processes, including mitosis during growth and development. They are also upregulated in response to various stressors, such as osmotic, hypoxic and thermal stress (liver: Cowan and Storey 2003; gill: Tian et al. 2019), and upregulation of these proteins has been observed in Atlantic salmon and *Oncorhynchus* spp. with thermal stress (liver: Akbarzadeh et al. 2018; liver: Shi et al. 2019). MAPKs are associated with the induction of HSPs, for example, *MAPK3* has been shown to induce expression of *HSP70* in zebrafish embryos under thermal stress (Keller et al. 2008). Both *MAPK3* and *HSP70* were upregulated in warm-acclimated lake trout offspring with warm-acclimated mothers, suggesting that these fish relied on MAPK signaling cascades for resilience to warming. This is perhaps not surprising, as MAPK cascades and the induction of HSPs are evolutionarily conserved across numerous taxa (Krens et al. 2006; Kostenko et al. 2011), and upregulation of these genes has been observed previously in other taxa following heat stress (whole embryo: Keller et al. 2008; liver: Liu et al. 2021). Although, mRNA transcription does not guarantee that translation will also occur which may explain why warm acclimated lake trout offspring did not show differences in thermal

tolerance at the whole animal level despite expression of MAPK and HSP genes (Chapter 2; Penney et al. 2021). Other affected pathways indicating upper thermal stress included peroxisome activity (*HMGCLL1*), DNA damage (*DDIT4*) and apoptotic signaling (*pycard*), though whether these DEGs were up- or downregulated depended on whether it was the parental or offspring generation that had experienced warming. Taken together, these DEGs indicate increasing temperatures caused a stress response in lake trout, which we would expect given that each offspring was subjected to an acute temperature increase (+2°C/h) and these DEGs were likely upregulated to respond to cellular/protein damage occurring at their upper critical thermal limit (Chapter 2; Penney et al. 2021).

There was a general enrichment of pathways associated with metabolism, including genes involved in mitochondrial oxidative phosphorylation (e.g., *COX1*, *COX2*) and glycolysis (e.g., *GAPDH* and *ALDOB)*. In general, *GAPDH* and *ALDOB* were downregulated with within- or transgenerational warm acclimation, however, *COX1* and *COX2* were upregulated in warm-acclimated offspring with warm-acclimated mothers following an acute temperature challenge. The effect of acute warming on differential expression of cytochrome c oxidase subunits is equivocal in fish: for example, acute exposure to warmer temperatures resulted in downregulation of *COX1* and *COX2* in the liver of yellow croaker (Qian and Xue 2016) but upregulation of *COX1* in the liver of juvenile lake whitefish (Zak and Manzon 2019). For salmonids in particular, upregulation *COX2* also occurred with heat stress in the gill of chinook salmon (*O. tshawytscha*) likely in response to the increased metabolic demands associated with warming (Tomalty et al. 2015). Upregulation cytochrome c oxidase subunit 6B1 (*COX6B1*) has been shown to occur with heat stress in the gills of Pacific salmon (Akbarzadeh et al. 2018), though we

did not detect expression of this subunit in our study. In our experiment, the matching of offspring and maternal thermal experience increased COX gene expression to a greater extent compared to other groups, potentially allowing offspring to meet the energetic demands associated with acute warming. We did not see upregulation of any other COX subunits, but it seems that expression of COX subunits does not follow stoichiometric expectations (Bremer and Moyes 2014) which may explain why we observed upregulation of only two of the 14 subunits that make up the COX holoenzyme.

We found some indication that warm acclimation and upper thermal stress affected the growth of the lake trout offspring. We expected to see upregulation of some growth-related genes given that the warm-acclimated offspring were heavier than the cold-acclimated offspring at the time of sampling (Chapter 2; Penney et al. 2021). Common molecular indicators of growth in fish include genes encoding growth hormone (*GH*) and insulin-like growth factor-1 (*IGF-1*), and genes related to thyroid function (liver: Hevrøy et al. 2015; liver: Li et al. 2021). We did not observe differential expression of *IGF-1* in the liver of our lake trout, but the thyroid receptors *TRα* and *TRβ* were downregulated in offspring that experienced warm acclimation. Thyroid hormones have important roles in growth and development (reviewed by Deal and Volkoff 2020), however, it is curious that *TRα* and *TRβ* were downregulated given we showed previously that warm-acclimated lake trout grew larger than the cold-acclimated group (Chapter 2; Penney et al. 2021). Indicators of growth, measured as expression of *GH* and *IGF-1*, increased in *Labeo rohita* at slightly warmer acclimation temperatures but was suppressed at higher acclimation temperatures (liver: Shahjahan et al. 2021). We collected liver samples at each fish's CTMax, so it is possible that fish were responding

to the stress of the acute temperature increase and may have suppressed growth at this point in favour of a heat stress response. Lastly, there is evidence suggesting that growth hormone signaling pathways in the muscle are more sensitive to elevated temperature than in the liver of Atlantic salmon and rainbow trout (Hevrøy et al. 2015), thus it is possible that we may have seen a stronger transcriptional response for growth pathways in the muscle compared to the liver.

Transcriptomic responses to warm acclimation across generations

We found that an offspring's thermal acclimation often had a greater influence on the level (LFC) of gene expression than did the thermal experience of its parents and in some cases multigenerational (offspring and parental) warm acclimation had an additive effect. For example, cold-acclimated offspring downregulated the molecular chaperone *HYOU1* when either parent was warm-acclimated (LFC maternal: -1.21, paternal: -0.94; Table A4.17), however, a combination of offspring and parental warm acclimation upregulated *HYOU1* (LFC offspring & maternal: 0.72, offspring & paternal 0.80, offspring & both parents 1.14), though to a lesser extent than when offspring were warm-acclimated in isolation (LFC 1.29; Table A4.17). This trend suggests that within- and transgenerational warm acclimation had an additive effect on the expression level of *HYOU1*. The gene *MAPK14a* was upregulated whenever the offspring were warm-acclimated, regardless of parental thermal experience (LFC ranged from 1.19 to 2.06) and appeared to override the isolated effect of transgenerational (parental) warm acclimation (LFC ranged from -0.67 to 0.52; Table A4.17). The idea of within-generation plasticity (WGP) overriding TGP has been presented before (Shama 2017), and our experiment

provides evidence of this phenomenon at the transcriptomic level. It has been suggested that transgenerational effects are strongest in early-juvenile life stages (Yin et al. 2019) and it would be interesting to see if this is reflected at the transcriptomics level by looking at gene expression at different life stages following within- and transgenerational warm acclimation.

Compared to within-generation (offspring) warm acclimation, transgenerational warm acclimation (parents only) had a smaller effect on differential gene expression in lake trout offspring, supporting our previous study of whole animal metabolic responses to acute warming (Chapter 2; Penney et al. 2021). Maternal and paternal warm acclimation separately elevated DEGs functioning in the DNA damage response (*DDIT4*) and heat shock proteins (maternal only: *HSPB1*, *HSPB7*). These transgenerationally elevated pathways associated with DNA damage and HSPs support the idea of an adaptive response whereby parents may improve offspring fitness when they correctly anticipate their offspring's environment (Bateson et al. 2014; Bernatchez 2016; Ashe et al. 2021).

Our prediction that transgenerational (parental) warm acclimation would upregulate metabolic processes in the lake trout offspring was partially supported. We observed downregulation of the genes for the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase and enolase in all groups except for the two groups that included the combined effect of offspring cold acclimation and paternal warm acclimation, which indicates a paternal transgenerational effect of increasing offspring metabolism (Table A4.17). Despite this evidence, we cannot be fully confident that glycolysis was upregulated in these offspring because, while the differential expression of

GAPDH, *ALDOB* and *ENOA* suggests an effect of warm acclimation on the rate of flux through glycolysis in the offspring, these three transcripts did not significantly enrich the 'glycolysis' pathway in our analysis. While the expression of these genes may vary depending on species, tissue type and treatment, the enzymes these genes encode are not considered rate-limiting enzymes, and these genes have even been suggested as housekeeping genes for experiments exploring differential gene expression (Hori et al. 2012; Purohit et al. 2016; Shekh et al. 2017).

Although transgenerational warming can result in larger offspring (Salinas and Munch 2012), we did not find support for a transgenerational effect of warm acclimation on growth in lake trout at the transcriptome level, consistent with a lack of response at the whole animal level (Chapter 2; Penney et al. 2021). Lastly, despite some genes being transgenerationally expressed in the same direction by either the maternal or paternal warm acclimation, none of these genes appeared to have an additive or synergistic effect on expression level when both parents were simultaneously warm-acclimated (Table A4.14).

The combination of offspring, maternal and paternal warm acclimation had the greatest effect on the overall number genes that were differentially expressed. This adds to a growing literature suggesting that transgenerational plasticity occurs when offspring and parental thermal experiences are coincident (Shama et al. 2014; Donelson et al. 2017; Yin et al. 2019). Among the groups compared, the combination of offspring and parent warm acclimation had the strongest effect on cytochrome c oxidase subunit upregulation. Differential expression of COX-related genes has elsewhere been recorded with transgenerational acclimation (Shama et al. 2016; Bernal et al. 2018). Also, the

expression level of *COX1* and *COX2* subunits (among 7 of 14 subunits tested) correlated with cytochrome c oxidase activity in muscle of fish (Bremer and Moyes 2014); although this can be species-specific (Linder et al. 1995). It is worth noting, however, that gene expression does not necessarily confer a protein to function given that post-transcriptional and post-translational activity could also tag a protein for degradation.

There were a few instances where some genes such as *GAPDH* and *CNOT1* appeared to be simultaneously upregulated and downregulated within treatments, though why this occurred is not immediately clear. One possible explanation is that the direction of the responses were sex-specific (Best et al. 2018; Bell and Hellmann 2019; Bautista et al. 2020). For example, in stickleback, mothers can influence the direction (up- or downregulation) of differentially expressed genes depending on whether the offspring is male or female (Metzger and Schulte 2016). Although we did not test for this, it's possible that these genes were differentially up- or downregulated in male vs. female offspring because each treatment group included representatives of both sexes. Alternatively, the simultaneous up- and downregulation of *GAPDH* and *CNOT1* in this study may be due to expression of paralogs. Lake trout, like other salmonids, have an ancestrally duplicated genome (Allendorf and Thorgaard 1984) which presents a challenge for *de novo* assembly of transcriptomes from non-model organisms. Mis-assembly of transcripts is possible because reads expressed from paralogs may be erroneously assigned to the incorrect transcript, leading to an over- or underestimation of the up- or downregulation of a gene (Raghavan et al. 2022). This could mean that the total number of significantly expressed genes or the magnitude of differential expression was inaccurate for some transcripts in our experiment, but it may also explain why some

genes (*GAPDH* and *CNOT1*) appeared to be both up- and downregulated. Aligning the reads to the recently available lake trout reference genome (Smith et al. 2022) will help to identify transcripts from paralogous genes (Raghavan et al. 2022;).

The offspring exhibited a stronger within-generation affect on gene expression transgenerational effects, with more DEGs in the offspring warm acclimation treatments than from parental warm acclimation. Some evidence suggests that transgenerational warming has a stronger effect on offspring differential gene expression compared to developmental warming (Veillleux et al. 2015; Shama et al. 2016). Prior to initiating warm acclimation, all offspring in our experiment were reared in the same environment and temperature regime to minimize differential developmental effects. The influence of thermal acclimation during early life stages on differential gene expression at subsequent life stages compared to within- and transgenerational acclimation may be a fruitful area for study.

Venney et al. (2022) used a similar breeding design as this study to assess temperature-related DNA methylation in adult brook trout and their offspring. In contrast to our findings, Venney et al. (2022) observed a stronger transgenerational effect from adults held at higher temperatures during gonad development compared with methylation from offspring rearing temperatures. Despite the apparent contrasts between the results of Venney et al. (2022) and our study, the findings from the two studies are complementary rather than contradictory. Some studies on salmonids have shown that changes in global methylation do not appear to influence differential gene expression (Christensen et al. 2021; Leitwein et al. 2022). DNA methylation can influence gene expression in an individual and subsequent generations, but gene expression is also a direct response to

physiological, metabolic, and bioenergetic requirements that are primarily determined by the environment. The greater methylation observed from reproductive adults by Venney at al. (2022) may reflect the lower temperature tolerances and scope of reproductive adults compared with other free-swimming life stages in many fish species (Dahlke et al. 2020). The lesser extent of DNA methylation observed in juvenile brook trout by Venney et al. may also reflect the ability of subadult brook trout to utilize warmer temperatures than adults (Smith and Ridgway 2019). By contrast, our study examined levels of gene expression by transcriptome sequencing and showed a greater effect of within-generation acclimation on gene expression, paralleling results from a previous study looking at whole organism respirometry in lake trout (Chapter 2; Penney at al. 2021).

Conclusions

Our study found that lake trout exhibit both transgenerational (both maternal and paternal) and within-generation effects of thermal acclimation, with both levels of acclimation influencing the strength and direction of differential expression of genes associated with metabolism and thermal stress/tolerance. Despite this, the limited transgenerational effect on DEGs indicates that adaptive transgenerational acclimation is unlikely to contribute sufficiently substantive benefits to enable lake trout to cope with rapidly changing environmental conditions related to global warming (Chapter 2; Penney et al. 2021). Instead, population-level responses to temperature-related stress will likely be limited to within-generation acclimation (Kelly et al. 2014) and longer-term adaptive responses. Beyond acclimation, persistence of cold-water species and populations may be reliant on existing genetic resources to cope with chronic warming (Comte and Olden

2017; Crozier and Hutchings 2014; Guzzo and Blanchfield 2017), which is particularly daunting for long-lived species (Willi et al. 2006). It therefore seems likely that lake trout and similarly vulnerable cold-water species may require increased management and conservation efforts to ensure their future in a rapidly warming world.

Tables

Table 4.1: Summary of the comparison treatments used for assessing differential gene expression in juvenile lake trout following within- and transgenerational thermal acclimation, showing treatment groups (offspring and parental treatments), numbers of male and female offspring sequenced (chosen at random from four offspring families per treatment), and measured effects.

	Treatment group (parent treatment)	**Sequence year (sample size)**	**Offspring sex (#♂, #♀)**	**Effect measured**
Control group	Cold-acclimated offspring (C♀xC♂)	2018 (n=6) & 2020 (n=6)	2, 4 5, 1	*No effect (all other groups are compared to the control group)*
Within-generation Warm acclimation	Warm-acclimated offspring (C♀xC♂)	2018 (n=6)	3, 3	Offspring warm acclimation
	Cold-acclimated offspring (W♀xC♂)	2020 (n=6)	4, 2	Maternal warm acclimation
Transgenerational Warm acclimation	Cold-acclimated offspring (C♀xW♂)	2020 (n=6)	4, 2	Paternal warm acclimation
	Cold-acclimated offspring (W♀xW♂)	2018 (n=6)	3, 3	Maternal & paternal warm acclimation
	Warm-acclimated offspring (W♀xC♂)	2020 (n=6)	2, 4	Offspring & maternal warm acclimation
Within- and transgenerational Warm acclimation	Warm-acclimated offspring (C♀xW♂)	2020 (n=6)	3, 3	Offspring & paternal warm acclimation
	Warm-acclimated offspring (W♀xW♂)	2018 (n=6)	5, 1	Offspring, maternal & paternal warm acclimation

Figures

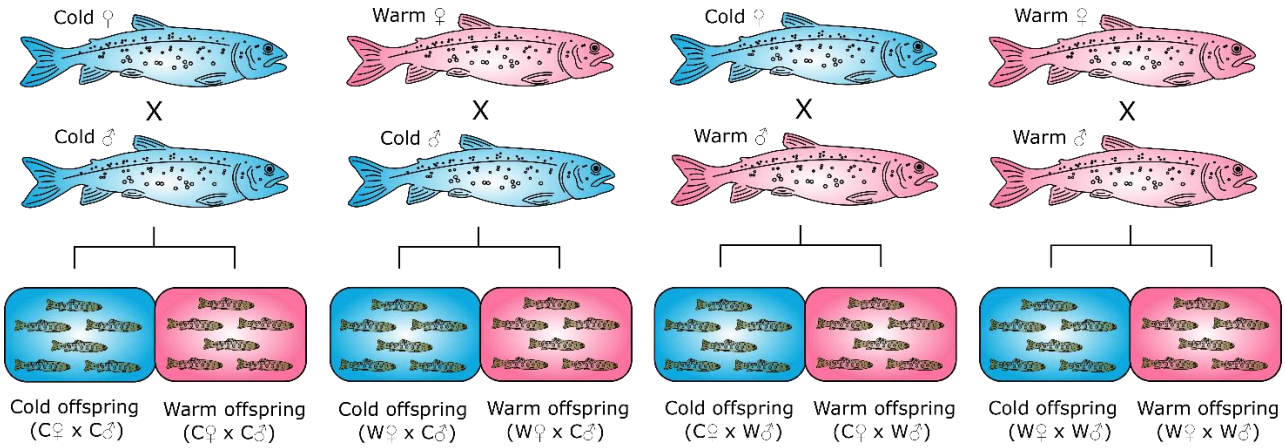

Figure 4.1: Experimental treatment groups of lake trout (*Salvelinus namaycush*). Two males and two females each from cold- and warm acclimation tanks were bred in a full factorial cross, resulting in four families per parental treatment group (C♀xC♂, W♀xC♂, C♀xW♂, W♀xW♂; one each depicted here for simplicity). Offspring from each family were divided and acclimated to either a cold (11°C) or warm (15°C) temperature.

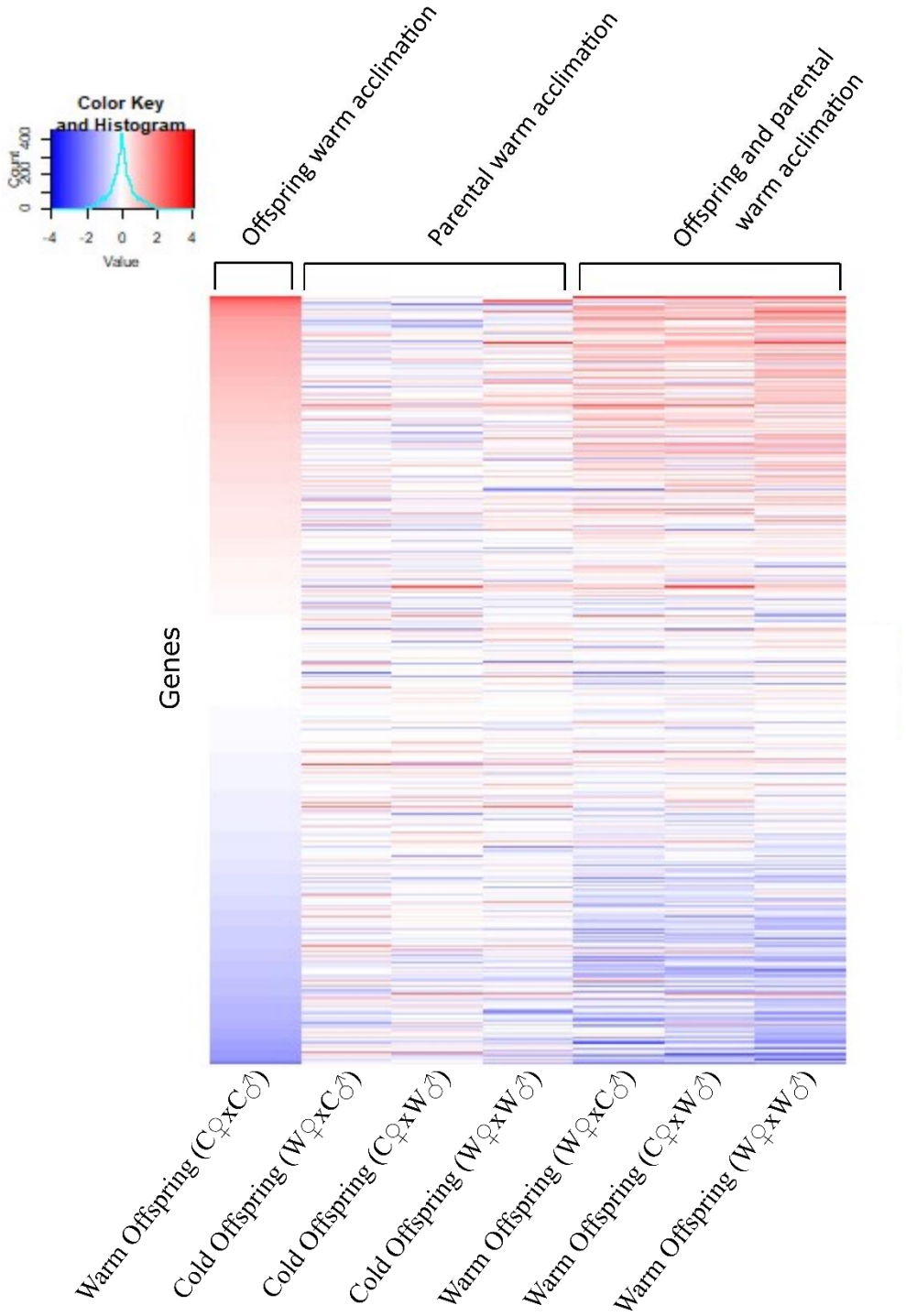

Figure 4.2: Heatmap comparing the effect of parental and offspring thermal acclimation on differential gene expression in juvenile lake trout, grouped by combined parental and offspring warm acclimation (transgenerational warming; top) and offspring acclimation treatment (within-generation; bottom). Each column represents a treatment group (n = 6

individuals) compared against the control group (11°C C♀xC♂; n = 6; not shown). Gene expression levels are represented as log2-fold change (LFC; blue = downregulate, red = upregulated). LFC is sorted according to gene expression level in warm-acclimated offspring to show that any given gene up- or downregulated by offspring warm acclimation could be expressed differently with the influence of maternal and/or paternal warm acclimation. The list of genes along with their LFC values and FDR (adjusted p-value) can be found in the appendix (Table A4.17).

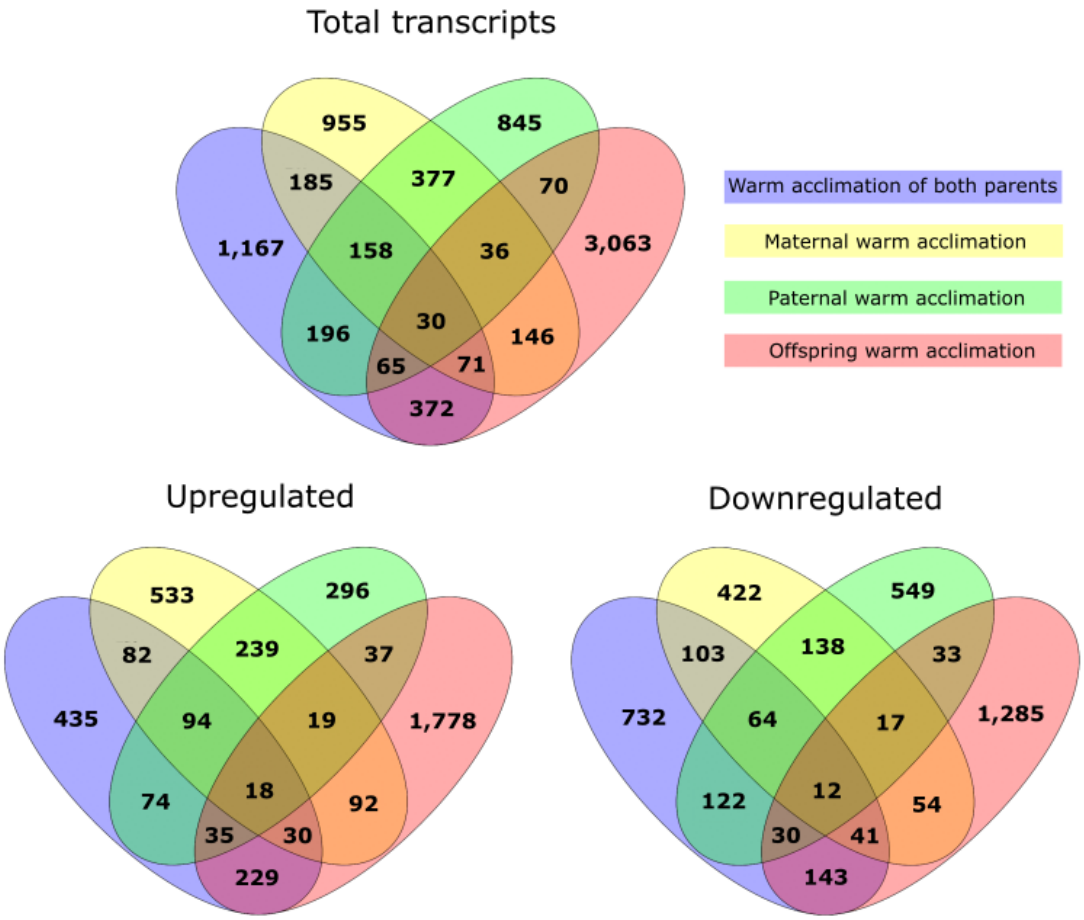

Figure 4.3: Number of transcripts that were differentially expressed in response to warm acclimation of juvenile lake trout offspring (n = 6) when compared against the control group (11°C C♀xC♂; n = 12). Genes were expressed at log2-fold change (LFC) of ≤ -1 or ≥ +1 and are presented to show the total differentially expressed transcripts (top) which were then separated to show the upregulated (bottom, left) and downregulated (bottom, right) transcripts.

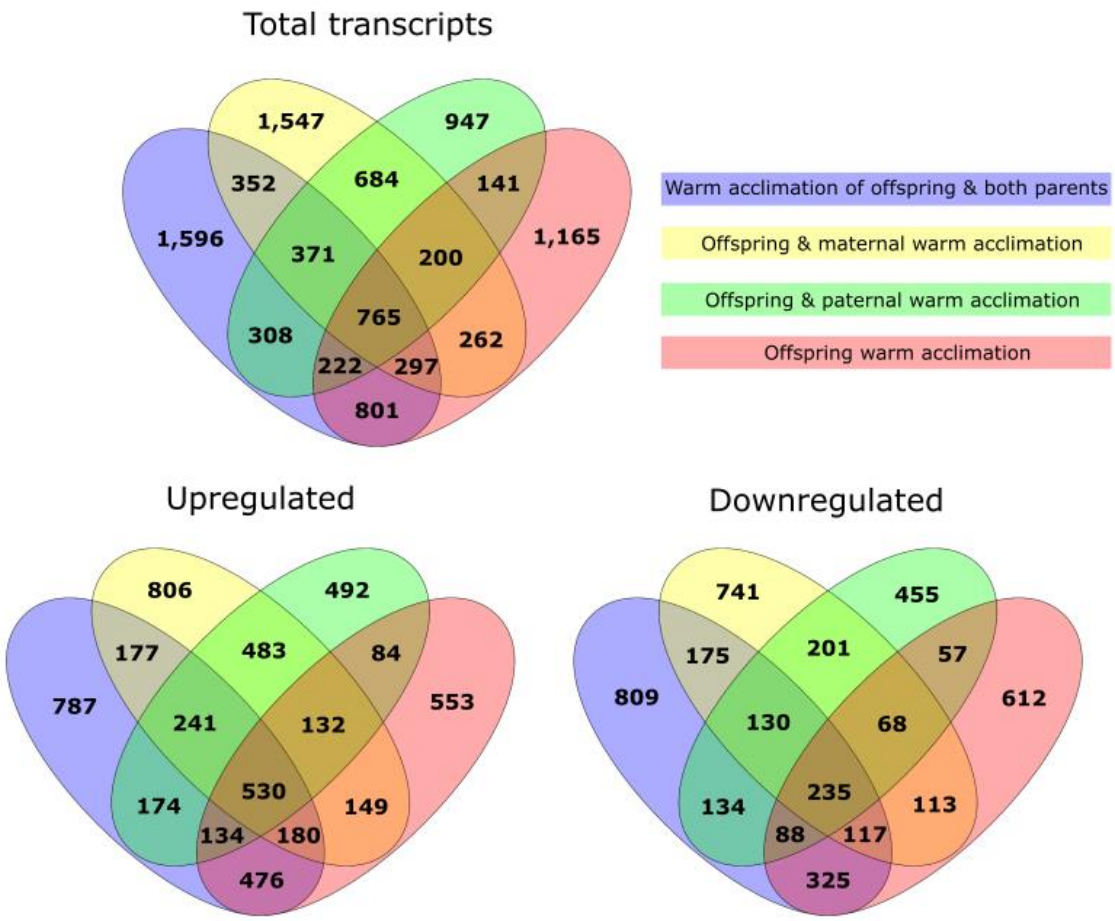

Figure 4.4: Number of transcripts that were differentially expressed in response to transgenerational (parental) warm acclimation of juvenile lake trout offspring when the mothers (A) or the fathers (B) were warm-acclimated, and when both parents (C) were warm-acclimated. Treatment groups (n = 6 each) were compared against control group (11°C C♀xC♂; n = 12). Genes were expressed at log2-fold change (LFC) of ≤ -1 or ≥ +1 and are presented to show the total differentially expressed transcripts (top) which were then separated to show the upregulated (bottom, left) and downregulated (bottom, right) transcripts.

Chapter 5: General Discussion

My research examined the capacity for thermal acclimation within- and across a generation in two cold-adapted freshwater fishes, lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*). I explored the effect of within- and transgenerational warm acclimation on offspring whole-animal metabolic rate and upper thermal tolerance to assess their comparative capacity for thermal plasticity within and across generations. Both species exhibited transgenerational thermal plasticity in addition to within-generation thermal plasticity, with the magnitude of the acclimation responses differing between the two species. Despite the difference in acclimation temperatures for each species, neither species increased their CTM by a large margin. Complementing my investigation of transgenerational plasticity in lake trout, liver RNA sequencing identified metabolic pathways influenced by within- and transgenerational warm acclimation.

Chapter summaries

In Chapter 2, I examined the effect of within- and transgenerational temperature acclimation on the upper thermal tolerance and whole animal metabolic rate in cold- and warm-acclimated lake trout offspring by measuring their critical thermal maximum (CTM) and rate of oxygen consumption (MO_2) during an acute temperature challenge. Offspring CTM and peak MO_2 did not vary substantially with either within- or transgenerational warm acclimation. By contrast, a transgenerational effect of parental environment on offspring metabolic rate was detected in cold-acclimated offspring, such that cold-acclimated offspring from two warm-acclimated parents showed an elevated resting MO_2, prior to the start of the acute thermal challenge. Further, this elevated

resting MO_2 did not translate into increased size or condition of these offspring, suggesting that this is an example of detrimental plasticity. Despite evidence from other works that transgenerational warm acclimation can improve fitness-related traits in offspring that experience warming (Salinas and Munch, 2012; Donelson et al., 2012; Shama et al., 2014; Munday et al., 2017), my results demonstrated that transgenerational effects of thermal acclimation may not always benefit the offspring. My work showed that matching of thermal environments across generations conferred little benefit to the offspring's response to warming. Instead, thermal environment mismatching across generations appears to be detrimental.

In Chapter 3, I tested the effect of within- and transgenerational temperature acclimation on the upper thermal tolerance and whole animal metabolic rate in cold- and warm-acclimated brook trout offspring, using a parallel study design to that used in Chapter 2. Brook trout make a useful comparison because more variation in upper thermal tolerance occurs among brook trout populations compared to lake trout populations (Stitt et al. 2011; McDermid et al 2013; Kelly et al. 2014); thus, this comparison gave me an opportunity to test whether the magnitude of the transgenerational response to warming is contingent on existing within-generation variation. Again, I measured their CTM and MO_2 during an acute temperature challenge. Within-generation warm acclimation elevated the offspring's CTM by 0.5°C and lowered their peak MO_2. Transgenerational warm acclimation had the general effect of elevating MO_2 throughout much of the acute temperature challenge except at higher temperatures that approached their CTM. Both parents contributed to the transgenerational effect, but surprisingly, the paternal contribution was larger than the maternal contribution. Similar

to what was observed for lake trout in Chapter 2, the transgenerational effect was not necessarily beneficial for brook trout as parental warm acclimation had the effect of lowering the condition of warm-acclimated offspring.

In Chapter 4, I used RNA-sequencing to explore the transcriptomic responses in the liver of lake trout offspring to within- and transgenerational warm acclimation, with the goal of determining which metabolic pathways were involved in thermal plasticity within- and across generations. Functions related to thermal stress responses, signaling processes, immune function, and transcription regulation were differentially expressed following warm acclimation of offspring and/or parents. A transgenerational effect on offspring gene expression was apparent in the offspring, and the size and direction (up- or downregulation) of the effect on differential gene expression depended on the combination of offspring and parental warm acclimation. Additionally, the offspring's (within-generation) warm acclimation had a larger effect on differential expression by affecting more genes and enriching more pathways compared to the contributions from parental warm acclimation.

Synthesis

With respect to the questions posed in Chapter 1, my research adds to the growing body of knowledge documenting the occurrence of transgenerational plasticity in aquatic organisms (Donelson et al. 2018; Bell and Hellman 2019; Yin et al. 2019; Rebolledo et al. 2023), and provides new information on under-represented longer-lived cool- or cold-adapted stenothermal fish (Venney et al. 2022; Houle et al. 2023). My results confirmed that transgenerational plasticity occurs in both species, but also showed that

transgenerational thermal plasticity in both species was limited with respect to within-generation plasticity and is potentially maladaptive in some cases. This latter finding lends support to conjectures that though transgenerational effects may be common, they are not likely to be meaningfully beneficial in responding to strong selective pressures (Uller et al. 2013, Sánchez-Tójar et al. 2020).

As demonstrated by the combined results from Chapters 2 and 4, studying transgenerational effects at multiple levels of biological organization provides a deeper understanding of the systems involved in transgenerational plasticity. Physiological modifications can occur through changes in gene expression, and transgenerational plasticity can, to some degree, regulate offspring gene expression (Veillleux et al. 2015; Shama et al. 2016; Oomen and Hutchings 2017; Chapter 4). In Chapter 2, I showed that the metabolic rate and upper thermal tolerance (MO_2 and CTM) between the cold- and warm-acclimated lake trout offspring demonstrated moderate within-generation plasticity whereas transgenerational plasticity was limited. These patterns at the whole animal level were reflected in liver differential gene expression in the lake trout offspring (Chapter 4); within-generation warm acclimation had the effect of up- or downregulating more genes compared to transgenerational (parental) warm acclimation. Other studies reported a sizeable contribution of transgenerational plasticity to offspring thermal tolerance and metabolic capacity which is reflected in gene expression, and it is not immediately clear why my results did not follow this trend seen in other fish such as *Gasterosteus aculeatus* (Shama et al. 2014, Shama et al. 2016)or the warm-adapted *Acanthochromis polyacanthus* (Donelson et al. 2012, Veilleux et al. 2015). Unlike in other studies, offspring in my experiments were not held at different temperatures during the winter

(during the egg and alevin stages) for the purpose of minimizing differences due to developmental plasticity. Offspring can be particularly sensitive to environmental changes during early development (Vagner et al. 2019; Bautista and Crespel 2021). How warm acclimation during earlier stages of development interacts with the effects of transgenerational acclimation is not clear, but if such an interaction can occur it may have influenced my results. Alternatively, limited transgenerational plasticity could be characteristic of cold-adapted, stenothermal fish, but more studies would be required to determine the existence of a relationship between transgenerational plasticity and environmental tolerances across populations or species.

An additive effect of parental warm acclimation was evident in the thermal physiology (MO_2 and CTM) and transcriptomics of the lake trout offspring. In Chapter 2, lake trout offspring displayed similar metabolic rate and upper thermal tolerance irrespective of parental acclimation temperature, with the exception of cold (11°C) acclimated offspring from warm-acclimated mothers and fathers, which had an elevated resting MO_2 compared to the other treatment groups. Nevertheless, statistical models detected additive effects of both maternal and paternal warm acclimation on the offspring's resting and peak MO_2, and CTM in Chapter 2. This supported findings at the transcriptomics level (Chapter 4), where the combined effect of warm acclimation of both parents resulted in more differentially expressed genes in the cold-acclimated offspring compared to when either mothers or fathers were warm-acclimated in isolation. Although my thesis did not investigate the epigenetic mechanism underlying transgenerational plasticity, gene expression in the latter can be achieved through DNA methylation, microRNAs and histone modification of parental genes (Dai et al. 2020; Spadafora 2020).

I did not measure epigenetic modification in the offspring groups used for my experiments, however, transgenerational thermal acclimation can increase DNA methylation of the genome in *Salvelinus*, as seen in brook trout (Venney et al. 2022). It would be interesting to measure DNA methylation in lake trout to see if differential methylation coincides with differential gene expression with within- and transgenerational thermal acclimation.

Transgenerational plasticity may be contingent on within-generation plasticity (Leimar and McNamara 2015), which is consistent with my experimental results. Both lake trout and brook trout are capable of within-generation thermal plasticity (Stitt et al. 2011; McDermid et al 2013; Kelly et al. 2014) and my work showed that transgenerational effects also occur in these fish (also see Venney et al. 2022; Houle et al. 2023).

How the strength of one form of plasticity compares to the other may well be situation-dependent and may vary from species to species, but likely depends on the strength and timing of environmental change relative to generation time. It is thought that within-generation plasticity is favoured when environmental variation occurs within an individual's lifetime, whereas transgenerational plasticity is favoured when environmental variation occurs across generations but is relatively stable within a generation (Leimar and McNamara 2015; Beaman et al. 2016). Comparing the whole animal responses of lake trout and brook trout (Chapters 2 and 3, respectively) allowed me to observe within-generation responses relative to transgenerational responses in these congeners. Compared to lake trout, the transgenerational effect was relatively weaker with respect to within-generation plasticity in brook trout, evidenced as a similar upper

thermal tolerance (CTM) and metabolic rates (resting and peak MO_2) among offspring groups irrespective of parental warm acclimation. In natural settings, these two salmonids also differ with regards to habitat instability. Lake trout populations are landlocked with very limited (if any) dispersal opportunities, and during the summer when lakes stratify lake trout retreat to the hypolimnion where water is cold and more oxygenated (Casselman 2008; Guzzo and Blanchfield 2017). In contrast, brook trout typically experience more within-generation thermal variation (Biro et al. 2008; Smith and Ridgway 2019) which is also reflected in the general observation that, among salmonids, brook trout may be thermal generalists (Durhack et al. 2021). Thus, the finding that transgenerational plasticity was relatively weaker than within-generation plasticity in brook trout may potentially reflect the habitat instability experienced by brook trout within a lifetime/generation. This lends support for the current theory that the strength of within-generation plasticity relative to transgenerational plasticity depends on the strength and timing of environmental change relative to generation time (Leimar and McNamara 2015; Beaman et al. 2016; Clement et al. 2023).

It is also possible that transgenerational plasticity had a relatively weaker effect in brook trout because within-generation plasticity was overriding transgenerational effects. Previous studies have shown that brook trout exhibit greater within-generation plasticity than lake trout, both within and among populations (Stitt et al. 2011; McDermid et al. 2012, 2013; Kelly et al. 2014). The idea that within-generation plasticity could override transgenerational plasticity in juveniles has been proposed before (Shama et al. 2014; Leimar and McNamara 2015; Donelson et al. 2018). I measured thermal plasticity at only one life stage, but it is possible that transgenerational plasticity is stronger at earlier

stages of development. Early life stages are particularly sensitive to temperature (Cook et al. 2018a and b), thus transgenerational plasticity may be more important for offspring survival at these earlier developmental stages (Leimar and McNamara 2015; Vagner et al. 2019; Bautista and Crespel 2021). To confirm this, further study would be required to track the strength of the transgenerational response relative to the within-generation response in brook trout from fertilization throughout early development.

Cold-adapted populations are particularly vulnerable to climate change, especially warming environments (IPCC 2022; Wu et al. 2022). Uncertainty remains with regards to what extent transgenerational plasticity will buffer the negative impacts of climate change on vulnerable species. Some studies point to the potential for adaptive or beneficial transgenerational plasticity (Donelson et al. 2018; Yin et al. 2019), but others argue that transgenerational or anticipatory effects are not widespread or are weak (Uller et al. 2013; Sánchez-Tójar et al. 2020). According to my results, transgenerational effects may not have a sufficiently meaningful impact on the ability of populations of cool- and cold-water stenotherms to cope with climate change, particularly for species with long generation times, as climate change effects may simply be too rapid for transgenerational responses to benefit offspring fitness. However, this is not to say that transgenerational plasticity will be detrimental or insufficient for all species or populations of cool- and cold-adapted populations. The strength or outcome of transgenerational plasticity may be dependent on interacting temporal and environmental factors (Leimar and McNamara 2015; Beaman et al. 2016; Colicchio and Herman 2020; Clement et al. 2023). If transgenerational plasticity is insufficient to buffer the negative impacts of environmental

warming due to climate change, then selection will act on those individuals or populations that are the most thermally tolerant (Morgan et al. 2020).

Future directions

Future work on transgenerational plasticity in cold-adapted species could expand to include the effect of combined abiotic factors. Transgenerational responses may depend on certain types of stressors (Burton et al. 2021) or the interaction of multiple stressors (Guillaume et al. 2016; Harmon and Pfenning 2021). For example, hypoxia can have negative consequences on the physiology of aquatic organisms (Earhart et al. 2022) and hypoxia often coincides with warming. Although I detected a transgenerational effect of temperature in lake trout and brook trout, habitat-limited hypoxia may be a more limiting stress, particularly for lake trout. Lake trout spend their summer months in the cooler hypolimnion below the thermocline (Casselman 2008; Guzzo and Blanchfield 2017) and the oxygen in the water below the thermocline is not replenished due to lack of mixing. Near the end of the summer, lake trout often experience hypoxia and as summers become hotter for longer periods due to climate change, lake trout will likely be limited to the hypolimnion for longer durations, increasing their exposure to hypoxic waters (Guzzo and Blanchfield 2017). For my experiments, I had supplemented oxygen to the holding tanks holding to avoid eliciting a hypoxia response in the fish. Future studies could investigate whether there is a transgenerational component to hypoxia acclimation. Specifically, does the response to hypoxia in offspring depend on a combination of within-generation and transgenerational exposure?

Conclusion

Cold-adapted stenotherms are capable of transgenerational plasticity, however, it was limited in both charr species. Unfortunately, this implies that neither lake trout nor brook trout is likely to significantly benefit from transgenerational plasticity under the threat of warming due to climate change. Distributional shifts towards cooler environments are unlikely for landlocked populations and climate change is quickly outpacing the ability of populations to adapt (Willi et al. 2006, Comte and Olden 2017). This leaves these two species of *Salvelinus* reliant on their already limited within-generation thermal plasticity to cope with warming freshwater habitats. In the long-term, without the buffering capacity of adaptive transgenerational plasticity, effective conservation and management policies will likely be required to intervene in preventing the extirpation of cold-adapted, stenothermal populations due to climate change.

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Appendices

Appendix – Chapter 2

Methods

Egg quality measurements

Preliminary analysis was conducted on the lake trout eggs to determine whether egg quality differed between the females of the two different acclimation temperatures. Ten eggs were randomly chosen from each of four cold-acclimated females and four warm-acclimated females (40 eggs per temperature treatment) and were measured for size and mass on the same day of spawning. Size was measured as diameter (mm) using digital calipers and mass (g) was measured via a microbalance. Water content was determined by measuring the wet and dry mass of an additional 10 eggs from each female. Eggs were pooled per female in aluminum weigh dishes (one dish per female) to measure wet mass, then eggs were placed in an oven at 60℃ for 24 hours to determine dry mass. Water content was expressed as the percentage of egg mass wet weight due to water. To measure energy content, 30 eggs from each female were placed into aluminum weigh dishes, twice rinsed with deionized water to wash away ovarian fluid, then dried in an oven at 60℃ for 24 hours. After drying, eggs were placed into sample tubes and frozen (-20℃) until analysis. Egg energy content was measured in calories per gram of dried eggs via bomb calorimetry with a Parr 6725 semi-micro calorimeter (Parr Instrument Company, Illinois, USA). The means of the mass, size, % water content and energy content between the two temperature treatments were analyzed using student's t-test in GraphPad Prism 5 (GraphPad Software Inc., La Jolla, California, USA).

DNA extraction and genotyping

Genomic DNA of offspring lake trout was extracted from caudal fin samples, lysing approximately 0.25 cm^2 of tissue in deep-well 96-well plates by adding approximately 10 mg of tissue to each well along with 250 µL lysis buffer (50 mM Tris pH 8, 1000 mM NaCl, 1 mM EDTA, 1% sodium dodecyl sulphate (SDS) weight per volume, and 1000 µg proteinase K). The plates were incubated for 16 hours at 37°C, after which DNA was precipitated by adding 500 µL of 80% isopropanol per well and centrifuging the plates at 2000 *g* for 45 minutes. Afterwards, the supernatant was removed and the remaining pellets were rinsed with 1 mL of 70% ethanol, followed by re-centrifugation for 45 minutes at 2000 *g*. DNA pellets were air dried in a 70°C incubator for 30 minutes, then dissolved in 150 µl 1x TE (10 mM Tris, 1 mM EDTA). Extraction yields and quality were tested using electrophoresis alongside a mass ladder (Bioshop, Burlington, Ontario) in 1.5% agarose TBE gels stained with Sybr Green (Cedar Lane Laboratories, Burlington, Ontario).

Lake trout DNA samples were amplified at 17 microsatellite loci: MSU01, MSU02, MSU03, MSU05, MSU06, MSU08, MSU09, MSU10, MSU11, MSU13 (Rollins et al. 2009), *Ogo*1a (Olsen *et al*. 1998), *Sco*19 (Taylor *et al*. 2001), *Sco*215 (DeHaan et al. 2005), *Sfo*1, *Sfo*12 (Angers *et al*. 1995), *Sfo*C88 (King et al. 2012), and *Ssa*85 (O'Reilly et al. 1996). Multiplex reactions were performed in 10 µl reactions containing the following: 2 µl DNA with approximately 6 ng/µl, 1x PCR buffer containing 1.5 mM $MgCl_2$ (Qiagen, Mississauga, Ontario), 2 mM each dNTP (Bioshop, Burlington, Ontario), 0.5 mM $MgCl_2$ (Qiagen, Mississauga, Ontario), 0.2 mg/ml BSA (Bioshop, Burlington, Ontario), 0.025 U *Taq* DNA polymerase (Qiagen, Mississauga, Ontario) and ddH_2O. PCR

cycling was carried out on Eppendorf Mastercycler Pro S thermal cyclers. Amplified products for all samples were run on an AB 3730 DNA analysis system with ROX 500 size standard (Applied Biosystems, Foster City, California). Allele sizes were scored using GeneMapper version 3.1 (Applied Biosystems, Foster City, California) and proofread with manual editing.

Sibling relationships were calculated for multilocus genotypes of the offspring using a maximum likelihood relatedness estimator in ML Relate (Kalinowski, Wagner and Taper 2006). The breeding design (small number of parents, known closed mating history, and equal offspring family sizes) negated the need for more complex analytical approaches, and all offspring were assigned to specific mating crosses with high confidence.

Results

Egg quality measurements

Eggs of warm-acclimated female were heavier by 0.006 g, however, no other metrics were significantly different between eggs of cold- and warm-acclimated females (Table A2.2).

Tables

Table A2.1: The lake trout crosses using cold- and warm-acclimated adults to generate families from parents of similar temperatures (C♀xC♂, W♀xW♂) and between temperatures (C♀xW♂, W♀xC♂).

	Female 1 Cold acclimated	Female 2 Cold acclimated	Female 3 Warm acclimated	Female 4 Warm acclimated
Male 1 Cold acclimated	Family 1 C♀xC♂	Family 5 C♀xC♂	Family 9 W♀xC♂	Family 13 W♀xC♂
Male 2 Cold acclimated	Family 2 C♀xC♂	Family 6 C♀xC♂	Family 10 W♀xC♂	Family 14 W♀xC♂
Male 3 Warm acclimated	Family 3 C♀xW♂	Family 7 C♀xW♂	Family 11 W♀xW♂	Family 15 W♀xW♂
Male 4 Warm acclimated	Family 4 C♀xW♂	Family 8 C♀xW♂	Family 12 W♀xW♂	Family 16 W♀xW♂

Table A2.2: Measurements of egg quality from cold-acclimated (10℃, n = 4) and warm-acclimated (17℃, n = 4) lake trout females. Asterix denotes a significant difference between the means ($p<0.05$).

Egg metric	**Cold-acclimated females**	**Warm-acclimated females**
Mass (g)	0.065 ± 0.001	0.059 ± 0.001*
Size (mm)	4.72 ± 0.03	4.69 ± 0.03
% Water content	150.1 ± 2.35	150.1 ± 7.88
Energy content (cal/g)	5703 ± 159	5803 ± 58

Appendix – Chapter 3

Methods

DNA extraction and genotyping

Genomic DNA of offspring lake trout was extracted from caudal fin samples, lysing approximately 0.25 cm^2 of tissue in deep-well 96-well plates by adding approximately 10 mg of tissue to each well along with 250 µL lysis buffer (50 mM Tris pH 8, 1000 mM NaCl, 1 mM EDTA, 1% sodium dodecyl sulphate (SDS) weight per volume, and 1000 µg proteinase K). The plates were incubated for 16 hours at 37°C, after which DNA was precipitated by adding 500 µL of 80% isopropanol per well and centrifuging the plates at 2000 *g* for 45 minutes. Afterwards, the supernatant was removed and the remaining pellets were rinsed with 1 mL of 70% ethanol, followed by re-centrifugation for 45 minutes at 2000 *g*. DNA pellets were air dried in a 70°C incubator for 30 minutes, then dissolved in 150 µl 1x TE (10 mM Tris, 1 mM EDTA). Extraction yields and quality were tested using electrophoresis alongside a mass ladder (Bioshop, Burlington, Ontario) in 1.5% agarose TBE gels stained with Sybr Green (Cedar Lane Laboratories, Burlington, Ontario).

Brook trout DNA samples were amplified at 14 microsatellite loci: *Sfo*12, *Sfo*18, *Sfo*23 (Angers et al. 1995), *Sfo*B52, *Sfo*C24, *Sfo*C28, SfoC38, *Sfo*C86, *Sfo*C88, *Sfo*C115, *Sfo*C129, *Sfo*D75, *Sfo*D100 and *Sfo*C113 (King et al. 2012). Multiplex reactions were performed in 10 µl reactions containing the following: 2 µl DNA (approximately 6 ng/µl), 1x PCR buffer containing 1.5 mM $MgCl_2$ (Qiagen, Mississauga, Ontario), 2 mM each dNTP (Bioshop, Burlington, Ontario), 0.5 mM $MgCl_2$ (Qiagen, Mississauga, Ontario), 0.2 mg/ml BSA (Bioshop, Burlington, Ontario), 0.025 U *Taq* DNA polymerase

(Qiagen, Mississauga, Ontario) and ddH_2O. PCR cycling was carried out on Eppendorf Mastercycler Pro S thermal cyclers. Amplified products for all samples were run on an AB 3730 DNA analysis system with ROX 500 size standard (Applied Biosystems, Foster City, California). Allele sizes were scored using GeneMapper version 3.1 (Applied Biosystems, Foster City, California) and proofread with manual editing.

Sibling relationships were calculated for multilocus genotypes of the offspring using a maximum likelihood relatedness estimator in ML Relate (Kalinowski, Wagner and Taper, 2006). The breeding design (small number of parents, known closed mating history, and equal offspring family sizes) negated the need for more complex analytical approaches, and all offspring were assigned to specific mating crosses with high confidence.

Tables

Table A3.1: The brook trout crosses using cold- and warm-acclimated adults to generate families from parents of similar temperatures (C♀xC♂, W♀xW♂) and between temperatures (C♀xW♂, W♀xC♂).

	Female 1 Cold-acclimated	Female 2 Cold-acclimated	Female 3 Warm-acclimated	Female 4 Warm-acclimated
Male 1 Cold-acclimated	Family 1 C♀xC♂	Family 5 C♀xC♂	Family 9 W♀xC♂	Family 13 W♀xC♂
Male 2 Cold-acclimated	Family 2 C♀xC♂	Family 6 C♀xC♂	Family 10 W♀xC♂	Family 14 W♀xC♂
Male 3 Warm-acclimated	Family 3 C♀xW♂	Family 7 C♀xW♂	Family 11 W♀xW♂	Family 15 W♀xW♂
Male 4 Warm-acclimated	Family 4 C♀xW♂	Family 8 C♀xW♂	Family 12 W♀xW♂	Family 16 W♀xW♂

Figures

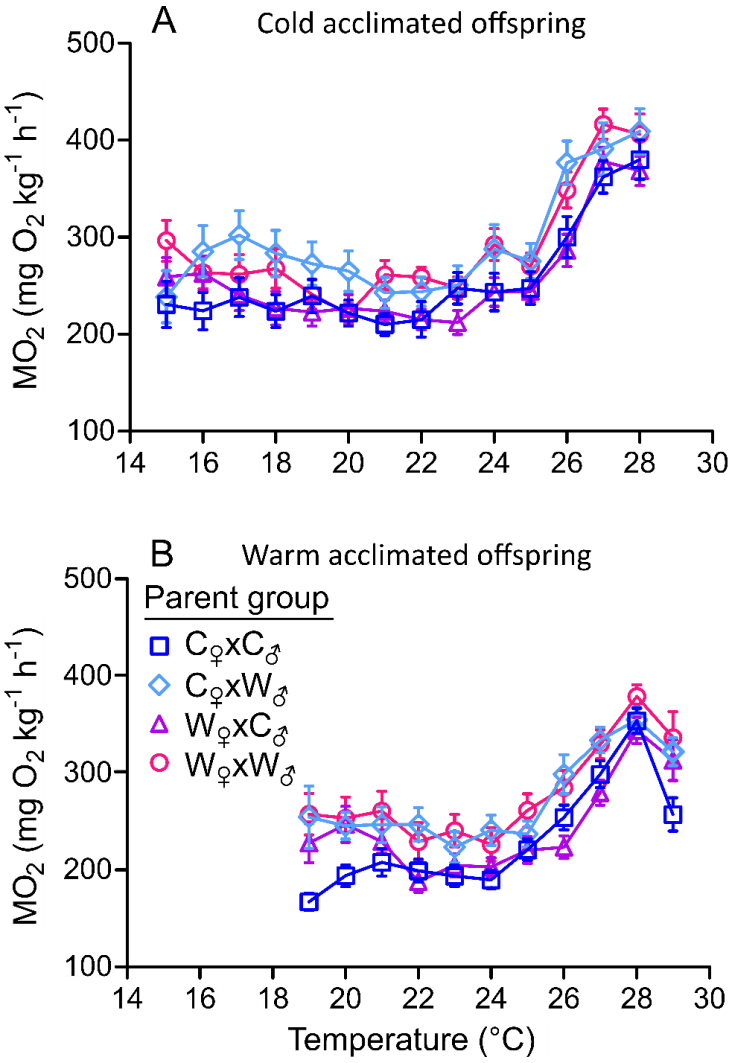


Figure A3.1: The change in the rate of oxygen consumption (MO_2) of A) cold- (15°C, n = 105) and B) warm- (19°C, n = 125) acclimated brook trout offspring (age: 5 months) in response to an acute temperature challenge of +2°C·h^{-1}. Parental groups are represented as C♀xC♂, C♀xW♂, W♀xC♂ and W♀xW♂ where C = cold and W = warm. Plotted values are mass-specific means ± standard error. Mass-specific values are shown for visual purposes only; statistical analyses were performed on whole animal oxygen consumption rates.

Appendix – Chapter 4

Tables

Table A4.1: Summary of the number of reads before and after trimming, and the post-trimming read survival (%) for each individual lake trout offspring sent for RNA-sequencing in years 2018 and 2020.

Batch year	Sample ID	Group	Offspring treatment	Parent treatment (♀x♂)	# Reads pre-trimming	# Reads post-trimming	% Read survival
2018	B1_1	4	11°C	WxW	54415550	52051250	95.6551
2018	B1_2	4	11°C	WxW	55018790	52625054	95.6492
2018	B1_3	4	11°C	WxW	54593506	52030764	95.3058
2018	B1_4	4	11°C	WxW	58782044	56098804	95.4353
2018	B1_5	4	11°C	WxW	56954940	54513606	95.7136
2018	B1_6	4	11°C	WxW	52312326	50135038	95.8379
2018	B1_7	1	11°C	CxC	54037544	51545332	95.388
2018	B1_8	1	11°C	CxC	55760172	53327836	95.6379
2018	B1_9	1	11°C	CxC	54473050	52262540	95.942
2018	B1_10	1	11°C	CxC	53661458	51320672	95.6379
2018	B1_11	1	11°C	CxC	55696120	53211448	95.5389
2018	B1_12	1	11°C	CxC	59913290	57144614	95.3789
2018	B1_13	8	15°C	WxW	54205960	52042214	96.0083
2018	B1_14	8	15°C	WxW	54860404	52493848	95.6862
2018	B1_15	8	15°C	WxW	54427454	52316180	96.1209
2018	B1_16	8	15°C	WxW	58684700	56084056	95.5684
2018	B1_17	8	15°C	WxW	46907110	44895946	95.7125
2018	B1_18	8	15°C	WxW	53466488	51387144	96.1109
2018	B1_19	5	15°C	CxC	55405244	53288246	96.1791

2018	B1_20	5	15°C	CxC	57421840	55123654	95.9977
2018	B1_21	5	15°C	CxC	56203518	54050520	96.1693
2018	B1_22	5	15°C	CxC	54358726	52048880	95.7507
2018	B1_23	5	15°C	CxC	56946592	54680038	96.0199
2018	B1_24	5	15°C	CxC	57348232	54752160	95.4731
2020	B2_1	1	11°C	CxC	63129608	62008762	98.2245
2020	B2_2	1	11°C	CxC	69852174	68758114	98.4337
2020	B2_3	1	11°C	CxC	56719790	55835768	98.4414
2020	B2_4	1	11°C	CxC	76433472	75014210	98.1431
2020	B2_5	1	11°C	CxC	58037958	57063776	98.3215
2020	B2_6	1	11°C	CxC	72412002	71543834	98.8011
2020	B2_7	2	11°C	CxW	73687114	72624372	98.5578
2020	B2_8	2	11°C	CxW	105492966	104254538	98.8261
2020	B2_9	2	11°C	CxW	87214684	86122112	98.7473
2020	B2_10	2	11°C	CxW	70431612	69222750	98.2836
2020	B2_11	2	11°C	CxW	66201500	65105912	98.3451
2020	B2_12	2	11°C	CxW	74245746	72826088	98.0879
2020	B2_13	3	11°C	WxC	67782674	66498102	98.1049
2020	B2_14	3	11°C	WxC	83478412	82035068	98.271
2020	B2_15	3	11°C	WxC	69670738	68801456	98.7523
2020	B2_16	3	11°C	WxC	67479924	66412604	98.4183
2020	B2_17	3	11°C	WxC	77674722	76558144	98.5625
2020	B2_18	3	11°C	WxC	80533370	79167884	98.3044
2020	B2_19	6	15°C	CxW	68483562	67535738	98.616
2020	B2_20	6	15°C	CxW	77243614	75602038	97.8748
2020	B2_21	6	15°C	CxW	81632490	80288422	98.3535
2020	B2_22	6	15°C	CxW	63142390	62275614	98.6273
2020	B2_23	6	15°C	CxW	74797822	73503334	98.2694

2020	B2_24	6	15°C	CxW	76285274	75057198	98.3902
2020	B2_25	7	15°C	WxC	68160270	66675054	97.821
2020	B2_26	7	15°C	WxC	64218652	63259522	98.5065
2020	B2_27	7	15°C	WxC	62820230	61897476	98.5311
2020	B2_28	7	15°C	WxC	72867056	71744330	98.4592
2020	B2_29	7	15°C	WxC	70275382	69254936	98.5479
2020	B2_30	7	15°C	WxC	77016664	75977628	98.6509

‘Offspring treatment’ refers to the acclimation temperature of the offspring and ‘parent treatment’ refers to the acclimation temperature of the parents which depicted as crosses (mothers listed first) where ‘C’ refers to ‘Cold’ (10°C) and ‘W’ refers to ‘Warm’ (17°C).

Table A4.2: Trinity statistics for *de novo* assembly of sequenced lake trout RNA.

Sequencing year (facility)	2018 (TCAG)	2020 (C3G)
Total assembled bases	437989618	401467999
Total transcripts	524988	413029
Total genes	261620	269149
Average contig length (bp)	834.29	972.01
Median contig length (bp)	417	502
GC content (%)	46.7	47.46
N10 (bp)	4254	4805
N20 (bp)	3210	3590
N30 (bp)	2553	2832
N40 (bp)	2026	2256
N50 (bp)	1563	1763

Table A4.3: The function of differentially expressed genes in lake trout offspring with warm acclimation (within-generation).

UP-REGULATED FUNCTIONS: Offspring warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Signal	29	LICH, TM2D2, SAA5, TPMT, PTPRN, IFNA2, CO7, MIME, LIPE, I12R2, ERLN2, ISK1, CD276, CASR, HCE1, IL1R2, CLM7, C1QL4, PDIA5, SUMF2, MFAP4, IL8, TNR5, BPI, MCP, COEA1, CBLN3, RL6, PVRL1	**0.017**
Cytoplasm (GO:0005737)	14	GNPI, TCPD, SYK, SYFB, SYFA, PHLA2, IF2A, KAD, DDT4L, TBA, MTAP, IF6, SYNC	**0.021**
Immunoglobulin-like fold (IPR013783)	9	TGM1, I12R2, MAG, IL1R2, BT2A2, CLM7, COEA1, PVRL1, CD276	**0.004**
Cytoplasm	9	GNPI, MTAP, TBA, NDOR1, TCPD, IF6, PHLA2, KAD	0.097
Immunoglobulin subtype (IPR003599)	6	MAG, IL1R2, BT2A2, CLM7, PVRL1, CD276	**0.004**
IG (SM00409)	6	MAG, IL1R2, BT2A2, CLM7, PVRL1, CD276	**0.008**
Immunoglobulin-like domain (IPR007110)	6	MAG, IL1R2, BT2A2, CLM7, PVRL1, CD276	**0.024**
Immunoglobulin V-set (IPR013106)	4	BT2A2, CLM7, PVRL1, CD276	**0.023**
Methyltransferase	4	AS3MT, NSUN4, TPMT, TRM61	0.095
Igv (SM00406)	3	BT2A2, CLM7, PVRL1	**0.026**
Metalloendopeptidase activity (GO:0004222)	3	MMP25, YBEY, HCE1	**0.049**
Aminoacyl-tRNA synthetase	3	SYFB, SYFA, SYNC	0.050
Flavin adenine dinucleotide binding (GO:0050660)	3	NDOR1, ETFA, SDHA	0.069
Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	0.051
Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	0.053
Nucleoside triphosphate biosynthetic process (GO:0009142)	2	KAD	0.053
Adenylate kinase, isozyme 1 (IPR006267)	2	KAD	0.054

PB1 (SM00666)	2	TFG, SQSTM	0.065
Lipoxygenase, LH2 (IPR001024)	2	LIPE, LOX5	0.079
Phox/Bem1p (IPR000270)	2	TFG, SQSTM	0.079
LH2 (SM00308)	2	LIPE, LOX5	0.095
DOWN-REGULATED FUNCTIONS: Offspring warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Metal-binding	19	ALB1, RNF13, FHL1, HBB1, TRF, CRIP2, TR-ALPHA, ADHX, LCE, CAHZ, 5NTC, RIR2, RN151, RL37, COX1, HBAD, DUS3L, LOC100136922	**0.009**
(SM01332)	3	CCNA2, CCNB3, CCNE2	**0.009**
Transferase	17	BUB1B, ESCO2, CDN2B, TGM1, GNPTG, KITH, TOPK, NAR5, PINK1, UPP, PGK, P4K2A, CKS1, UD2A2, ELOV7, TGM2, OTC	**0.096**
Metal ion binding (GO:0046872)	11	TGM1, 5NTC, RIR2, ALB1, BCL6, RL37, TRF, KLF4, DUS3L, LOC100136922, TGM2	**0.018**
Zinc	10	RNF13, RN151, FHL1, RL37, CRIP2, TR-ALPHA, ADHX, DUS3L, LCE, CAHZ	**0.045**
Iron	6	RIR2, HBB1, TRF, COX1, HBAD	0.063
Flavoprotein	4	IVD, PNPO, ACOX3, DUS3L	**0.011**
Heme	4	HBB1, COX1, HBAD	0.067
Heme binding (GO:0020037)	4	HBB1, COX1, HBAD	0.069
Cyclin, C-terminal domain (IPR004367)	3	CCNA2, CCNB3, CCNE2	**0.011**
CYCLIN (SM00385)	3	CCNA2, CCNB3, CCNE2	**0.042**
Cyclin, N-terminal (IPR006671)	3	CCNA2, CCNB3, CCNE2	**0.044**
Cyclin	3	CCNA2, CCNB3, CCNE2	0.051
glycosylation site:N-linked (GlcNAc...)	3	ALB1, TRF, LOC100136922	0.054
Cyclin-like (IPR013763)	3	CCNA2, CCNB3, CCNE2	0.056
Hemoglobin complex (GO:0005833)	3	HBB1, HBAD	0.072
Flavin adenine dinucleotide binding (GO:0050660)	3	IVD, ACOX3, DUS3L	0.073

disulfide bond	3	ALB1, TRF, LOC100136922	0.074
Tryp_SPc (SM00020)	3	FA10, PRS27, HGFA	0.076
DNA replication	3	PSF2, SLD5, MCM2	0.087
Peptidase S1 (IPR001254)	3	FA10, PRS27, HGFA	0.090
Globin (IPR000971)	3	HBB1, HBAD	0.098
Globin, structural domain (IPR012292)	3	HBB1, HBAD	0.098
Globin-like (IPR009050)	3	HBB1, HBAD	0.098
ALBUMIN (SM00103)	2	ALB1, LOC100136922	**0.044**
Chromosome, centromeric region (GO:0000775)	2	MIS12, CENPN	**0.044**
Serum albumin, N-terminal (IPR014760)	2	ALB1, LOC100136922	**0.048**
Serum albumin/Alpha-fetoprotein (IPR021177)	2	ALB1, LOC100136922	**0.048**
ALB/AFP/VDB (IPR000264)	2	ALB1, LOC100136922	**0.048**
Serum albumin, conserved site (IPR020857)	2	ALB1, LOC100136922	**0.048**
Regulation of G2/M transition of mitotic cell cycle (GO:0010389)	2	CCNA2, CCNB3	0.051
TGc (SM00460)	2	TGM1, TGM2	0.065
Transglutaminase, C-terminal (IPR008958)	2	TGM1, TGM2	0.071
Transglutaminase, N-terminal (IPR001102)	2	TGM1, TGM2	0.071
Serum albumin-like (IPR020858)	2	ALB1, LOC100136922	0.071
Angiotensin II receptor family (IPR000248)	2	APJA, AGTR2	0.071
Transglutaminase-like (IPR002931)	2	TGM1, TGM2	0.071
Protein-glutamine gamma-glutamyltransferase, eukaryota (IPR023608)	2	TGM1, TGM2	0.071

domain:Albumin 2	2	ALB1, LOC100136922	0.075
domain:Albumin 1	2	ALB1, LOC100136922	0.075
domain:Albumin 3	2	ALB1, LOC100136922	0.075
Regulation of cyclin-dependent protein serine/threonine kinase activity (GO:0000079)	2	CCNA2, CCNB3	0.076
Peptide cross-linking (GO:0018149)	2	TGM1, TGM2	0.076
Serum albumin (PIRSF002520)	2	ALB1, LOC100136922	0.077
Angiotensin type II receptor activity (GO:0004945)	2	APJA, AGTR2	0.078
Protein-glutamine gamma-glutamyltransferase activity (GO:0003810)	2	TGM1, TGM2	0.078
U6 snRNA-associated Sm-like protein LSm7 (IPR017132)	2	LSM7	0.093

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.4: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to warm acclimation (within-generation).

UP-REGULATED PATHWAYS: Offspring warm-acclimated		
ID	**Gene Name**	**KEGG**
HMGCLL1	3-hydroxymethyl-3-methylglutaryl-CoA lyase-like 1(hmgcll1)	dre00072:Synthesis and degradation of ketone bodies dre00280:Valine, leucine and isoleucine degradation dre00650:Butanoate metabolism dre01100:Metabolic pathways dre04146:Peroxisome
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
CD99L2	CD99 molecule-like 2(cd99l2)	dre04514:Cell adhesion molecules (CAMs)
JAK1	Janus kinase 1(jak1)	dre04630:Jak-STAT signaling pathway dre05168:Herpes simplex infection
pycard	PYD and CARD domain containing(pycard)	dre04621:NOD-like receptor signaling pathway dre04623:Cytosolic DNA-sensing pathway dre05132:Salmonella infection
BHMT	betaine-homocysteine methyltransferase(bhmt)	dre00260:Glycine, serine and threonine metabolism dre00270:Cysteine and methionine metabolism dre01100:Metabolic pathways
CTNNA2	catenin (cadherin-associated protein), alpha 2(ctnna2)	dre04520:Adherens junction
CHD8	chromodomain helicase DNA binding protein 8(chd8)	dre04310:Wnt signaling pathway
CYP26B1	cytochrome P450, family 26, subfamily b, polypeptide 1(cyp26b1)	dre00830:Retinol metabolism dre01100:Metabolic pathways
GRID2	glutamate receptor, ionotropic, delta 2(grid2)	dre04080:Neuroactive ligand-receptor interaction

hsp90aa1.1	heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 1(hsp90aa1.1)	dre04141:Protein processing in endoplasmic reticulum dre04621:NOD-like receptor signaling pathway dre04914:Progesterone-mediated oocyte maturation
mapk14a	mitogen-activated protein kinase 14a(mapk14a)	dre04010:MAPK signaling pathway dre04068:FoxO signaling pathway dre04261:Adrenergic signaling in cardiomyocytes dre04370:VEGF signaling pathway dre04620:Toll-like receptor signaling pathway dre04621:NOD-like receptor signaling pathway dre04622:RIG-I-like receptor signaling pathway dre04912:GnRH signaling pathway dre04914:Progesterone-mediated oocyte maturation dre05132:Salmonella infection
MTMR8	myotubularin related protein 8(mtmr8)	dre00562:Inositol phosphate metabolism dre01100:Metabolic pathways dre04070:Phosphatidylinositol signaling system
thbs4b	thrombospondin 4b(thbs4b)	dre04145:Phagosome dre04510:Focal adhesion dre04512:ECM-receptor interaction

DOWN-REGULATED PATHWAYS: Offspring warm-acclimated

ID	Gene Name	KEGG
hpda	4-hydroxyphenylpyruvate dioxygenase a(hpda)	dre00130:Ubiquinone and other terpenoid-quinone biosynthesis dre00350:Tyrosine metabolism dre00360:Phenylalanine metabolism dre01100:Metabolic pathways
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
DDIT4	DNA-damage-inducible transcript 4(ddit4)	dre04150:mTOR signaling pathway
FBXO5	F-box protein 5(fbxo5)	dre04114:Oocyte meiosis
gnai3	Gi1 alpha subunit(gnai3)	ola04261:Adrenergic signaling in cardiomyocytes

		ola04540:Gap junction ola04914:Progesterone-mediated oocyte maturation ola04916:Melanogenesis
MPV17L2	MPV17 mitochondrial membrane protein-like 2(mpv17l2)	dre04146:Peroxisome
rasgrf2b	Ras protein-specific guanine nucleotide-releasing factor 2b(rasgrf2b)	dre04010:MAPK signaling pathway
ADRA2C	adrenoceptor alpha 2C(adra2c)	dre04080:Neuroactive ligand-receptor interaction
ALDOB	aldolase b, fructose-bisphosphate(aldob)	dre00010:Glycolysis / Gluconeogenesis dre00030:Pentose phosphate pathway dre00051:Fructose and mannose metabolism dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
aplnra	apelin receptor a(aplnra)	dre04080:Neuroactive ligand-receptor interaction
cahz	carbonic anhydrase(cahz)	dre00910:Nitrogen metabolism
ERO1A	endoplasmic reticulum oxidoreductase alpha(ero1a)	dre04141:Protein processing in endoplasmic reticulum
eif4ebp3l	eukaryotic translation initiation factor 4E binding protein 3, like(eif4ebp3l)	dre03013:RNA transport
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
nkd2b	naked cuticle homolog 2b(nkd2b)	dre04310:Wnt signaling pathway
plcd3a	phospholipase C, delta 3a(plcd3a)	dre00562:Inositol phosphate metabolism dre01100:Metabolic pathways dre04020:Calcium signaling pathway

		dre04070:Phosphatidylinositol signaling system
ppp1r3cb	protein phosphatase 1, regulatory subunit 3Cb(ppp1r3cb)	dre04910:Insulin signaling pathway dre04931:Insulin resistance
RRM2	ribonucleotide reductase regulatory subunit M2(rrm2)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways dre04115:p53 signaling pathway
thbs3a	thrombospondin 3a(thbs3a)	dre04145:Phagosome dre04510:Focal adhesion dre04512:ECM-receptor interaction
UBE2T	ubiquitin-conjugating enzyme E2T (putative)(ube2t)	dre03460:Fanconi anemia pathway

Table A4.5: The function of differentially expressed genes in lake trout offspring with maternal (transgenerational) warm acclimation.

UP-REGULATED FUNCTIONS: Mothers warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Transferase	13	NDKA, RPB2, KGUA, HNMT, FPPS, TRM61, GNPTG, PINK1, UPP, UD2A2, MGT5B, BCAT1, TGM2	**0.041**
Allergen	3	LOC100194623, LOC100137051, PRVB	**0.004**
Troponin complex (GO:0005861)	3	TNNI1, TNNI2, TNNI3	**0.018**
Troponin (IPR001978)	3	TNNI1, TNNI2, TNNI3	**0.021**
Glycolytic process (GO:0006096)	3	LOC100194623, ENOA, ALDOA	**0.049**
Lyase	3	LOC100194623, DCUP, ALDOA	0.083
calcium-binding region:1	2	LOC100137051, PRVB	**0.019**
calcium-binding region:2	2	LOC100137051, PRVB	**0.019**
domain:EF-hand 2	2	LOC100137051, PRVB	**0.019**
domain:EF-hand 1	2	LOC100137051, PRVB	**0.019**
Glycerol channel activity (GO:0015254)	2	AQP8	**0.032**
Aquaporin 8 (IPR023277)	2	AQP8	**0.045**
Muscle protein	2	LOC100137051, PRVB	**0.048**
Urea channel activity (GO:0015265)	2	AQP8	**0.048**
ZM (SM00735)	2	PDLI3, LDB3	0.055
ZASP (IPR006643)	2	PDLI3, LDB3	0.059
Alpha crystallin/Heat shock protein (IPR001436)	2	HSPB7, HSPB1	0.059
CCP (SM00032)	2	CO7, MCP	0.069
Sushi/SCR/CCP (IPR000436)	2	CO7, MCP	0.073
Water channel activity (GO:0015250)	2	AQP8	0.079
DOWN-REGULATED FUNCTIONS: Mothers warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**

Signal	14	LGI1, TM2D2, HCE1, LAMB1, EMP3, CBPA1, CTRL, APOH, TNR5, ERP27, ZPLD1, TRP-III, TSSP, CTRB	**0.002**
Hydrolase	10	CTRL, HCE1, ERCC2, LONP2, TRP-III, TSSP, CTRB, HYEP, CBPA1, ITPA	**<0.001**
Protease	7	CTRL, HCE1, LONP2, TRP-III, TSSP, CTRB, CBPA1	**0.001**
Disulfide bond	5	CTRL, APOH, TRP-III, CTRB, CBPA1	0.057
Serine protease	4	CTRL, LONP2, TRP-III, CTRB	**<0.001**
Serine-type endopeptidase activity (GO:0004252)	4	CTRL, LONP2, TRP-III, CTRB	**0.001**
Tryp_SPc (SM00020)	3	CTRL, TRP-III, CTRB	**0.011**
Peptidase S1, trypsin family, active site (IPR018114)	3	CTRL, TRP-III, CTRB	**0.012**
Peptidase S1A, chymotrypsin-type (IPR001314)	3	CTRL, TRP-III, CTRB	**0.013**
Peptidase S1 (IPR001254)	3	CTRL, TRP-III, CTRB	**0.014**
Trypsin-like cysteine/serine peptidase domain (IPR009003)	3	CTRL, TRP-III, CTRB	**0.018**
Nucleotide metabolic process (GO:0009117)	2	5NT1A, ITPA	**0.023**
Serine-type peptidase activity (GO:0008236)	2	TSSP, PEPE	**0.043**

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.6: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to maternal warm acclimation (transgenerational).

UP-REGULATED PATHWAYS: Mothers warm-acclimated		
ID	**Gene Name**	**KEGG**
DDIT4	DNA-damage-inducible transcript 4(ddit4)	dre04150:mTOR signaling pathway
ddb2	damage-specific DNA binding protein 2(ddb2)	dre03420:Nucleotide excision repair dre04115:p53 signaling pathway dre04120:Ubiquitin mediated proteolysis
ERO1A	endoplasmic reticulum oxidoreductase alpha(ero1a)	dre04141:Protein processing in endoplasmic reticulum
fgf1a	fibroblast growth factor 1a(fgf1a)	dre04010:MAPK signaling pathway dre04810:Regulation of actin cytoskeleton
hsd17b12a	hydroxysteroid (17-beta) dehydrogenase 12a(hsd17b12a)	dre00062:Fatty acid elongation dre00140:Steroid hormone biosynthesis dre01040:Biosynthesis of unsaturated fatty acids dre01100:Metabolic pathways dre01212:Fatty acid metabolism
zgc:101663	zgc:101663(zgc:101663)	dre00510:N-Glycan biosynthesis dre01100:Metabolic pathways
DOWN-REGULATED PATHWAYS: Mothers warm-acclimated		
ID	**Gene Name**	**KEGG**
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
FBXO5	F-box protein 5(fbxo5)	dre04114:Oocyte meiosis
EIF3C	eukaryotic translation initiation factor 3, subunit C(eif3c)	dre03013:RNA transport

GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
urah	urate (5-hydroxyiso-) hydrolase(urah)	dre00230:Purine metabolism dre01100:Metabolic pathways

Table A4.7: The function of differentially expressed genes in lake trout offspring with paternal (transgenerational) warm acclimation.

UP-REGULATED FUNCTIONS: Fathers warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Transferase	10	PARP3, PINK1, UPP, SYK, RPB2, NDKA, UD2A2, ALG3, TGM2	**0.022**
Glycosyltransferase	5	PARP3, UPP, UD2A2, ALG3	**0.001**
Allergen	3	LOC100194623, LOC100137051, PRVB	**0.001**
Parvalbumin (IPR008080)	3	LOC100137051, PRVA, PRVB	**0.005**
Glycolytic process (GO:0006096)	3	LOC100194623, ENOA, ALDOA	**0.035**
Muscle protein	2	LOC100137051, PRVB	**0.029**
calcium-binding region:1	2	LOC100137051, PRVB	0.057
domain:EF-hand 1	2	LOC100137051, PRVB	0.057
calcium-binding region:2	2	LOC100137051, PRVB	0.057
domain:EF-hand 2	2	LOC100137051, PRVB	0.057
Fructose-bisphosphate aldolase, class-I (IPR000741)	2	LOC100194623, ALDOA	0.067
Fructose-bisphosphate aldolase activity (GO:0004332)	2	LOC100194623, ALDOA	0.071
Schiff base	2	LOC100194623, ALDOA	0.076
Transferase activity, transferring glycosyl groups (GO:0016757)	2	UPP, ALG3	0.091
DOWN-REGULATED FUNCTIONS: Fathers warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Signal	16	CALCA, TM2D2, PON2, HCE1, TRP-I, SERPH, LAMB1, EMP3, CBPA1, SUMF2, MIME, PYY, NAR5, CTRL, IL8, TSSP	**0.011**
Hydrolase	8	CTRL, HCE1, ERCC2, TRP-I, TSSP, CBPA2, CBPA1, LKHA4	0.053
Protease	7	CTRL, HCE1, TRP-I, TSSP, CBPA2, CBPA1, LKHA4	**0.006**

Zinc ion binding (GO:0008270)	6	RABX5, HCE1, TRI39, CBPA2, CBPA1, LKHA4	0.089
Metalloprotease	4	HCE1, CBPA2, CBPA1, LKHA4	**0.002**
Magnesium ion binding (GO:0000287)	3	SYFB, THG1, PGM1	0.057
Carbohydrate metabolic process (GO:0005975)	3	CHID1, GFPT1, PGM1	0.091
Proteinase inhibitor, carboxypeptidase propeptide (IPR003146)	2	CBPA2, CBPA1	0.054
Metallocarboxypeptidase activity (GO:0004181)	2	CBPA2, CBPA1	0.063
Peptidase M14, carboxypeptidase A (IPR000834)	2	CBPA2, CBPA1	0.064
Proteinase inhibitor, propeptide (IPR009020)	2	CBPA2, CBPA1	0.075
Endocytosis (GO:0006897)	2	NECP1, SNX9	0.080
Zn_pept (SM00631)	2	CBPA2, CBPA1	0.084

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.8: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to paternal warm acclimation (transgenerational).

UP-REGULATED PATHWAYS: **Fathers warm-acclimated**		
Gene ID	**Gene Name**	**KEGG**
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
DDIT4	DNA-damage-inducible transcript 4(ddit4)	dre04150:mTOR signaling pathway
ERO1A	endoplasmic reticulum oxidoreductase alpha(ero1a)	dre04141:Protein processing in endoplasmic reticulum
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
PPP2R2D	protein phosphatase 2, regulatory subunit B, delta(ppp2r2d)	dre03015:mRNA surveillance pathway dre04261:Adrenergic signaling in cardiomyocytes dre04530:Tight junction,
DOWN-REGULATED PATHWAYS: Fathers warm-acclimated		
Gene ID	**Gene Name**	**KEGG**
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids

Table A4.9: The function of differentially expressed genes in lake trout offspring with offspring (within-generation) and parental (both parents; transgenerational) warm acclimation.

UP-REGULATED FUNCTIONS: Both parents warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Signal	16	ETBR2, ALB1, TRP-II, SPHM, TM2D2, TRP-I, CBPA1, SUMF2, GNPTG, CTRL, TNR6, FXYD6, TNR5, OX2G, RL6, FOLR1	**0.017**
Hydrolase	9	UCHL1, CTRL, TRP-II, SPHM, ERCC2, TRP-I, LONP2, CBPA2, CBPA1	**0.024**
Protease	7	UCHL1, CTRL, TRP-II, TRP-I, LONP2, CBPA2, CBPA1	**0.008**
Disulfide bond	6	CTRL, ALB1, TRP-II, TRP-I, CBPA2, CBPA1	**0.065**
Serine-type endopeptidase activity (GO:0004252)	5	TRYP, CTRL, TRP-II, TRP-I, LONP2	**0.001**
Tryp_SPc (SM00020)	4	TRYP, CTRL, TRP-II, TRP-I	**0.001**
Serine protease	4	CTRL, TRP-II, TRP-I, LONP2	**0.002**
Peptidase S1A, chymotrypsin-type (IPR001314)	4	TRYP, CTRL, TRP-II, TRP-I	**0.002**
Peptidase S1 (IPR001254)	4	TRYP, CTRL, TRP-II, TRP-I	**0.002**
Trypsin-like cysteine/serine peptidase domain (IPR009003)	4	TRYP, CTRL, TRP-II, TRP-I	**0.003**
Peptidase S1, trypsin family, active site (IPR018114)	3	CTRL, TRP-II, TRP-I	**0.022**
disulfide bond	3	ALB1, TRP-II, TRP-I	**0.040**
Peroxisome	2	LONP2, PEX12	**0.023**
Digestion	2	TRP-II, TRP-I	**0.046**
Digestion (GO:0007586)	2	TRP-II, TRP-I	**0.048**
Proteinase inhibitor, carboxypeptidase propeptide (IPR003146)	2	CBPA2, CBPA1	0.059

Zn_pept (SM00631)	2	CBPA2, CBPA1	0.063
Peptidase M14, carboxypeptidase A (IPR000834)	2	CBPA2, CBPA1	0.070
Metallocarboxypeptidase activity (GO:0004181)	2	CBPA2, CBPA1	0.080
Proteinase inhibitor, propeptide (IPR009020)	2	CBPA2, CBPA1	0.082
DOWN-REGULATED FUNCTIONS: Both parents warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Metal ion binding (GO:0046872)	7	TGM1, ZN271, CATA, BCL6, RL37, TRF, DUS3L	0.083
Translation (GO:0006412)	7	RS8, RS11, RL37, RL3, RL2, RL10	0.090
Ribosome (GO:0005840)	6	RS8, RS11, RL37, RL3, RL2, RL10	0.090
Glycosyltransferase	4	PARP3, NAR5, GALT2, UD2A2	**0.022**
Lectin	3	LEG3, GALT2, FEL	**0.039**
Carbohydrate binding (GO:0030246)	3	LEG3, GALT2, FEL	0.050
Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	0.030
Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	**0.034**
Ribosomal protein S17 (IPR000266)	2	RS11	**0.043**
Ribosomal protein S17, conserved site (IPR019979)	2	RS11	**0.043**
Protein ADP-ribosylation (GO:0006471)	2	PARP3, NAR5	0.074

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.10: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to warm acclimation of both parents (transgenerational).

UP-REGULATED PATHWAYS: Both parents warm-acclimated		
Gene ID	**Gene Name**	**KEGG**
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
DDIT4	DNA-damage-inducible transcript 4(ddit4)	dre04150:mTOR signaling pathway
SEC61A1	Sec61 translocon alpha 1 subunit(sec61a1)	dre03060:Protein export dre04141:Protein processing in endoplasmic reticulum dre04145:Phagosome
SEPSECS	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase(sepsecs)	dre00450:Selenocompound metabolism dre00970:Aminoacyl-tRNA biosynthesis
BHMT	betaine-homocysteine methyltransferase(bhmt)	dre00260:Glycine, serine and threonine metabolism dre00270:Cysteine and methionine metabolism dre01100:Metabolic pathways
ERO1A	endoplasmic reticulum oxidoreductase alpha(ero1a)	dre04141:Protein processing in endoplasmic reticulum
GRID2	glutamate receptor, ionotropic, delta 2(grid2)	dre04080:Neuroactive ligand-receptor interaction
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)(gatm)	dre00260:Glycine, serine and threonine metabolism dre00330:Arginine and proline metabolism dre01100:Metabolic pathways

GAMT	guanidinoacetate N-methyltransferase(gamt)	dre00260:Glycine, serine and threonine metabolism dre00330:Arginine and proline metabolism dre01100:Metabolic pathways
LIPT2	lipoyl(octanoyl) transferase 2 (putative)(lipt2)	dre00785:Lipoic acid metabolism dre01100:Metabolic pathways
thbs4b	thrombospondin 4b(thbs4b)	dre04145:Phagosome dre04510:Focal adhesion dre04512:ECM-receptor interaction
tigara	tp53-induced glycolysis and apoptosis regulator a(tigara)	dre00051:Fructose and mannose metabolism
DOWN-REGULATED PATHWAYS: Both parents warm-acclimated		
Gene ID	**Gene Name**	**KEGG**
HMGCLL1	3-hydroxymethyl-3-methylglutaryl-CoA lyase-like 1(hmgcll1)	dre00072:Synthesis and degradation of ketone bodies dre00280:Valine, leucine and isoleucine degradation dre00650:Butanoate metabolism dre01100:Metabolic pathways,dre04146:Peroxisome
APH1B	APH1B gamma secretase subunit(aph1b)	dre04330:Notch signaling pathway
pycard	PYD and CARD domain containing(pycard)	dre04621:NOD-like receptor signaling pathway dre04623:Cytosolic DNA-sensing pathway dre05132:Salmonella infection
cldnd	claudin d(cldnd)	dre04514:Cell adhesion molecules (CAMs) dre04530:Tight junction
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
PPP2R2D	protein phosphatase 2, regulatory subunit B, delta(ppp2r2d)	dre03015:mRNA surveillance pathway dre04261:Adrenergic signaling in cardiomyocytes dre04530:Tight junction

zgc:101663	zgc:101663(zgc:101663)	dre00510:N-Glycan biosynthesis dre01100:Metabolic pathways

Table A4.11: The function of differentially expressed genes in lake trout offspring in response to the combined effect of offspring (within-generation) and maternal (transgenerational) warm acclimation.

UP-REGULATED FUNCTIONS: Offspring & mothers warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Transferase	20	PARP3, SYK, PIMT, NDKA, RPB2, F262, HNMT, FPPS, TRM61, TGM1, GNPTG, KITH, AS3MT, MTAP, CDK6, PGK, STT3A, PLCD, BCAT1, MAPK3	**0.042**
Immunoglobulin-like fold (IPR013783)	7	TGM1, MAG, IL1R2, IL31R, COEA1, PVRL1, CD276	0.056
Protein folding (GO:0006457)	5	TCPD, FKBP8, PPIA, DNJB1, FKBP5	0.086
Metalloendopeptidase activity (GO:0004222)	4	MMP25, MMP13, YBEY, MMP9	**0.006**
Metalloprotease	4	MMP25, MMP13, YBEY, MMP9	**0.022**
PDZ domain (IPR001478)	4	PDLI3, RHG21, PDLI1, LDB3	**0.033**
Lyase	4	HEM2, DCUP, GADL1, PISD	0.055
Zinc finger C2H2-type/integrase DNA-binding domain (IPR013087)	4	ZN271, PRDM9, ZN214, ZN391	0.062
Stress response	4	HSP70, HSPB1, HSP7C, HSP30	0.075
Zinc finger, C2H2-like (IPR015880)	4	ZN271, PRDM9, ZN214, ZN391	0.083
Immunoglobulin subtype (IPR003599)	4	MAG, IL1R2, PVRL1, CD276	0.100
ZASP (IPR006643)	3	PDLI3, PDLI1, LDB3	**0.005**
Double-strand break repair (GO:0006302)	3	PARP3, MRE11, CCD98	**0.006**
ZM (SM00735)	3	PDLI3, PDLI1, LDB3	**0.006**
Peptidase M10A (IPR021190)	3	MMP25, MMP13, MMP9	**0.008**
Peptidase M10, metallopeptidase (IPR001818)	3	MMP25, MMP13, MMP9	**0.008**
Hemopexin-like domain (IPR000585)	3	MMP25, MMP13, MMP9	**0.008**

Peptidoglycan binding-like (IPR002477)	3	MMP25, MMP13, MMP9	**0.008**
Hemopexin-like repeats (IPR018487)	3	MMP25, MMP13, MMP9	**0.008**
HX (SM00120)	3	MMP25, MMP13, MMP9	**0.010**
Extracellular matrix (GO:0031012)	3	MMP25, MMP13, MMP9	**0.012**
SMAD domain-like (IPR017855)	3	IRF4, IRF7, SMAD5	**0.026**
Peptidase, metallopeptidase (IPR006026)	3	MMP25, MMP13, MMP9	**0.032**
Metallopeptidase, catalytic domain (IPR024079)	3	MMP25, MMP13, MMP9	**0.038**
ZnMc (SM00235)	3	MMP25, MMP13, MMP9	**0.039**
SMAD/FHA domain (IPR008984)	3	IRF4, IRF7, SMAD5	0.059
Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	0.054
Porphyrin biosynthesis	2	HEM2, DCUP	0.057
Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	0.061
ER-associated ubiquitin-dependent protein catabolic process (GO:0030433)	2	ERLN2, UBXN8	0.061
Respiratory chain complex IV (GO:0045277)	2	COX2, COX1	0.074
Peptidase M10A, metazoans (IPR016293)	2	MMP25, MMP13	0.083
Peptidase M10A, cysteine switch, zinc binding site (IPR021158)	2	MMP13, MMP9	0.083
Decarboxylase	2	DCUP, PISD	0.085
Matrix metalloproteinase, stromelysin type (PIRSF001191)	2	MMP25, MMP13	0.097

DOWN-REGULATED FUNCTIONS: Offspring & mothers warm-acclimated

Function (Term)	**Count**	**Genes**	***p*-value**
Transferase	21	ACVR1, SRPK3, ALAT2, BUB1B, ESCO2, TGM1, KITH, TOPK, GLYG, PINK1, FUT7, UPP, STK35, GBP, PGK, CDK2, CKS1, UD2A2, LRAT, CDKN3, OTC	**0.018**

Nucleus (GO:0005634)	20	AP2A, RFA1, THRB, HLF, MIS12, TAF11, SSU72, PSF2, SLD5, TR-ALPHA, CCNA2, CCNB3, CCNB1, NFIL3, CCNE2, MSTN1, IRF2, MCM3, MCM2	**0.043**
ATP binding (GO:0005524)	19	ACVR1, SRPK3, UBE2C, SYFB, SYFA, RIR1, KITH, TOPK, PINK1, RAD51, P2RX4, STK35, PGK, CDK2, MCM3, LONP2, MYH7, ABCG2, MCM2	**0.001**
Kinase	12	ACVR1, SRPK3, KITH, TOPK, PINK1, STK35, GBP, PGK, CDK2, CKS1, BUB1B, CDKN3	**0.035**
Receptor	11	OGFR, ACVR1, I13R2, THRB, P2RX4, PAR2, ERD22, ARH, CCR9, TR-ALPHA, LRP11	0.085
ATP-binding	11	ACVR1, KITH, RAD51, STK35, PGK, UBE2C, CDK2, MCM3, LONP2, ABCG2, MCM2	0.093
Serine/threonine-protein kinase, active site (IPR008271)	6	ACVR1, SRPK3, TOPK, PINK1, STK35, CDK2	**0.017**
S_TKc (SM00220)	6	ACVR1, SRPK3, TOPK, PINK1, STK35, CDK2	**0.034**
Protein kinase, catalytic domain (IPR000719)	6	ACVR1, SRPK3, TOPK, PINK1, STK35, CDK2	0.086
Cyclin, N-terminal (IPR006671)	5	CCNA2, CCNB3, CCNB1, CCNE2, CCNG2	**<0.001**
CYCLIN (SM00385)	5	CCNA2, CCNB3, CCNB1, CCNE2, CCNG2	**<0.001**
Cyclin	5	CCNA2, CCNB3, CCNB1, CCNE2, CCNG2	**<0.001**
Cyclin-like (IPR013763)	5	CCNA2, CCNB3, CCNB1, CCNE2, CCNG2	**0.001**
DNA replication	5	RIR1, MCM3, PSF2, SLD5, MCM2	**0.002**
Serine-type endopeptidase activity (GO:0004252)	5	PPCE, TRP-I, LONP2, FA10, TRP-III	**0.009**
(SM01332)	4	CCNA2, CCNB3, CCNB1, CCNE2	**0.001**
Cyclin, C-terminal domain (IPR004367)	4	CCNA2, CCNB3, CCNB1, CCNE2	**0.001**
disulfide bond	4	TRP-I, SPP2, TRP-III, LOC100136922	**0.013**
DNA replication (GO:0006260)	4	RIR1, RFA1, PSF2, SLD5	**0.017**
Cell division (GO:0051301)	4	CDC20, CDK2, CKS1, SKA1	0.056
Cell cycle	4	CDC20, CDK2, CKS1, CDKN3	0.076

Zymogen	3	DCAM, TRP-I, TRP-III	**0.031**
Chromosome, centromeric region (GO:0000775)	2	MIS12, CENPN	**0.048**
Thyroid hormone receptor (IPR001728)	2	THRB, TR-ALPHA	0.054
Thyroid hormone receptor activity (GO:0004887)	2	THRB, TR-ALPHA	0.055
Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	0.055
Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	0.058
Regulation of G2/M transition of mitotic cell cycle (GO:0010389)	2	CCNA2, CCNB3	0.058
Mitotic spindle assembly checkpoint (GO:0007094)	2	BUB1B, MD2L1	0.058
Chromosome segregation (GO:0007059)	2	CENPN, SKA1	0.085
Regulation of cyclin-dependent protein serine/threonine kinase activity (GO:0000079)	2	CCNA2, CCNB3	0.085

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.12: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to the combined effect of offspring (within-generation) and maternal (transgenerational) warm acclimation.

UP-REGULATED PATHWAYS: Offspring & mothers warm-acclimated		
Gene ID	**Gene Name**	**KEGG**
HMGCLL1	3-hydroxymethyl-3-methylglutaryl-CoA lyase-like 1(hmgcll1)	dre00072:Synthesis and degradation of ketone bodies dre00280:Valine, leucine and isoleucine degradation dre00650:Butanoate metabolism dre01100:Metabolic pathways dre04146:Peroxisome
C1D	C1D nuclear receptor corepressor(c1d)	dre03018:RNA degradation
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
JAK1	Janus kinase 1(jak1)	dre04630:Jak-STAT signaling pathway dre05168:Herpes simplex infection
SMAD5	SMAD family member 5(smad5)	dre04350:TGF-beta signaling pathway
BHMT	betaine-homocysteine methyltransferase(bhmt)	dre00260:Glycine, serine and threonine metabolism dre00270:Cysteine and methionine metabolism dre01100:Metabolic pathways
FLAD1	flavin adenine dinucleotide synthetase 1(flad1)	dre00740:Riboflavin metabolism dre01100:Metabolic pathways
hsc70	heat shock protein 70 cognate(hsc70)	ola03040:Spliceosome ola04010:MAPK signaling pathway ola04141:Protein processing in endoplasmic reticulum ola04144:Endocytosis
hsp90aa1.1	heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 1(hsp90aa1.1)	dre04141:Protein processing in endoplasmic reticulum dre04621:NOD-like receptor signaling pathway dre04914:Progesterone-mediated oocyte maturation

mapk14a	mitogen-activated protein kinase 14a(mapk14a)	dre04010:MAPK signaling pathway dre04068:FoxO signaling pathway dre04261:Adrenergic signaling in cardiomyocytes dre04370:VEGF signaling pathway dre04620:Toll-like receptor signaling pathway dre04621:NOD-like receptor signaling pathway dre04622:RIG-I-like receptor signaling pathway dre04912:GnRH signaling pathway dre04914:Progesterone-mediated oocyte maturation dre05132:Salmonella infection
mapk14b	mitogen-activated protein kinase 14b(mapk14b)	dre04010:MAPK signaling pathway dre04068:FoxO signaling pathway dre04261:Adrenergic signaling in cardiomyocytes dre04370:VEGF signaling pathway dre04620:Toll-like receptor signaling pathway dre04621:NOD-like receptor signaling pathway dre04622:RIG-I-like receptor signaling pathway dre04912:GnRH signaling pathway dre04914:Progesterone-mediated oocyte maturation dre05132:Salmonella infection
nog3	noggin 3(nog3)	dre04350:TGF-beta signaling pathway
rdh10a	retinol dehydrogenase 10a(rdh10a)	dre00830:Retinol metabolism dre01100:Metabolic pathways

DOWN-REGULATED PATHWAYS: Offspring & mothers warm-acclimated

Gene ID	Gene Name	KEGG
hpda	4-hydroxyphenylpyruvate dioxygenase a(hpda)	dre00130:Ubiquinone and other terpenoid-quinone biosynthesis dre00350:Tyrosine metabolism dre00360:Phenylalanine metabolism dre01100:Metabolic pathways
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation

ELOVL6	ELOVL fatty acid elongase 6(elovl6)	dre00062:Fatty acid elongation dre01040:Biosynthesis of unsaturated fatty acids dre01212:Fatty acid metabolism
FBXO5	F-box protein 5(fbxo5)	dre04114:Oocyte meiosis
rasgrf2b	Ras protein-specific guanine nucleotide-releasing factor 2b(rasgrf2b)	dre04010:MAPK signaling pathway
UAP1L1	UDP-N-acetylglucosamine pyrophosphorylase 1, like 1(uap1l1)	dre00520:Amino sugar and nucleotide sugar metabolism dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics
AACS	acetoacetyl-CoA synthetase(aacs)	dre00280:Valine, leucine and isoleucine degradation dre00650:Butanoate metabolism
alcama	activated leukocyte cell adhesion molecule a(alcama)	dre04514:Cell adhesion molecules (CAMs)
ALDOB	aldolase b, fructose-bisphosphate(aldob)	dre00010:Glycolysis / Gluconeogenesis dre00030:Pentose phosphate pathway dre00051:Fructose and mannose metabolism dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
cldnd	claudin d(cldnd)	dre04514:Cell adhesion molecules (CAMs) dre04530:Tight junction
EIF3C	eukaryotic translation initiation factor 3, subunit C(eif3c)	dre03013:RNA transport
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
MRPS7	mitochondrial ribosomal protein S7(mrps7)	dre03010:Ribosome

phospho1	phosphatase, orphan 1(phospho1)	dre00564:Glycerophospholipid metabolism dre01100:Metabolic pathways
plcd3a	phospholipase C, delta 3a(plcd3a)	dre00562:Inositol phosphate metabolism dre01100:Metabolic pathways dre04020:Calcium signaling pathway dre04070:Phosphatidylinositol signaling system
RRM1	ribonucleotide reductase M1 polypeptide(rrm1)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways
RRM2	ribonucleotide reductase regulatory subunit M2(rrm2)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways dre04115:p53 signaling pathway
RIMKLA	ribosomal modification protein rimK-like family member A(rimkla)	dre00250:Alanine, aspartate and glutamate metabolism dre01100:Metabolic pathways
tdo2a	tryptophan 2,3-dioxygenase a(tdo2a)	dre00380:Tryptophan metabolism dre01100:Metabolic pathways
TTK	ttk protein kinase(ttk)	dre04110:Cell cycle
UBE2T	ubiquitin-conjugating enzyme E2T (putative)(ube2t)	dre03460:Fanconi anemia pathway
zgc:153916	zgc:153916(zgc:153916)	dre05132:Salmonella infection

Table A4.13: The function of differentially expressed genes in lake trout offspring in response to the combined effect of offspring (within-generation) and paternal (transgenerational) warm acclimation.

UP-REGULATED FUNCTIONS: Offspring & father warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Membrane (GO:0016020)	5	COG2, PE2R4, VEGFC, S12A1, CRLS1	0.074
Metalloendopeptidase activity (GO:0004222)	4	MMP25, MMP13, YBEY, MMP9	**0.004**
Metalloprotease	4	MMP25, MMP13, YBEY, MMP9	**0.017**
Peptidase M10A (IPR021190)	3	MMP25, MMP13, MMP9	**0.006**
Peptidoglycan binding-like (IPR002477)	3	MMP25, MMP13, MMP9	**0.006**
Peptidase M10, metallopeptidase (IPR001818)	3	MMP25, MMP13, MMP9	**0.006**
Hemopexin-like domain (IPR000585)	3	MMP25, MMP13, MMP9	**0.006**
Hemopexin-like repeats (IPR018487)	3	MMP25, MMP13, MMP9	**0.006**
HX (SM00120)	3	MMP25, MMP13, MMP9	**0.007**
Extracellular matrix (GO:0031012)	3	MMP25, MMP13, MMP9	**0.011**
Complement C1q protein (IPR001073)	3	C1QL4, CBLN3, C1QL2	**0.022**
C1Q (SM00110)	3	C1QL4, CBLN3, C1QL2	**0.024**
Peptidase, metallopeptidase (IPR006026)	3	MMP25, MMP13, MMP9	**0.027**
ZnMc (SM00235)	3	MMP25, MMP13, MMP9	**0.030**
Metallopeptidase, catalytic domain (IPR024079)	3	MMP25, MMP13, MMP9	**0.032**
TNFR/NGFR cysteine-rich region (IPR001368)	3	TNR6, TNR5, TNR14	**0.032**
TNFR (SM00208)	3	TNR6, TNR5, TNR14	**0.036**

Tumour necrosis factor-like domain (IPR008983)	3	C1QL4, CBLN3, C1QL2	0.071
6-phosphofructo-2-kinase activity (GO:0003873)	2	F262, F264	**0.048**
6-phosphofructo-2-kinase (IPR013079)	2	F262, F264	0.052
Fructose-2,6-bisphosphatase (IPR003094)	2	F262, F264	0.052
Fructose metabolic process (GO:0006000)	2	F262, F264	0.059
Fructose 2,6-bisphosphate metabolic process (GO:0006003)	2	F262, F264	0.059
Respiratory chain complex IV (GO:0045277)	2	COX2, COX1	0.069
Matrix metalloproteinase, stromelysin type (PIRSF001191)	2	MMP25, MMP13	0.071
Peptidase M10A, metazoans (IPR016293)	2	MMP25, MMP13	0.076
Sedlin (IPR006722)	2	TPC2L, LOC106562066	0.076
Peptidase M10A, cysteine switch, zinc binding site (IPR021158)	2	MMP13, MMP9	0.076
Decarboxylase	2	DCUP, PISD	0.078
DOWN-REGULATED PATHWAYS: Offspring & father warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
ATP binding (GO:0005524)	14	SYK, UBE2C, SYFB, SYFA, RIR1, KITH, TOPK, RAD51, STK35, ERCC2, CDK2, LONP2, EPHB1, MCM2	**0.013**
Hydrolase	13	HCE1, TRP-I, CBPA2, CBPA1, LCE, YBEY, CTRL, ERCC2, LONP2, TRP-III, CTRB, HYEP, MCM2	**0.046**
Protease	10	YBEY, CTRL, HCE1, TRP-I, LONP2, TRP-III, CBPA2, CTRB, CBPA1, LCE	**0.007**

Disulfide bond	9	PDIA3, CTRL, APOH, TRP-I, FA10, TRP-III, CBPA2, CTRB, CBPA1	0.062
Serine-type endopeptidase activity (GO:0004252)	7	CTRL, PPCE, TRP-I, LONP2, FA10, TRP-III, CTRB	**<0.001**
Peptidase S1 (IPR001254)	5	CTRL, TRP-I, FA10, TRP-III, CTRB	**0.001**
Tryp_SPc (SM00020)	5	CTRL, TRP-I, FA10, TRP-III, CTRB	**0.001**
Serine protease	5	CTRL, TRP-I, LONP2, TRP-III, CTRB	**0.001**
Metalloprotease	5	YBEY, HCE1, CBPA2, CBPA1, LCE	**0.001**
Trypsin-like cysteine/serine peptidase domain (IPR009003)	5	CTRL, TRP-I, FA10, TRP-III, CTRB	**0.001**
Calcium	5	TRP-I, RECO, FA10, TRP-III, PRVB	0.081
Cyclin, C-terminal domain (IPR004367)	4	CCNA2, CCNB3, CCNB1, CCNE2	**<0.001**
(SM01332)	4	CCNA2, CCNB3, CCNB1, CCNE2	**<0.001**
Cyclin, N-terminal (IPR006671)	4	CCNA2, CCNB3, CCNB1, CCNE2	**0.003**
CYCLIN (SM00385)	4	CCNA2, CCNB3, CCNB1, CCNE2	**0.004**
Cyclin	4	CCNA2, CCNB3, CCNB1, CCNE2	**0.004**
Cyclin-like (IPR013763)	4	CCNA2, CCNB3, CCNB1, CCNE2	**0.004**
Peptidase S1, trypsin family, active site (IPR018114)	4	CTRL, TRP-I, TRP-III, CTRB	**0.007**
Peptidase S1A, chymotrypsin-type (IPR001314)	4	CTRL, TRP-I, TRP-III, CTRB	**0.008**
DNA replication	4	RIR1, DPOD1, PSF2, MCM2	**0.009**
Zymogen	3	DCAM, TRP-I, TRP-III	**0.021**
Metalloendopeptidase activity (GO:0004222)	3	YBEY, HCE1, LCE	**0.037**
DNA replication (GO:0006260)	3	RIR1, DPOD1, PSF2	0.065
Chromosome, centromeric region (GO:0000775)	2	MIS12, CENPN	**0.036**

Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	**0.044**
Regulation of G2/M transition of mitotic cell cycle (GO:0010389)	2	CCNA2, CCNB3	**0.044**
Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	**0.044**
Peroxisome	2	LONP2, PEX12	**0.046**
Regulation of cyclin-dependent protein serine/threonine kinase activity (GO:0000079)	2	CCNA2, CCNB3	0.065
Chromosome segregation (GO:0007059)	2	CENPN, SKA1	0.065
Site:Required for specificity	2	TRP-I, TRP-III	0.075
Metal ion-binding site:Calcium	2	TRP-I, TRP-III	0.075
Metal ion-binding site:Calcium; via carbonyl oxygen	2	TRP-I, TRP-III	0.075
Domain:Peptidase S1	2	TRP-I, TRP-III	0.075
Propeptide:Activation peptide	2	TRP-I, TRP-III	0.075
Active site:Charge relay system	2	TRP-I, TRP-III	0.075
Digestion (GO:0007586)	2	TRP-I, TRP-III	0.086
Digestion	2	TRP-I, TRP-III	0.089

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.14: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to the combined effect of offspring (within-generation) and paternal (transgenerational) warm acclimation.

UP-REGULATED PATHWAYS: Offspring & fathers warm-acclimated		
Gene ID	**Gene Name**	**KEGG**
HMGCLL1	3-hydroxymethyl-3-methylglutaryl-CoA lyase-like 1(hmgcll1)	dre00072:Synthesis and degradation of ketone bodies dre00280:Valine, leucine and isoleucine degradation dre00650:Butanoate metabolism dre01100:Metabolic pathways dre04146:Peroxisome
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
alcama	activated leukocyte cell adhesion molecule a(alcama)	dre04514:Cell adhesion molecules (CAMs)
cldnd	claudin d(cldnd)	dre04514:Cell adhesion molecules (CAMs) dre04530:Tight junction
FLAD1	flavin adenine dinucleotide synthetase 1(flad1)	dre00740:Riboflavin metabolism dre01100:Metabolic pathways
foxo1a	forkhead box O1 a(foxo1a)	dre04068:FoxO signaling pathway dre04910:Insulin signaling pathway dre04931:Insulin resistance
GRID2	glutamate receptor, ionotropic, delta 2(grid2)	dre04080:Neuroactive ligand-receptor interaction
HYOU1	hypoxia up-regulated 1(hyou1)	dre04141:Protein processing in endoplasmic reticulum
mapk14a	mitogen-activated protein kinase 14a(mapk14a)	dre04010:MAPK signaling pathway dre04068:FoxO signaling pathway dre04261:Adrenergic signaling in cardiomyocytes dre04370:VEGF signaling pathway dre04620:Toll-like receptor signaling pathway dre04621:NOD-like receptor signaling pathway

		dre04622:RIG-I-like receptor signaling pathway dre04912:GnRH signaling pathway dre04914:Progesterone-mediated oocyte maturation dre05132:Salmonella infection
MYLK3	myosin light chain kinase 3(mylk3)	dre04020:Calcium signaling pathway dre04270:Vascular smooth muscle contraction dre04510:Focal adhesion dre04810:Regulation of actin cytoskeleton
MTMR8	myotubularin related protein 8(mtmr8)	dre00562:Inositol phosphate metabolism dre01100:Metabolic pathways dre04070:Phosphatidylinositol signaling system

DOWN-REGULATED PATHWAYS: Offspring & fathers warm-acclimated

Gene ID	Gene Name	KEGG
hpda	4-hydroxyphenylpyruvate dioxygenase a(hpda)	dre00130:Ubiquinone and other terpenoid-quinone biosynthesis dre00350:Tyrosine metabolism dre00360:Phenylalanine metabolism dre01100:Metabolic pathways
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
ELOVL6	ELOVL fatty acid elongase 6(elovl6)	dre00062:Fatty acid elongation dre01040:Biosynthesis of unsaturated fatty acids dre01212:Fatty acid metabolism
FBXO5	F-box protein 5(fbxo5)	dre04114:Oocyte meiosis
SEC23A	Sec23 homolog A, COPII coat complex component(sec23a)	dre04141:Protein processing in endoplasmic reticulum
SEPSECS	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase(sepsecs)	dre00450:Selenocompound metabolism dre00970:Aminoacyl-tRNA biosynthesis
UAP1L1	UDP-N-acetylglucosamine pyrophosphorylase 1, like 1(uap1l1)	dre00520:Amino sugar and nucleotide sugar metabolism dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics

ALDH4A1	aldehyde dehydrogenase 4 family, member A1(aldh4a1)	dre00250:Alanine, aspartate and glutamate metabolism dre00330:Arginine and proline metabolism dre01100:Metabolic pathways
ALDOB	aldolase b, fructose-bisphosphate(aldob)	dre00010:Glycolysis / Gluconeogenesis dre00030:Pentose phosphate pathway dre00051:Fructose and mannose metabolism dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
eif4ebp3l	eukaryotic translation initiation factor 4E binding protein 3, like(eif4ebp3l)	dre03013:RNA transport
GAPDH	glyceraldehyde-3-phosphate dehydrogenase(gapdh)	dre00010:Glycolysis / Gluconeogenesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
NAPRT	nicotinate phosphoribosyltransferase(naprt)	dre00760:Nicotinate and nicotinamide metabolism dre01100:Metabolic pathways
RRM1	ribonucleotide reductase M1 polypeptide(rrm1)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways
RRM2	ribonucleotide reductase regulatory subunit M2(rrm2)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways dre04115:p53 signaling pathway
tdo2a	tryptophan 2,3-dioxygenase a(tdo2a)	dre00380:Tryptophan metabolism dre01100:Metabolic pathways
UBE2T	ubiquitin-conjugating enzyme E2T (putative)(ube2t)	dre03460:Fanconi anemia pathway

Table A4.15: The function of differentially expressed genes in lake trout offspring in response to the combined effect of offspring (within-generation) and parental (both parents; transgenerational) warm acclimation.

UP-REGULATED FUNCTIONS: Offspring & both parents warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Signal	41	TRP-II, SAA5, SPHM, TPMT, SERPH, CBPB1, I12R2, ERLN2, ISK1, LAMP1, CLUS, SDF1, SMS1A, CD276, CASR, CYSP2, HCE1, IL1R2, ANGL1, VEGFC, LAMB1, FEL, PDIA5, SUMF2, CBPA1, MFAP4, PYY, GNPTG, SSRD, P4HA1, IL8, MXRA8, TNR6, TNR5, CD226, MCP, TSSP, COEA1, CBLN3, RL6, PVRL1	**<0.001**
Immunoglobulin-like fold (IPR013783)	9	TGM1, I12R2, IL1R2, MXRA8, CD226, COEA1, PVRL1, NFKB2, CD276	**0.010**
Metalloprotease	5	YBEY, HCE1, CBPA2, CBPA1, CBPB1	**0.004**
Immunoglobulin subtype (IPR003599)	5	IL1R2, MXRA8, CD226, PVRL1, CD276	**0.032**
IG (SM00409)	5	IL1R2, MXRA8, CD226, PVRL1, CD276	0.053
Immunoglobulin V-set (IPR013106)	4	MXRA8, CD226, PVRL1, CD276	0.033
Proteinase inhibitor, carboxypeptidase propeptide (IPR003146)	3	CBPA2, CBPA1, CBPB1	**0.009**
Metallocarboxypeptidase activity (GO:0004181)	3	CBPA2, CBPA1, CBPB1	**0.013**
Peptidase M14, carboxypeptidase A (IPR000834)	3	CBPA2, CBPA1, CBPB1	**0.013**
Proteinase inhibitor, propeptide (IPR009020)	3	CBPA2, CBPA1, CBPB1	**0.018**
Zn_pept (SM00631)	3	CBPA2, CBPA1, CBPB1	**0.018**
Carboxypeptidase	3	CBPA2, CBPA1, CBPB1	**0.044**
SMAD/FHA domain (IPR008984)	3	IRF4, IRF7, APTX	0.069

Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	0.055
Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	0.060
Glycerol channel activity (GO:0015254)	2	AQP8	0.060
Respiratory chain complex IV (GO:0045277)	2	COX2, COX1	0.076
Urea channel activity (GO:0015265)	2	AQP8	0.089
Aquaporin 8 (IPR023277)	2	AQP8	0.091
DOWN-REGULATED FUNCTIONS: Offspring & both parents warm-acclimated			
Function (Term)	**Count**	**Genes**	***p*-value**
Transferase	24	PARP3, SYK, PIMT, BUB1B, SAT2, ESCO2, FPPS, TRM61, CDN2B, KITH, TOPK, FUT7, DPOD1, UPP, STK35, KPYK, PGK, STT3A, CKS1, UD2A2, THG1, LRAT, TGM2, CDKN3	**0.002**
DNA replication	6	RIR1, DPOD1, MCM3, PSF2, SLD5, MCM2	**<0.001**
Iron	6	RIR2, HBB1, TRF, COX1, HBAD	0.080
DNA replication (GO:0006260)	5	RIR1, RFA1, DPOD1, PSF2, SLD5	**0.002**
Magnesium ion binding (GO:0000287)	5	KPYK, SYFB, THG1, ENOA, ENOPH	**0.025**
Nucleic acid-binding, OB-fold (IPR012340)	5	RFA1, MCM3, RL2, IF2A, MCM2	**0.043**
Iron ion binding (GO:0005506)	5	HBB1, SC5D, COX1, HBAD	**0.044**
Ligase	5	HERC4, RNF34, ACSL1, SYFB, SYFA	0.061
(SM01332)	4	CCNA2, CCNB3, CCNE2, CCND1	**<0.001**
Cyclin, C-terminal domain (IPR004367)	4	CCNA2, CCNB3, CCNE2, CCND1	**0.001**
CYCLIN (SM00385)	4	CCNA2, CCNB3, CCNE2, CCND1	**0.004**
Cyclin, N-terminal (IPR006671)	4	CCNA2, CCNB3, CCNE2, CCND1	**0.006**
Cyclin	4	CCNA2, CCNB3, CCNE2, CCND1	**0.006**
Cyclin-like (IPR013763)	4	CCNA2, CCNB3, CCNE2, CCND1	**0.009**
Ligase activity (GO:0016874)	4	HERC4, UCHL1, RNF34, ACSL1	0.055

Magnesium	4	5NTC, KPYK, THG1, ENOPH	0.074
Heme	4	HBB1, COX1, HBAD	0.079
ZnF_C2HC (SM00343)	3	CNBP, ZCRB1	**0.010**
S-adenosyl-L-methionine	3	DCAM, PIMT, TRM61	0.050
Zinc finger, CCHC-type (IPR001878)	3	CNBP, ZCRB1	0.056
Hemoglobin complex (GO:0005833)	3	HBB1, HBAD	0.092
Chromosome, centromeric region (GO:0000775)	2	MIS12, CENPN	0.051
Zinc knuckle CX2CX3GHX4C (IPR025829)	2	CNBP	0.055
Mitotic spindle assembly checkpoint (GO:0007094)	2	BUB1B, MD2L1	0.061
Regulation of G2/M transition of mitotic cell cycle (GO:0010389)	2	CCNA2, CCNB3	0.061
Phenylalanyl-tRNA aminoacylation (GO:0006432)	2	SYFB, SYFA	0.061
Phenylalanine-tRNA ligase activity (GO:0004826)	2	SYFB, SYFA	0.061
Respiratory chain complex IV (GO:0045277)	2	COX3, COX1	0.075
Peptidase C12, ubiquitin carboxyl-terminal hydrolase 1 (IPR001578)	2	UCHL1, UCHL3	0.081
Decarboxylase	2	DCAM, DCUP	0.082
Ion transport (GO:0006811)	2	TRF, S12A1	0.091
Regulation of cyclin-dependent protein serine/threonine kinase activity (GO:0000079)	2	CCNA2, CCNB3	0.091
Chromosome segregation (GO:0007059)	2	CENPN, SKA1	0.091
Posttranslational modification, protein turnover, chaperones / Intracellular trafficking and secretion	2	CNBP	0.098

Included with the function are the database terms in parentheses where applicable. The *p*-values are derived from Fisher's Exact tests modified for enrichment analysis (i.e. EASE score) and range from 0 to 1 with $p = 0.05$ representing a significantly enriched function and $p = 0$ being perfectly enriched.

Table A4.16: The up- and downregulated KEGG pathways associated with differentially expressed genes from lake trout offspring in response to the combined effect of offspring (within-generation) and parental (both parents; transgenerational) warm acclimation.

UP-REGULATED PATHWAYS: Offspring & both parents warm-acclimated		
Gene ID	**Gene Name**	**KEGG**
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
CD99L2	CD99 molecule-like 2(cd99l2)	dre04514:Cell adhesion molecules (CAMs)
JAK1	Janus kinase 1(jak1)	dre04630:Jak-STAT signaling pathway dre05168:Herpes simplex infection
UAP1L1	UDP-N-acetylglucosamine pyrophosphorylase 1, like 1(uap1l1)	dre00520:Amino sugar and nucleotide sugar metabolism dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics
BHMT	betaine-homocysteine methyltransferase(bhmt)	dre00260:Glycine, serine and threonine metabolism dre00270:Cysteine and methionine metabolism dre01100:Metabolic pathways
CASC3	cancer susceptibility candidate 3(casc3)	dre03013:RNA transport dre03015:mRNA surveillance pathway
CTNNA2	catenin (cadherin-associated protein), alpha 2(ctnna2)	dre04520:Adherens junction
CHD8	chromodomain helicase DNA binding protein 8(chd8)	dre04310:Wnt signaling pathway
CYP26B1	cytochrome P450, family 26, subfamily b, polypeptide 1(cyp26b1)	dre00830:Retinol metabolism dre01100:Metabolic pathways
CYFIP1	cytoplasmic FMR1 interacting protein 1(cyfip1)	dre03013:RNA transport dre04810:Regulation of actin cytoskeleton
EIF6	eukaryotic translation initiation factor 6(eif6)	dre03008:Ribosome biogenesis in eukaryotes
fsta	follistatin a(fsta)	dre04350:TGF-beta signaling pathway

foxo1a	forkhead box O1 a(foxo1a)	dre04068:FoxO signaling pathway dre04910:Insulin signaling pathway dre04931:Insulin resistance
GRID2	glutamate receptor, ionotropic, delta 2(grid2)	dre04080:Neuroactive ligand-receptor interaction
GAMT	guanidinoacetate N-methyltransferase(gamt)	dre00260:Glycine, serine and threonine metabolism dre00330:Arginine and proline metabolism dre01100:Metabolic pathways
hsp90aa1.1	heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 1(hsp90aa1.1)	dre04141:Protein processing in endoplasmic reticulum dre04621:NOD-like receptor signaling pathway dre04914:Progesterone-mediated oocyte maturation
HYOU1	hypoxia up-regulated 1(hyou1)	dre04141:Protein processing in endoplasmic reticulum
mapk14a	mitogen-activated protein kinase 14a(mapk14a)	dre04010:MAPK signaling pathway dre04068:FoxO signaling pathway dre04261:Adrenergic signaling in cardiomyocytes dre04370:VEGF signaling pathway dre04620:Toll-like receptor signaling pathway dre04621:NOD-like receptor signaling pathway dre04622:RIG-I-like receptor signaling pathway dre04912:GnRH signaling pathway dre04914:Progesterone-mediated oocyte maturation dre05132:Salmonella infection
mapk14b	mitogen-activated protein kinase 14b(mapk14b)	dre04010:MAPK signaling pathway dre04068:FoxO signaling pathway dre04261:Adrenergic signaling in cardiomyocytes dre04370:VEGF signaling pathway dre04620:Toll-like receptor signaling pathway dre04621:NOD-like receptor signaling pathway dre04622:RIG-I-like receptor signaling pathway dre04912:GnRH signaling pathway dre04914:Progesterone-mediated oocyte maturation dre05132:Salmonella infection

thbs4b	thrombospondin 4b(thbs4b)	dre04145:Phagosome dre04510:Focal adhesion dre04512:ECM-receptor interaction

DOWN-REGULATED PATHWAYS: Offspring & both parents warm-acclimated

Gene ID	Gene Name	KEGG
DHCR7	7-dehydrocholesterol reductase(dhcr7)	dre00100:Steroid biosynthesis dre01100:Metabolic pathways
CNOT1	CCR4-NOT transcription complex, subunit 1(cnot1)	dre03018:RNA degradation
DCLRE1C	DNA cross-link repair 1C, PSO2 homolog (S. cerevisiae)(dclre1c)	dre03450:Non-homologous end-joining
DDIT4	DNA-damage-inducible transcript 4(ddit4)	dre04150:mTOR signaling pathway
ELOVL6	ELOVL fatty acid elongase 6(elovl6)	dre00062:Fatty acid elongation dre01040:Biosynthesis of unsaturated fatty acids dre01212:Fatty acid metabolism
FBXO5	F-box protein 5(fbxo5)	dre04114:Oocyte meiosis
MPV17L2	MPV17 mitochondrial membrane protein-like 2(mpv17l2)	dre04146:Peroxisome
pycard	PYD and CARD domain containing(pycard)	dre04621:NOD-like receptor signaling pathway dre04623:Cytosolic DNA-sensing pathway dre05132:Salmonella infection
AACS	acetoacetyl-CoA synthetase(aacs)	dre00280:Valine, leucine and isoleucine degradation dre00650:Butanoate metabolism
ALDH4A1	aldehyde dehydrogenase 4 family, member A1(aldh4a1)	dre00250:Alanine, aspartate and glutamate metabolism dre00330:Arginine and proline metabolism dre01100:Metabolic pathways
ALDOB	aldolase b, fructose-bisphosphate(aldob)	dre00010:Glycolysis / Gluconeogenesis dre00030:Pentose phosphate pathway dre00051:Fructose and mannose metabolism dre01100:Metabolic pathways

		dre01130:Biosynthesis of antibiotics dre01200:Carbon metabolism dre01230:Biosynthesis of amino acids
cldnd	claudin d(cldnd)	dre04514:Cell adhesion molecules (CAMs) dre04530:Tight junction
CYB5R2	cytochrome b5 reductase 2(cyb5r2)	dre00520:Amino sugar and nucleotide sugar metabolism
MSMO1	methylsterol monooxygenase 1(msmo1)	dre00100:Steroid biosynthesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics
mvda	mevalonate (diphospho) decarboxylase a(mvda)	dre00900:Terpenoid backbone biosynthesis dre01100:Metabolic pathways dre01130:Biosynthesis of antibiotics
MRPS7	mitochondrial ribosomal protein S7(mrps7)	dre03010:Ribosome
nkd2b	naked cuticle homolog 2b(nkd2b)	dre04310:Wnt signaling pathway
nrxn1a	neurexin 1a(nrxn1a)	dre04514:Cell adhesion molecules (CAMs)
ppp1r3cb	protein phosphatase 1, regulatory subunit 3Cb(ppp1r3cb)	dre04910:Insulin signaling pathway dre04931:Insulin resistance
RRM1	ribonucleotide reductase M1 polypeptide(rrm1)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways
RRM2	ribonucleotide reductase regulatory subunit M2(rrm2)	dre00230:Purine metabolism dre00240:Pyrimidine metabolism dre00480:Glutathione metabolism dre01100:Metabolic pathways dre04115:p53 signaling pathway
UBE2T	ubiquitin-conjugating enzyme E2T (putative)(ube2t)	dre03460:Fanconi anemia pathway
zgc:101723	zgc:101723(zgc:101723)	dre00040:Pentose and glucuronate interconversions dre00980:Metabolism of xenobiotics by cytochrome P450

Table A4.17: The level of expression represented as the log2 fold change (LFC) and adjusted *p*-value (Benjamini-Hochberg method with a FDR of 0.1) of genes included in the heatmap. Column headings describe the treatment of the offspring (cold- or warm-acclimated: 11 or 15°C) with the parental treatment in parentheses (cold- or warm-acclimated: C=10°C or W=17°C; mothers: ♀, fathers: ♂). Each group in the table was compared to a control group where offspring and parents were cold-acclimated.

	Warm offspring (C♀xC♂)		**Cold offspring (W♀xC♂)**		**Cold offspring (C♀xW♂)**		**Cold offspring (W♀xW♂)**		**Warm offspring (W♀xC♂)**		**Warm offspring (C♀xW♂)**		**Warm offspring (W♀xW♂)**	
GENE	**LFC**	***p***	**LFC**	***p***	**LFC**	***p***	**LFC**	***p***	**LFC**	***p***	**LFC**	***p***	**LFC**	***p***
NCCRP1	3.99	0.35	1.75	0.902	1.53	1	-0.59	0.993	1.07	0.601	1.7	0.464	-2.03	0.291
NOSIP	3.01	0.0516	-1.19	0.931	-0.72	1	0.48	0.993	3.72	0.0109	3.18	0.0571	3.49	0.0292
IL8	3	0.0114	-0.29	0.982	-1.22	1	-0.63	0.98	2	0.0697	1.07	0.462	2.27	0.0475
bt2a2	2.93	0.206	-0.12	0.977	0.1	1	-0.32	0.96	-0.11	0.827	-0.29	0.583	-0.43	0.298
AS3MT	2.7	0.0505	0.8	0.945	-0.12	1	1.98	0.724	2.33	0.0722	1.07	0.546	2.68	0.0468
saa5	2.7	0.0192	0.43	0.967	0.2	1	-0.23	0.998	1.16	0.311	1.69	0.197	1.82	0.112
JUN	2.69	0.00394	-0.21	0.949	-0.17	1	0.21	0.98	0.55	0.134	0.37	0.433	0.22	0.633
si:ch211-163l21.7	2.56	0.0819	-0.06	0.996	0.44	1	-0.7	0.983	3.05	0.0262	2.22	0.178	1.31	0.403
TIMM8A	2.46	0.45	4.97	0.672	1.56	1	3.77	0.8	5.27	0.0506	-1.3	0.756	3.67	0.203
cox20	2.42	0.119	0.06	0.996	0.08	1	2.71	0.527	0.43	0.822	-0.34	0.895	0.38	0.854
CASR	2.41	0.0607	0.08	0.996	-0.29	1	-0.35	0.993	2.15	0.0735	2.06	0.15	1.6	0.217
TGM1	2.33	0.0338	-1.63	0.938	0.22	1	0.57	0.926	-2.67	0.243	-1.94	0.517	-0.57	0.857
ica69	2.23	0.057	0.38	0.929	0.13	1	0.06	0.999	0.23	0.701	-0.01	0.995	0.12	0.86
tnr5	2.23	0.0465	-1.17	0.882	0.34	1	1.48	0.792	-0.82	0.482	1.16	0.392	1.8	0.103
SUMF2	2.13	0.0773	-0.25	0.898	-0.09	1	0.06	0.995	-0.09	0.781	-0.07	0.88	-0.04	0.91
MEGF6	2.12	0.0274	-0.52	0.953	0.35	1	-0.03	0.999	0.92	0.339	2.02	0.053	1.64	0.0835

pacsin1b	2.11	0.0818	-0.48	0.963	-1.57	1	0.11	0.999	1.21	0.312	1.03	0.495	1.8	0.132
LAS1L	2.04	0.0148	0.32	0.892	-0.23	1	1.43	0.575	0.4	0.669	0.25	0.54	2.13	0.00884
EPG5	1.94	0.234	0.22	0.989	0.73	1	-0.32	0.979	1.45	0.351	-0.32	0.694	-1.27	0.0248
ankrd46b	1.91	0.163	-0.29	0.983	-0.58	1	-0.27	0.998	1.76	0.166	1.46	0.362	1.07	0.463
CD276	1.91	0.0163	-0.28	0.97	-0.3	1	0.42	0.981	1.44	0.0545	0.74	0.45	0.94	0.246
CBLN3	1.89	0.0658	-0.05	0.991	-0.64	1	-0.44	0.917	1.59	0.098	0.55	0.254	2.11	0.0342
4f2	1.87	0.0147	0.34	0.96	0.52	1	-0.46	0.977	1.21	0.0925	1.49	0.0732	2.21	0.00325
IRF7	1.87	0.0405	-0.27	0.977	-0.38	1	-0.29	0.993	1.57	0.0691	1.31	0.209	1.57	0.0832
TNIP2	1.83	0.0102	0.14	0.984	-0.03	1	-0.42	0.977	1.5	0.0242	0.99	0.219	1.55	0.0247
coea1	1.81	0.106	-0.67	0.943	-0.81	1	-0.62	0.978	1.32	0.211	0.35	0.841	1.4	0.208
kad	1.77	0.0282	0.11	0.976	-0.02	1	-0.35	0.921	0.1	0.809	0.41	0.311	0.09	0.844
pvrl1	1.77	0.0478	0.69	0.927	0.56	1	-0.15	0.999	1.33	0.114	0.83	0.453	1.1	0.222
DOHH	1.76	0.0205	0.19	0.982	0.36	1	2.06	0.0781	0.76	0.315	0.89	0.321	2.53	0.000751
gp132	1.73	0.0872	0.25	0.981	0.05	1	-0.22	0.997	0.43	0.708	0.72	0.583	0.99	0.349
ikbe	1.73	0.0157	-0.08	0.991	-0.27	1	-0.15	0.998	1.13	0.0942	1.43	0.0652	1.63	0.0193
IL18	1.73	0.0208	0.56	0.929	0.23	1	0.24	0.993	1.09	0.126	1.01	0.242	1.4	0.0578
JAK1	1.73	0.0239	0.29	0.971	0.38	1	0.29	0.992	1.32	0.0991	1.28	0.182	1.16	0.16
lyz2	1.7	0.00357	-0.41	0.933	0.92	1	0	0.999	0.99	0.0653	1.64	0.00663	1.17	0.0344
EIF3C	1.68	0.0619	-0.18	0.894	-0.27	1	0.57	0.973	-0.27	0.122	0.42	0.747	1.1	0.379
prdm9	1.67	0.113	0.71	0.914	-0.05	1	0.06	0.999	1.25	0.0976	0.86	0.368	1.15	0.0151
WDR92	1.66	0.556	-0.14	0.943	-0.08	1	-0.02	0.999	0.12	0.642	0.05	0.899	0.1	0.717
SZRD1	1.65	0.238	-0.03	0.994	0.24	1	0.42	0.949	-0.13	0.822	0.1	0.893	-0.06	0.924
HSPB11	1.63	0.0173	0.83	0.912	0.48	1	-0.68	0.966	1.86	0.0338	2.06	0.0404	0.52	0.636
LOC100136747	1.62	0.529	4.93	0.325	-0.39	1	-0.13	0.999	4.42	0.0267	1.41	0.616	1.56	0.509
CNN1	1.61	0.0239	0.2	0.986	-0.21	1	0.78	0.97	0.86	0.454	0.04	0.987	0.62	0.64
cnrg	1.61	0.00942	-0.66	0.967	-0.23	1	-1.95	0.892	-0.52	0.812	-0.4	0.89	-1.5	0.428

syfa	1.59	0.0876	-0.04	0.996	-0.64	1	-0.75	0.938	1.56	0.0334	1.47	0.0834	1.11	0.153
CD99L2	1.58	0.135	-0.21	0.939	0.04	1	-0.1	0.993	-0.65	0.0299	-0.33	0.385	-0.8	0.0103
hsp30	1.57	0.0162	-0.64	0.897	-0.8	1	-0.53	0.956	1.46	0.0172	1.52	0.03	1.36	0.0315
slc5a8l	1.57	0.404	-1.04	0.943	0.49	1	0.08	0.999	-0.74	0.703	-1.01	0.661	-0.65	0.752
KDELC1	1.56	0.0112	-0.15	0.982	-0.09	1	0.49	0.958	1.08	0.0576	0.77	0.273	1.5	0.0115
mapk14a	1.52	0.322	-0.33	0.982	0.52	1	-0.67	0.984	2.06	0.125	1.19	0.507	1.86	0.191
APMAP	1.51	0.0187	-0.15	0.982	0.42	1	0.74	0.871	0.84	0.17	0.95	0.188	0.85	0.186
IFI44	1.51	0.441	0.59	0.82	0.39	1	-0.2	0.988	-0.62	0.751	0.04	0.96	1.27	0.488
TBC1D7	1.5	0.423	-0.66	0.965	0.32	1	1.27	0.961	-3.01	0.0549	-1.46	0.484	2.33	0.161
HAUS1	1.5	0.0114	-0.37	0.942	0.47	1	-0.43	0.964	1.66	0.00324	2.22	0.000234	1.7	0.00315
TFG	1.48	0.0444	-0.72	0.898	-0.24	1	0.45	0.976	1.04	0.138	0.52	0.587	0.98	0.184
s35b1	1.47	0.188	-1.15	0.872	-1.57	1	-0.32	0.993	0.9	0.416	0.16	0.933	1	0.382
CXADR	1.46	0.108	0.18	0.983	-0.05	1	-1.07	0.857	0.12	0.92	0.58	0.624	0.7	0.478
tax1bp1b	1.46	0.0131	0.18	0.976	0.22	1	0.89	0.682	0.4	0.517	0.76	0.261	1.2	0.0356
SSU72	1.46	0.286	-1.01	0.927	-0.96	1	-0.05	0.999	-1.11	0.391	0.37	0.604	1.25	0.35
PTPRN	1.43	0.158	-0.86	0.917	-0.95	1	-0.6	0.977	0.71	0.491	-0.24	0.882	0.08	0.959
uk114	1.43	0.611	-0.04	0.997	0.34	1	1.1	0.95	0.45	0.761	0.54	0.767	2.35	0.0525
fa86a	1.43	0.0308	-0.03	0.996	0.07	1	0.32	0.984	0.6	0.371	0.25	0.804	1.49	0.0214
syub	1.43	0.0251	-0.52	0.925	-0.56	1	-0.16	0.995	0.49	0.457	0.05	0.966	1.1	0.0807
GFPT1	1.42	0.28	-0.27	0.983	-1.39	1	0.25	0.998	0.3	0.852	0.39	0.842	1.22	0.184
BHMT	1.4	0.169	-0.15	0.977	-0.14	1	-0.3	0.977	0.4	0.45	0.54	0.384	0.08	0.915
IL1R2	1.38	0.0139	-0.27	0.958	-0.43	1	0.11	0.999	1.31	0.0126	1.11	0.0667	1.21	0.0265
TPMT	1.38	0.0614	-0.28	0.829	-0.14	1	0.01	0.999	-0.41	0.0468	-0.19	0.484	-0.14	0.593
plbl1	1.38	0.0621	0.22	0.927	0.05	1	0.04	0.999	0.05	0.906	0.01	0.99	0.04	0.923
LDB3	1.37	0.0768	-0.01	0.998	0.5	1	0.44	0.921	-0.23	0.627	0.62	0.204	-0.1	0.868
zbtb16a	1.37	0.0291	-0.06	0.989	0	1	-0.17	0.988	0.06	0.913	-0.13	0.835	0.27	0.561
ubp5	1.36	0.207	-0.23	0.957	0.33	1	0.97	0.946	0.77	0.481	0.74	0.588	0.88	0.434
MBL2	1.36	0.182	-0.74	0.959	-1.35	1	-1.12	0.891	0.47	0.818	-0.3	0.915	0.08	0.974

pls2	1.36	0.107	-0.19	0.982	-0.24	1	0.23	0.993	1.35	0.0856	0.85	0.402	0.85	0.326
hsp90aa1.1	1.36	0.1	-0.22	0.979	-0.64	1	0.16	0.998	0.89	0.268	0.85	0.386	1.09	0.183
GNA13	1.36	0.246	1.21	0.909	1.58	1	-1.47	0.892	1.23	0.354	1.64	0.287	-0.83	0.588
MAG	1.34	0.145	0.23	0.949	-0.08	1	-0.08	0.999	0.1	0.847	0.16	0.796	0.49	0.252
ccd98	1.34	0.105	0.8	0.895	0.12	1	0.54	0.97	1.65	0.031	1.4	0.12	1.79	0.0232
MTMR8	1.33	0.0956	0.66	0.922	0.37	1	-0.97	0.845	0.8	0.301	1.32	0.13	0.58	0.51
gnpi	1.33	0.178	0.14	0.989	0.19	1	0.45	0.984	0.72	0.468	1.18	0.29	1.33	0.164
DKC1	1.33	0.0335	0.12	0.982	-0.13	1	0.56	0.95	1.1	0.0625	0.8	0.273	1.3	0.034
BAG3	1.32	0.00642	-0.54	0.855	-0.44	1	0	1	1.06	0.0179	1	0.0499	1.12	0.0157
DNAJC27	1.3	0.0192	-0.07	0.991	-0.02	1	0.66	0.857	-0.06	0.943	0.22	0.796	0.95	0.0826
lich	1.3	0.542	0.13	0.994	0.1	1	0.34	0.999	0.22	0.932	0.18	0.957	-0.77	0.728
IRF4	1.3	0.136	1.26	0.797	0.04	1	-1.24	0.711	1.45	0.0707	1.4	0.139	1.23	0.147
TMEM53	1.29	0.203	-0.16	0.987	0.18	1	1.29	0.792	0.15	0.912	1.11	0.337	0.9	0.383
lhpl4	1.29	0.168	0.02	0.998	0.07	1	-0.08	0.999	0.32	0.774	0.49	0.699	1.25	0.172
ISM1	1.29	0.0299	0.58	0.929	0.08	1	-0.01	0.999	1.18	0.101	1.11	0.197	1.14	0.131
HYOU1	1.29	0.0609	-1.21	0.902	-0.94	1	0.44	0.992	0.72	0.991	0.8	0.575	1.14	0.319
HMGCLL1	1.28	0.279	0.13	0.991	-0.01	1	-1.77	0.657	1.61	0.123	1.72	0.17	0.38	0.784
C1QL4	1.28	0.327	0.33	0.979	0.09	1	-1.18	0.933	1.78	0.119	1.07	0.479	0.68	0.634
RBM19	1.28	0.000263	0.06	0.983	0.12	1	-0.15	0.983	0.34	0.26	0.27	0.494	0.34	0.285
nfm	1.27	0.28	0	0.999	0.1	1	0.1	0.999	0.04	0.965	0.75	0.302	0.2	0.8
DHTKD1	1.26	0.302	-0.31	0.985	0.88	1	2.1	0.835	0.26	0.908	1.89	0.341	2.09	0.215
UTP15	1.26	0.354	0.06	0.996	-0.31	1	0.3	0.995	0.26	0.871	-0.15	0.948	1.57	0.207
egr2b	1.26	0.221	-1.18	0.85	-1.33	1	0.46	0.984	0.95	0.337	1.24	0.28	1.53	0.118
MMP25	1.25	0.261	0.82	0.927	0.28	1	0.59	0.978	1.24	0.221	1.45	0.223	0.99	0.37
GRID2	1.25	0.608	0.55	0.979	-0.41	1	3.34	0.594	0.03	0.992	1.39	0.592	3.09	0.116
si:dkey-189g17.2	1.24	0.116	0.15	0.984	0.01	1	1.78	0.214	0.42	0.626	1.1	0.21	1.81	0.0163

PIR	1.24	0.137	1.24	0.892	0.1	1	1.01	0.956	0.18	0.918	0.36	0.855	0.99	0.472
FBXO5	1.24	0.672	-1.52	0.872	-0.96	1	-2.08	0.949	-1.51	0.281	-0.66	0.752	-1.41	0.594
CXCR3	1.23	0.0599	-0.19	0.977	-0.14	1	-0.13	0.998	0.67	0.299	0.91	0.216	0.99	0.125
NSUN4	1.23	0.139	-0.41	0.956	-0.69	1	0.22	0.993	0.7	0.401	0.53	0.626	0.52	0.583
phla2	1.23	0.251	0.1	0.993	-0.61	1	0.4	0.991	1.29	0.185	0.86	0.506	0.64	0.588
DDIT4L	1.22	0.1	-0.06	0.994	-0.23	1	0.66	0.949	0.87	0.219	0.58	0.537	0.96	0.192
ddt4l	1.22	0.1	-0.06	0.994	-0.23	1	0.66	0.949	0.87	0.219	0.58	0.537	0.96	0.192
sqstm	1.22	0.00791	-0.46	0.884	-0.26	1	-0.31	0.97	0.92	0.0294	0.67	0.188	1.06	0.0156
kctd6b	1.21	0.0162	0.15	0.982	0.24	1	0.68	0.892	0.71	0.237	0.63	0.4	0.79	0.203
KCTD6	1.21	0.0162	0.15	0.982	0.24	1	0.68	0.892	0.71	0.237	0.63	0.4	0.79	0.203
CYP26B1	1.21	0.0433	0.38	0.938	0.16	1	0.44	0.959	-0.18	0.792	0.21	0.808	0.11	0.89
SYK	1.2	0.154	-0.65	0.927	-0.1	1	0.59	0.966	-0.21	0.838	0.33	0.784	0.2	0.853
rl3	1.2	0.0156	0.01	0.998	-0.89	1	-0.05	0.999	0.15	0.866	0.11	0.928	-0.26	0.762
DDX4	1.19	0.199	-0.73	0.925	-0.2	1	0.1	0.999	1.08	0.205	1.68	0.0856	1.57	0.0722
RASEF	1.19	0.0464	0.35	0.945	0.28	1	-0.37	0.974	0.78	0.173	0.82	0.231	1.41	0.0158
LOC100136024	1.19	0.0187	-0.09	0.99	0.11	1	-0.46	0.973	0.65	0.377	1.02	0.222	0.28	0.757
btg3	1.18	0.0292	0.07	0.986	-0.18	1	0.28	0.97	-0.26	0.55	-0.29	0.587	0.48	0.243
SRP19	1.18	0.115	-0.16	0.938	-0.09	1	0.03	0.999	-0.14	0.594	-0.18	0.563	-0.16	0.565
CEBPB	1.18	0.057	-0.12	0.985	0.25	1	-0.01	0.999	0.8	0.175	0.83	0.238	1.33	0.0265
NBEAL2	1.18	0.241	1.66	0.672	1.03	1	-0.6	0.973	2	0.0267	1.85	0.0784	1.29	0.181
CCM2	1.18	0.551	0.48	0.919	0.04	1	0.26	0.984	0.12	0.872	0.31	0.688	0.46	0.453
RGS14	1.17	0.098	0.64	0.909	0.44	1	0.81	0.964	1.78	0.0831	1.57	0.205	0.3	0.722
COX2	1.17	0.155	0.41	0.956	0.22	1	-0.3	0.961	-0.02	0.97	-0.59	0.177	1.41	0.074
NR2C2AP	1.16	0.0471	0.29	0.956	-0.61	1	0.69	0.858	0.58	0.322	0.4	0.602	0.21	0.771
zn214	1.16	0.0205	-0.04	0.997	-1	1	-0.56	0.979	-0.54	0.622	-0.49	0.73	-0.34	0.785
RPP38	1.16	0.147	0.4	0.955	0.68	1	-0.07	0.999	1.62	0.0267	1.26	0.149	0.8	0.323
CHP2	1.16	0.442	-0.99	0.929	0.86	1	0.88	0.97	0.28	0.874	0.31	0.888	1.26	0.37

gdnfa	1.15	0.341	0.8	0.931	-0.19	1	0.65	0.977	0.47	0.712	0.82	0.567	0.93	0.438
LOX5	1.15	0.18	-0.4	0.958	0.41	1	0.52	0.973	0.56	0.53	0.58	0.595	0.8	0.361
clm7	1.15	0.239	-0.18	0.984	-0.68	1	-0.64	0.97	-0.75	0.431	0.09	0.96	-0.84	0.399
RFX3	1.15	0.0222	-0.56	0.859	-0.34	1	0.03	0.999	0.35	0.511	0.16	0.845	0.84	0.0921
mrp	1.15	0.0852	0.18	0.971	-0.1	1	-0.11	0.998	-0.19	0.759	-0.08	0.927	0.1	0.893
sft2b	1.15	0.131	-0.13	0.967	-0.04	1	0.15	0.988	0.16	0.7	-0.01	0.994	0.25	0.528
ERCC2	1.14	0.557	-1.17	0.933	-1.39	1	1.76	0.905	-0.86	0.639	-1.77	0.365	1.15	0.531
LPXN	1.13	0.0649	0.19	0.976	0.2	1	-0.02	0.999	0.41	0.526	0.43	0.589	0.68	0.281
HEBP1	1.13	0.422	-0.16	0.963	-0.17	1	0.32	0.981	-0.25	0.552	-0.18	0.752	-0.4	0.323
hamp	1.12	0.0502	0.1	0.986	0.5	1	0.61	0.908	0.86	0.113	1.06	0.0905	0.82	0.152
sc61g	1.12	0.465	-0.15	0.962	-0.17	1	-0.15	0.989	0.19	0.628	0.01	0.989	-0.14	0.737
MLLT11	1.12	0.0299	-0.33	0.941	-0.24	1	-0.08	0.999	1.42	0.0043	1.58	0.00397	1.3	0.0106
co7	1.12	0.0769	0.21	0.965	0.02	1	0.07	0.999	0.31	0.603	0.07	0.942	-0.55	0.324
ifna2	1.11	0.0779	-0.18	0.978	-0.52	1	0.27	0.988	0.86	0.147	0.05	0.969	0.59	0.38
CFAP36	1.11	0.119	0.4	0.946	0.82	1	0.48	0.97	1.25	0.0582	1.19	0.0195	1.11	0.0163
hce	1.11	0.219	-1.9	0.932	-1.41	1	0.89	0.993	-0.71	0.83	-1.82	0.601	1.02	0.754
hce1	1.11	0.219	-1.9	0.932	-1.41	1	0.89	0.993	-0.71	0.83	-1.82	0.601	1.02	0.754
at1b4	1.1	0.176	0.29	0.968	0.33	1	-0.51	0.972	1.47	0.049	1.34	0.127	0.94	0.245
THAP4	1.1	0.144	0.06	0.994	-0.14	1	0.2	0.993	1.54	0.0262	1.3	0.111	1.42	0.0489
BABAM1	1.1	0.679	-0.08	0.973	-0.02	1	-0.03	0.999	-0.01	0.975	-0.25	0.426	-0.15	0.613
isk1	1.09	0.0239	-0.57	0.961	0.03	1	0.84	0.973	1.23	0.357	1.03	0.55	1	0.5
MECR	1.09	0.0657	-0.03	0.994	0.09	1	0.17	0.984	-0.42	0.22	-0.19	0.708	-0.28	0.468
LIPE	1.09	0.189	-0.16	0.98	0.08	1	-0.84	0.788	0.23	0.763	-0.43	0.604	-0.37	0.614
MFAP4	1.09	0.0876	0.34	0.987	1.78	1	-0.19	0.999	2.12	0.289	1.28	0.639	-1.58	0.481
NDOR1	1.08	0.0977	-0.12	0.971	-0.31	1	0.18	0.981	-0.32	0.362	-0.23	0.627	0.28	0.47
MDM1	1.08	0.286	0.22	0.982	-0.19	1	1.04	0.892	0.1	0.941	-0.13	0.941	1.09	0.259
CARM1	1.08	0.157	0.64	0.919	0.22	1	-1.03	0.915	1.7	0.0175	1.76	0.0311	0.8	0.311
jarid2b	1.08	0.0573	-0.22	0.983	-1.24	1	-0.74	0.97	0.1	0.947	-0.46	0.764	0.23	0.871

FAM46C	1.07	0.015	-0.02	0.996	-0.08	1	-0.08	0.999	0.82	0.0481	0.91	0.0558	0.58	0.192
UNC45B	1.07	0.0949	-0.69	0.809	-0.41	1	-0.33	0.972	0.34	0.539	0.52	0.405	0.47	0.396
SYNC	1.07	0.196	-0.25	0.962	-0.42	1	0.23	0.992	-0.01	0.995	-0.27	0.763	-0.46	0.486
DNLZ	1.07	0.169	-0.01	0.998	-0.16	1	0.6	0.96	0.46	0.575	0.43	0.682	1	0.189
specc1la	1.07	0.123	-0.54	0.959	-0.95	1	-0.6	0.981	-0.39	0.782	-0.28	0.885	0.85	0.521
cyp3a40	1.07	0.297	0.09	0.993	-0.01	1	1.18	0.845	-0.53	0.614	-0.28	0.855	1.13	0.242
DSTYK	1.06	0.0958	-0.03	0.996	-0.43	1	0.15	0.995	0.69	0.26	0.4	0.638	0.66	0.31
i12r2	1.06	0.218	-0.41	0.957	-0.41	1	0.5	0.977	0.18	0.867	0.15	0.918	1.15	0.162
UQCC2	1.06	0.0293	-0.05	0.991	0	1	0.27	0.97	0.06	0.919	0.09	0.889	0.09	0.862
BPI	1.05	0.15	0.32	0.961	-0.06	1	-0.67	0.95	-0.22	0.811	-0.15	0.905	-0.24	0.798
MTAP	1.05	0.0722	0.52	0.943	0.33	1	-0.16	0.999	0.86	0.319	0.46	0.71	0.74	0.432
lat3	1.05	0.426	-0.06	0.984	-0.14	1	0.01	0.999	0.12	0.761	0.17	0.714	-0.19	0.611
PISD	1.05	0.233	0.4	0.958	0.46	1	-0.32	0.992	1.48	0.0615	1.19	0.213	1.45	0.0787
TIA1	1.05	0.292	-0.06	0.971	0.07	1	0.01	0.999	-0.18	0.334	-0.17	0.472	-0.08	0.723
ETFA	1.05	0.0872	-0.1	0.961	0	1	0.24	0.915	-0.34	0.112	-0.27	0.32	-0.52	0.0192
TENM4	1.05	0.291	-0.18	0.983	-0.46	1	-0.26	0.993	-1.5	0.073	-1.13	0.274	-2.77	0.00146
CTNNA2	1.04	0.0297	-0.22	0.959	-0.19	1	0.27	0.978	0.64	0.164	0.41	0.505	1.11	0.0185
PKD1L1	1.04	0.217	-0.5	0.919	-0.31	1	-0.15	0.986	-0.04	0.962	-0.58	0.415	-0.31	0.655
ytx2	1.04	0.176	-0.64	0.927	0.13	1	-0.67	0.953	-0.04	0.97	-0.43	0.704	0.22	0.832
SPG20	1.03	0.114	-0.16	0.971	0.26	1	-0.13	0.993	0.79	0.202	0.53	0.516	0.78	0.232
PDIA5	1.03	0.0251	0.34	0.977	0.07	1	-0.36	0.993	1.46	0.13	1	0.494	1.04	0.404
DERL2	1.03	0.0589	-0.03	0.994	-0.15	1	-0.03	0.999	0.38	0.254	0.41	0.309	0.45	0.193
ENKUR	1.02	0.159	0.03	0.996	0.53	1	-0.37	0.981	1.08	0.105	0.64	0.468	0.87	0.226
kazna	1.02	0.0107	0.37	0.909	0.19	1	0.18	0.987	1.19	0.00202	1.18	0.00498	1.11	0.00393
LOC106602754	1.01	0.114	0.06	0.993	0.1	1	0.19	0.993	0.8	0.182	0.48	0.562	0.56	0.408
TKT	1.01	0.0763	-0.43	0.929	-0.19	1	0.75	0.798	0.44	0.45	0.5	0.481	0.74	0.196
tcpd	1.01	0.0232	0.08	0.99	-0.19	1	0.12	0.999	0.42	0.549	0.3	0.747	0.66	0.33

LRRC59	1.01	0.0489	-0.42	0.963	0.31	1	0.69	0.97	0.97	0.362	0.41	0.798	1.32	0.216
trioa	1.01	0.156	0.85	0.837	0.82	1	0.64	0.947	0.73	0.286	0.75	0.368	1.31	0.0525
PEX12	1	0.48	-0.15	0.99	0.49	1	1.55	0.835	-0.56	0.695	-1.11	0.468	0.62	0.675
fam213aa	0.99	0.0306	-0.15	0.969	-0.05	1	0.13	0.993	-0.06	0.927	-0.27	0.651	0.61	0.158
TXN	0.98	0.0916	0.18	0.988	-1.79	1	0.84	0.966	0.67	0.594	0.24	0.9	0.54	0.703
ylat1	0.98	0.0869	0.16	0.979	-0.09	1	0.25	0.987	0.17	0.804	0.9	0.155	0.92	0.0991
MMP13	0.98	0.384	-0.24	0.982	0.37	1	0.06	0.999	1.3	0.179	1.42	0.219	0.56	0.644
rl6	0.97	0.0557	0.15	0.967	0.06	1	-0.48	0.851	0.4	0.302	0.46	0.324	0.12	0.82
st2b1	0.96	0.22	0.11	0.965	0.23	1	0.08	0.993	-0.01	0.972	-0.27	0.433	-0.59	0.034
EXOC8	0.96	0.216	0.45	0.943	0.22	1	1.24	0.599	0.21	0.82	0	0.998	1.27	0.083
SPNS3	0.96	0.311	0.06	0.995	-0.3	1	0.12	0.999	-0.04	0.976	-0.03	0.985	1.2	0.171
STT3A	0.96	0.125	-0.3	0.957	-0.07	1	0.32	0.981	1.03	0.072	0.54	0.482	0.71	0.255
IMP3	0.96	0.0647	-0.06	0.982	-0.03	1	-0.01	0.999	0.06	0.86	0	0.994	0.08	0.831
ndua4	0.96	0.541	0.07	0.977	0.07	1	0.15	0.974	0.08	0.796	0.14	0.674	0.01	0.987
EGR1	0.96	0.383	-1.46	0.769	-0.73	1	-0.11	0.999	0.72	0.491	0.54	0.694	0.92	0.387
LIPT2	0.95	0.114	-0.12	0.984	-0.2	1	1.2	0.347	-0.22	0.756	0.24	0.786	0.93	0.112
FLAD1	0.95	0.0168	0.35	0.912	0.31	1	0.32	0.956	1.49	0.000148	1.53	0.000187	0.97	0.0125
myclb	0.95	0.0702	0.11	0.983	-0.04	1	0.46	0.95	0.54	0.297	0.56	0.376	1.45	0.0045
mcl1b	0.95	0.0702	0.11	0.983	-0.04	1	0.46	0.95	0.54	0.297	0.56	0.376	1.45	0.0045
zn391	0.95	0.157	-0.53	0.925	-0.53	1	0.39	0.976	0.12	0.89	-0.2	0.848	0.84	0.197
snx10a	0.95	0.0137	-0.25	0.965	0.47	1	0.03	0.999	0.22	0.783	0.43	0.614	0.59	0.39
glci1	0.94	0.378	0.84	0.918	0	1	0.47	0.983	1.38	0.129	-0.53	0.696	0.82	0.429
LAMP1	0.94	0.0611	0.41	0.925	0.1	1	0.79	0.67	-0.4	0.484	-0.42	0.158	1.01	0.0385
SEC61A1	0.93	0.313	-0.45	0.953	-0.07	1	1.04	0.849	-0.26	0.803	0.06	0.973	0.73	0.429
s61a1	0.93	0.313	-0.45	0.953	-0.07	1	1.04	0.849	-0.26	0.803	0.06	0.973	0.73	0.429
zn271	0.93	0.0618	0.14	0.991	-1.21	1	-1.59	0.787	1.08	0.369	-0.29	0.882	-0.3	0.849
DNM1L	0.91	0.222	1.27	0.872	-0.4	1	1.14	0.918	-0.5	0.703	-0.7	0.538	-0.68	0.494
tigara	0.9	0.4	0.74	0.927	0.48	1	1.06	0.892	-0.28	0.813	-0.33	0.822	0.37	0.756

RCL1	0.9	0.0209	0.15	0.988	-0.09	1	0.17	0.986	0.83	0.023	0.76	0.0718	0.96	0.0113
NFKB2	0.9	0.0288	0.5	0.929	0.19	1	-0.04	0.999	0.52	0.443	0.3	0.761	0.42	0.587
FKBP8	0.9	0.0359	-0.3	0.936	-0.53	1	0.27	0.974	0.28	0.549	0.36	0.507	0.31	0.526
HERC4	0.9	0.053	0.34	0.962	0	1	-0.53	0.97	0.28	0.77	-0.08	0.956	-0.21	0.844
etbr2	0.89	0.105	0.14	0.98	0.09	1	-0.19	0.992	1.07	0.0347	0.35	0.625	0.72	0.182
RPS13	0.89	0.0148	0	0.999	0.15	1	-0.4	0.982	0.03	0.981	-0.58	0.579	-0.6	0.507
CCNG2	0.88	0.0344	-0.4	0.898	-0.53	1	-0.24	0.977	1.09	0.00638	0.65	0.163	0.7	0.0863
hnrll	0.88	0.327	1	0.843	0.67	1	0.23	0.993	0.23	0.821	1.12	0.235	0.8	0.356
pur9	0.87	0.0856	0.21	0.962	0.15	1	0.38	0.961	0.92	0.0507	0.51	0.396	0.26	0.66
sema4e	0.87	0.0653	-0.26	0.953	0.06	1	0.13	0.993	0.61	0.178	0.4	0.495	-0.07	0.911
SRGAP2	0.87	0.373	0.67	0.826	1	1	0.18	0.993	0.66	0.19	1.09	0.0568	0.28	0.657
COX1	0.86	0.184	0.35	0.962	0.33	1	-3.3	0.813	0.15	0.898	1.08	0.0877	1.02	0.0743
SDHB	0.85	0.125	-0.67	0.897	0.49	1	0.43	0.979	-1.17	0.115	-0.33	0.733	0.34	0.675
CD226	0.85	0.286	0.48	0.94	0.34	1	-0.11	0.999	0.67	0.375	0.83	0.349	1.05	0.157
IMPA2	0.85	0.76	1.86	0.909	1.03	1	1.27	0.973	2.35	0.23	2.15	0.379	0.24	0.936
AGR2	0.84	0.553	0.93	0.927	0.2	1	0.33	0.993	1.37	0.239	0.18	0.934	-0.46	0.754
il31r	0.84	0.147	0.88	0.719	0.92	1	0.46	0.957	1.42	0.00832	1.06	0.0864	0.68	0.236
tax1bp1a	0.84	0.124	0.05	0.994	0.12	1	0.24	0.988	0.2	0.752	0.89	0.141	1.17	0.0254
CYFIP1	0.84	0.374	-0.11	0.931	-0.05	1	-0.06	0.992	-0.24	0.102	-0.16	0.396	-0.14	0.385
rs16	0.84	0.297	0.1	0.981	0.08	1	0.11	0.993	0.3	0.466	0.19	0.729	0.17	0.73
m3k8	0.83	0.119	-0.17	0.983	-0.42	1	-1.05	0.815	0.48	0.589	0.12	0.933	-0.03	0.977
nog3	0.83	0.222	0.31	0.958	0.42	1	0.49	0.963	1.16	0.0588	0.63	0.434	0.9	0.166
nogg3	0.83	0.222	0.31	0.958	0.42	1	0.49	0.963	1.16	0.0588	0.63	0.434	0.9	0.166
UBXN8	0.83	0.113	-0.22	0.859	-0.13	1	0.25	0.982	1.03	0.0336	0.64	0.286	0.87	0.0853
klhl41b	0.83	0.213	0.83	0.816	0.66	1	1.18	0.499	0.87	0.152	1.11	0.117	1.71	0.00613
msi2h	0.83	0.257	-0.68	0.898	-0.08	1	0.55	0.961	0.35	0.65	-0.17	0.882	1.59	0.0187
SURF2	0.83	0.0291	0.04	0.993	-0.15	1	0.54	0.74	0.3	0.437	0.44	0.324	1.12	0.00265

ATF5	0.83	0.114	-0.4	0.927	-0.33	1	-0.55	0.905	0.24	0.677	0.18	0.82	0.26	0.672
slc25a38b	0.82	0.115	0.49	0.95	0.25	1	-1.06	0.856	0.41	0.679	-0.3	0.826	-0.22	0.851
mpp7a	0.82	0.44	0.95	0.884	-0.73	1	-0.34	0.992	1.33	0.13	0.77	0.516	0.64	0.545
GADL1	0.82	0.398	-0.94	0.939	0.29	1	0.44	0.981	1.16	0.161	1.69	0.0761	0.61	0.539
rl28	0.82	0.612	-0.09	0.993	-0.45	1	-0.57	0.971	-0.51	0.596	0.22	0.879	-0.98	0.275
scn4ab	0.81	0.145	0.08	0.988	0.1	1	-0.34	0.973	0.97	0.0582	1.15	0.0505	1.23	0.0199
BRAT1	0.81	0.16	0.09	0.988	0.41	1	-0.08	0.999	1.1	0.0394	1.05	0.0884	1.13	0.0413
ATG5	0.8	0.601	-0.05	0.984	0.1	1	-0.14	0.974	0.08	0.781	-0.01	0.982	0.02	0.965
sdf1	0.8	0.259	-0.33	0.957	-0.34	1	0.27	0.99	0.62	0.353	0.77	0.329	1.38	0.0349
cysp2	0.8	0.196	-0.34	0.946	-0.07	1	0	1	0.6	0.306	0.6	0.404	1.07	0.0671
c3-1	0.8	0.203	-0.22	0.936	0.26	1	-0.3	0.941	0.12	0.743	0.3	0.438	0.03	0.946
msrb1a	0.8	0.0251	-0.07	0.973	-0.03	1	0.21	0.95	-0.23	0.31	-0.33	0.205	-0.22	0.356
thg1	0.8	0.639	0.81	0.929	-0.43	1	0.14	0.999	0.92	0.401	0.54	0.729	-1.71	0.109
MYLK3	0.77	0.46	0.06	0.994	0.53	1	-0.78	0.95	0.11	0.929	1.14	0.286	0.25	0.833
TXNIP	0.77	0.194	0.37	0.94	0.01	1	-0.14	0.995	0.78	0.152	0.67	0.322	1.16	0.0385
GGT1	0.77	0.467	0.09	0.992	-0.26	1	1.05	0.84	-0.11	0.923	-0.76	0.465	1.15	0.174
si:ch211-238n5.4	0.77	0.0573	-0.21	0.953	-0.19	1	-0.09	0.996	0.64	0.0927	0.16	0.792	0.5	0.224
TONSL	0.77	0.0843	-0.61	0.952	-0.27	1	-0.93	0.953	-1.14	0.306	-0.97	0.488	-2.71	0.0132
P2RY8	0.77	0.409	1.57	0.583	0.43	1	-0.06	0.999	1.42	0.0681	0.91	0.355	-0.14	0.905
mapk14b	0.76	0.524	-0.3	0.977	0.79	1	0.35	0.992	1.07	0.285	0.92	0.467	1.07	0.309
HNMT	0.76	0.0383	0.24	0.959	-0.26	1	0.52	0.921	1.02	0.00384	-0.26	0.715	0.05	0.951
SKA1	0.76	0.325	-1.97	0.843	-0.03	1	-0.85	0.981	-2.09	0.176	-1.44	0.368	-1.12	0.544
mime	0.75	0.137	-0.11	0.982	-0.49	1	-0.04	0.999	0.44	0.378	0.5	0.401	0.7	0.158
UBR7	0.75	0.502	0.89	0.907	0.58	1	1.15	0.856	-0.41	0.754	-0.29	0.681	0.66	0.544
samm50l	0.75	0.487	-0.25	0.979	-0.48	1	0.67	0.962	0	0.999	0.05	0.979	-0.87	0.353
klh13	0.75	0.455	0.02	0.994	0.09	1	0.05	0.998	-0.1	0.761	-0.32	0.313	-0.09	0.79
NCF1	0.74	0.28	0.19	0.977	0.59	1	-0.13	0.999	0.87	0.152	0.66	0.392	0.52	0.464

thbs4b	0.73	0.381	0.48	0.983	-0.25	1	2.81	0.82	0.19	0.953	-0.91	0.779	1.65	0.497
EXD1	0.72	0.612	-0.3	0.98	-1.18	1	0.63	0.98	0.45	0.742	0.82	0.586	0.26	0.868
EXOG	0.72	0.477	-0.49	0.884	-0.19	1	-0.13	0.993	-0.26	0.624	-0.21	0.768	-0.28	0.626
eki1	0.72	0.0226	0.09	0.979	-0.18	1	0.22	0.97	0.5	0.0931	0.5	0.163	0.56	0.0718
MIDN	0.71	0.313	0.6	0.913	-0.19	1	0.81	0.856	1.01	0.099	0.32	0.729	0.33	0.674
zgc:101723	0.71	0.346	-0.3	0.957	-0.26	1	0.39	0.972	0.09	0.914	-0.19	0.84	-0.45	0.503
MPV17L2	0.71	0.137	-0.37	0.95	-0.14	1	0.01	0.999	-0.66	0.322	-0.55	0.521	-1.52	0.0202
tbb3	0.7	0.302	-0.13	0.983	-0.13	1	-0.57	0.949	1.22	0.0386	0.63	0.407	0.68	0.293
CCR9	0.7	0.435	-0.41	0.953	-0.65	1	-0.31	0.992	-1.69	0.0229	-1.63	0.0571	0.44	0.635
loxl2b	0.7	0.601	0.37	0.915	0.04	1	0.01	0.999	0.47	0.25	0.38	0.465	0.43	0.329
TOE1	0.68	0.273	-0.14	0.946	0	1	0.16	0.972	-0.12	0.66	-0.03	0.937	0.12	0.689
RS2	0.68	0.0318	0.77	0.943	-0.01	1	-0.02	0.999	2.15	0.0683	0.84	0.609	0.9	0.523
AAR2	0.68	0.531	-0.29	0.973	-0.45	1	-0.39	0.988	0.95	0.295	0.2	0.894	1.34	0.143
si:ch211-157p22.10	0.68	0.186	0.01	0.998	0.15	1	-0.07	0.999	0.53	0.281	0.54	0.368	1.14	0.0191
clus	0.68	0.155	0.19	0.982	1.11	1	-0.36	0.988	0.28	0.779	0.26	0.842	0.01	0.996
psmb9-a	0.67	0.475	-0.21	0.98	0.11	1	-1.02	0.843	0.52	0.559	0.52	0.64	-0.44	0.653
nmes1	0.67	0.585	-0.69	0.938	-0.56	1	-0.25	0.995	0.44	0.707	-1.02	0.406	0.64	0.581
APTX	0.67	0.135	-0.21	0.957	-0.23	1	0.22	0.981	0.03	0.959	-0.31	0.587	1.06	0.0141
ITM2A	0.67	0.208	0.34	0.939	0.25	1	0.07	0.999	1.2	0.0126	1.36	0.013	1.02	0.0414
AEP1	0.66	0.612	0.47	0.96	0.83	1	0.83	0.961	0.82	0.454	1.24	0.322	0.42	0.747
nattl	0.66	0.612	0.47	0.96	0.83	1	0.83	0.961	0.82	0.454	1.24	0.322	0.42	0.747
ATG3	0.66	0.438	0.12	0.945	-0.04	1	-0.02	0.999	-0.04	0.897	-0.12	0.678	-0.03	0.902
NFIL3	0.66	0.0682	-0.02	0.996	0.09	1	0.42	0.921	-0.59	0.131	-0.28	0.606	0.02	0.98
CCNB1	0.66	0.366	-0.39	0.974	-0.61	1	-0.78	0.973	-1.34	0.275	-1.47	0.319	-0.96	0.484
si:dkey-22o12.2	0.66	0.432	0.15	0.983	0.18	1	0.01	0.999	0.03	0.973	0.25	0.828	1.37	0.0602
ima2	0.66	0.701	-0.46	0.969	-0.57	1	1.45	0.892	-0.48	0.755	-0.22	0.924	1.28	0.347
if6	0.66	0.0543	0.19	0.983	0.5	1	-0.13	0.999	1.51	0.0827	0.47	0.723	0.68	0.512

EIF6	0.66	0.0543	0.21	0.927	0.14	1	0.2	0.964	0.46	0.0654	0.38	0.219	0.31	0.252
METRNL	0.65	0.132	0.46	0.885	-0.33	1	0.35	0.961	0.22	0.663	-0.03	0.969	0.16	0.777
MRE11	0.65	0.198	-0.02	0.998	-0.17	1	0.38	0.978	0.77	0.248	0.62	0.459	-0.05	0.964
gtpb1	0.65	0.067	-0.1	0.991	0.22	1	1.13	0.835	0.14	0.908	0.29	0.839	1.25	0.165
ASCC2	0.65	0.0646	0.54	0.947	0.47	1	-0.16	0.999	1.55	0.0827	1.54	0.147	1.05	0.286
rabx5	0.64	0.0738	-0.03	0.996	-0.17	1	0.29	0.984	-0.59	0.337	-0.62	0.408	-0.34	0.644
arrd3	0.64	0.13	0.13	0.983	0.2	1	0.22	0.992	-1.04	0.0673	-0.7	0.329	-0.81	0.183
hsp70	0.64	0.0152	-0.48	0.941	-0.2	1	0.77	0.921	0.74	0.329	0.18	0.886	1.29	0.0837
rpb2	0.64	0.0762	-0.33	0.957	-0.48	1	0.43	0.97	-0.21	0.788	0.04	0.977	0.08	0.935
kgua	0.64	0.0521	-0.44	0.982	-0.88	1	0.76	0.989	0.65	0.778	0.44	0.888	1.44	0.49
necp1	0.63	0.587	-0.33	0.971	-1.05	1	0.24	0.994	0.26	0.827	-0.45	0.744	-0.54	0.635
RADIL	0.63	0.0623	0.44	0.813	0.25	1	0.44	0.812	0.88	0.00633	0.61	0.0975	1.04	0.00163
rhg21	0.62	0.218	0.03	0.994	0.08	1	-0.37	0.95	-0.36	0.404	-0.08	0.912	0.21	0.679
tsn16	0.62	0.506	-0.05	0.995	0.56	1	-0.13	0.999	0.78	0.329	1.31	0.142	1.23	0.121
LOC100137051	0.62	0.839	1.07	0.953	1.49	1	1.15	0.978	-0.53	0.838	-0.35	0.92	0.72	0.777
CCDC40	0.61	0.596	0.84	0.913	0.46	1	-0.12	0.999	1.71	0.0533	1.09	0.329	0.36	0.756
LOC100136565	0.61	0.02	0.45	0.69	0.17	1	0.16	0.977	0.48	0.0564	0.65	0.0252	0.48	0.0695
ugpa	0.61	0.282	-0.08	0.971	0.03	1	0.05	0.995	0.03	0.933	0.21	0.464	0.24	0.318
IER3IP1	0.61	0.0392	3.35	0.746	3.67	1	2.07	0.942	3.54	0.0896	3.43	0.169	1.93	0.418
NARF	0.6	0.4	-0.4	0.927	0	1	0.24	0.984	0.77	0.126	-0.28	0.686	-0.18	0.795
loxe3	0.6	0.72	0.12	0.993	1.17	1	0.45	0.992	-0.35	0.822	0.66	0.703	1.34	0.297
hsd17b12a	0.6	0.529	-0.12	0.97	-0.04	1	0.44	0.825	-0.39	0.244	-0.15	0.777	-0.08	0.856
VEGFC	0.59	0.384	0.37	0.943	0.09	1	0.8	0.812	0.51	0.415	1.42	0.0332	1.5	0.013
zgc:77849	0.59	0.525	0.53	0.939	0.55	1	0.91	0.876	0.24	0.804	0.6	0.558	1.01	0.205
DHDH	0.59	0.296	0.28	0.953	0.03	1	-0.37	0.97	0.79	0.11	0.42	0.529	0.56	0.3
k2c8	0.59	0.819	2.17	0.835	1.01	1	1.41	0.957	2.55	0.123	1.25	0.587	1.3	0.506

EIF2A	0.58	0.765	-0.17	0.99	-0.25	1	0.91	0.97	-1.14	0.425	-0.83	0.66	-0.17	0.933
f262	0.58	0.0843	0.06	0.987	-0.11	1	-0.01	0.999	0.52	0.0954	0.63	0.0833	0.59	0.0671
haaf	0.58	0.822	1.43	0.94	-1.16	1	0.46	0.998	0.58	0.835	0.53	0.885	-0.14	0.965
cbpa1	0.58	0.9	-1.04	0.961	-0.13	1	1.1	0.985	0.05	0.989	0	0.999	0.53	0.87
fel	0.57	0.862	0.79	0.968	0.33	1	-1.28	0.977	0.1	0.972	-0.65	0.848	1.04	0.689
CD82	0.57	0.307	-0.13	0.973	0.64	1	-0.01	0.999	-0.41	0.306	-0.45	0.364	-0.39	0.37
BNIP3	0.56	0.0322	0.48	0.952	0.32	1	-1.01	0.287	-0.26	0.809	-0.72	0.522	-0.08	0.907
btbd11a	0.56	0.559	0.1	0.991	0.3	1	0.64	0.96	1.18	0.124	0.67	0.512	0.81	0.342
tri39	0.56	0.0438	0.09	0.983	-0.29	1	-0.01	0.999	-0.74	0.0799	-0.37	0.52	0.12	0.841
PRPF39	0.55	0.634	0.71	0.929	0.61	1	0.47	0.981	0.17	0.895	1.27	0.235	0.74	0.472
CSRP2	0.55	0.431	0.3	0.957	0.28	1	0.22	0.992	0.6	0.336	0.71	0.334	1	0.101
CHD8	0.55	0.0135	-0.02	0.993	-0.05	1	0.12	0.979	0.5	0.0176	0.34	0.177	0.55	0.011
SPP2	0.55	0.244	0.31	0.936	0.06	1	-0.33	0.967	1	0.0188	0.94	0.0546	-1.2	0.174
PHF2	0.54	0.0727	0.37	0.916	0.13	1	-0.05	0.999	0.47	0.257	0.79	0.0935	0.34	0.467
tmem41aa	0.54	0.617	-0.14	0.958	0.07	1	-0.11	0.992	-0.05	0.896	-0.32	0.35	0.11	0.747
erd22	0.54	0.566	-0.27	0.929	0.02	1	0.04	0.999	-0.14	0.742	-0.06	0.923	-0.07	0.889
CASC3	0.54	0.585	0.12	0.988	0.33	1	-0.49	0.974	0.03	0.976	-0.04	0.98	1.07	0.189
acod	0.53	0.62	0.18	0.983	0.36	1	-0.14	0.999	-0.18	0.879	-0.02	0.993	0.11	0.933
CCNY	0.53	0.552	0.86	0.855	0.57	1	1.15	0.682	-0.44	0.378	1.06	0.218	0.96	0.205
ATF3	0.52	0.365	-0.36	0.938	-0.05	1	0.77	0.948	1.29	0.102	0.33	0.635	0.17	0.801
cbpb1	0.52	0.914	-0.65	0.982	-0.92	1	0.83	0.993	0.12	0.976	0.04	0.994	1.34	0.698
RP9	0.52	0.053	0.8	0.908	0.5	1	-0.1	0.999	0.89	0.292	1.65	0.0799	0.84	0.356
rdh10a	0.51	0.394	0.72	0.802	0.29	1	0.14	0.993	0.93	0.0638	0.68	0.271	0.5	0.379
hsp7c	0.51	0.0613	-0.07	0.982	0.07	1	-0.02	0.999	0.12	0.697	0.17	0.632	-0.12	0.714
MMP9	0.51	0.446	0.3	0.954	0.95	1	0.22	0.992	1.09	0.322	1.14	0.392	-0.25	0.727
rhg15	0.51	0.31	0.67	0.781	0.31	1	0.15	0.993	0.63	0.158	1.05	0.0396	0.58	0.224
dnjb1	0.5	0.0813	-0.19	0.929	-0.21	1	-0.09	0.992	0.3	0.222	0.28	0.358	0.18	0.519
slc25a36a	0.49	0.619	-0.16	0.984	-0.21	1	0.28	0.992	-0.86	0.271	0.14	0.919	0.96	0.237

icef1	0.49	0.397	0.51	0.897	-0.41	1	0.19	0.992	0.3	0.592	0.21	0.781	0.78	0.128
recO	0.49	0.473	0	0.998	0.08	1	-0.14	0.979	0.08	0.803	-0.3	0.302	-0.07	0.842
MED16	0.48	0.548	-0.37	0.973	0.4	1	-0.96	0.953	0.5	0.705	1.91	0.139	0.99	0.428
RIC8A	0.48	0.603	0.85	0.856	0.23	1	0.24	0.993	0.41	0.618	1.19	0.16	0.88	0.253
METTL13	0.48	0.412	0.15	0.97	0	1	0.13	0.993	0.22	0.649	0.36	0.521	-0.27	0.596
ppip2	0.47	0.541	0.87	0.813	0.21	1	-0.41	0.974	1.1	0.0739	0.43	0.623	-0.2	0.81
rpc4	0.47	0.0299	-0.33	0.958	-0.33	1	-0.05	0.999	1.02	0.12	-0.27	0.8	0.75	0.292
cks1	0.47	0.303	-0.61	0.954	-0.51	1	-0.9	0.961	-1.41	0.215	-1.27	0.374	-1.29	0.291
RPL30	0.46	0.599	-0.04	0.983	0.11	1	0.06	0.992	0.04	0.86	0.13	0.559	0.02	0.95
YBEY	0.46	0.229	-0.35	0.979	-0.02	1	-0.38	0.993	1.53	0.198	1.09	0.485	1.7	0.172
elov7	0.45	0.135	0.25	0.962	0.03	1	-0.05	0.999	0.39	0.534	0.5	0.491	0.33	0.63
f264	0.45	0.618	0.55	0.929	0.79	1	-0.86	0.869	0.9	0.202	1.4	0.0845	0.19	0.846
UNC93A	0.45	0.706	0.08	0.988	0.13	1	0.12	0.995	0.24	0.69	0.2	0.804	0.03	0.973
zgc:162879	0.45	0.728	0.38	0.964	-0.43	1	-0.72	0.963	0.74	0.454	0.3	0.844	-1.15	0.239
cldnd	0.45	0.845	-0.45	0.977	0.06	1	-2.13	0.749	-2.4	0.0976	1.04	0.607	-1.17	0.494
cldy	0.45	0.845	-0.45	0.977	0.06	1	-2.13	0.749	-2.4	0.0976	1.04	0.607	-1.17	0.494
fpps	0.45	0.796	2.49	0.549	0.41	1	1.63	0.779	2.15	0.0599	1.8	0.189	1.74	0.152
MAPK3	0.44	0.345	0.64	0.9	-0.04	1	-0.31	0.985	1.02	0.111	0.87	0.267	-0.15	0.874
gtr11	0.44	0.688	0.55	0.939	0.47	1	0	1	1.01	0.208	0.43	0.713	0.27	0.795
zgc:66447	0.44	0.671	0.02	0.994	-0.01	1	0.11	0.992	-0.14	0.704	-0.13	0.776	-0.07	0.861
TTC33	0.43	0.117	0.95	0.897	0.2	1	-0.15	0.999	0.33	0.781	0.95	0.428	0.72	0.509
asic1b	0.43	0.639	0.24	0.972	0.46	1	-0.3	0.989	-0.11	0.918	0.7	0.44	1.27	0.0783
SYBU	0.43	0.631	-0.56	0.927	-0.06	1	0.49	0.97	0.58	0.429	0.4	0.694	1.06	0.139
ppdpfb	0.43	0.54	0.33	0.939	0.19	1	0.36	0.964	-0.03	0.964	-0.03	0.978	0.07	0.921
clrn3	0.43	0.86	1.04	0.939	-0.44	1	0.98	0.974	1.55	0.33	1.11	0.595	1.41	0.409
fabpl	0.43	0.628	-0.01	0.998	-0.03	1	-0.01	0.999	0.22	0.425	0.11	0.779	0.13	0.685
COPA	0.42	0.106	0.3	0.953	0.31	1	0.04	0.999	-0.15	0.827	0.17	0.849	-0.2	0.779
CAPRIN2	0.42	0.859	0.03	0.998	-0.61	1	-1.94	0.833	0.32	0.873	-0.94	0.654	-2.61	0.0854

TNNI3	0.42	0.839	1.44	0.879	0.28	1	0.24	0.999	0.3	0.869	0.14	0.957	0.84	0.598
BCAT1	0.42	0.217	1.03	0.879	0.81	1	0.53	0.644	0.61	0.0492	0.63	0.632	0.6	0.0635
CDK6	0.41	0.438	0.32	0.938	0.39	1	0.39	0.935	1.11	0.0112	1	0.0477	0.14	0.782
LOC100136199	0.41	0.668	0.29	0.965	0.28	1	0.7	0.941	1.16	0.0985	1.14	0.173	1.07	0.15
143g2	0.41	0.668	0.29	0.965	0.28	1	0.7	0.941	1.16	0.0985	1.14	0.173	1.07	0.15
rpac1	0.4	0.104	-0.1	0.962	-0.02	1	0.03	0.999	0.22	0.362	0.26	0.365	0.34	0.155
ELOVL6	0.4	0.805	0.81	0.836	0.02	1	0.43	0.97	0.84	0.179	0.06	0.959	0.03	0.974
UBE2V2	0.4	0.121	-0.05	0.985	-0.09	1	-0.02	0.999	0.39	0.106	0.39	0.176	0.38	0.132
ub2v2	0.4	0.121	-0.05	0.985	-0.09	1	-0.02	0.999	0.39	0.106	0.39	0.176	0.38	0.132
COG6	0.39	0.256	-0.3	0.979	-0.52	1	1.31	0.868	0.22	0.884	-0.11	0.959	0.25	0.871
DCLRE1C	0.39	0.73	-0.04	0.996	-0.51	1	-0.08	0.999	0.13	0.911	-0.09	0.957	-1.3	0.121
lap2	0.39	0.105	1.18	0.859	0.03	1	-0.69	0.972	-0.04	0.978	0.38	0.819	-0.07	0.965
dmb	0.38	0.813	0.66	0.943	1.2	1	0.03	0.999	0.8	0.482	1.51	0.229	0	0.999
hsc70a	0.38	0.2	-0.1	0.962	0.06	1	0.04	0.999	0.06	0.859	0.07	0.851	-0.06	0.859
mk67i	0.38	0.455	-0.08	0.994	-0.05	1	-0.72	0.97	-0.38	0.771	0.2	0.914	-1.28	0.243
GPR18	0.37	0.729	0.19	0.982	0.49	1	-1	0.842	0.49	0.575	0.8	0.41	0.31	0.756
rnmtl1b	0.37	0.803	0.47	0.959	-0.17	1	0.22	0.996	0.71	0.509	0.09	0.962	1.33	0.193
nrxn3b	0.37	0.847	1.2	0.901	0.62	1	0.63	0.981	0.12	0.947	0.77	0.657	0.04	0.983
tnfaip8l2b	0.37	0.62	1.06	0.672	0.96	1	-0.13	0.998	0.69	0.24	0.67	0.355	-0.01	0.987
tomm20b	0.37	0.699	-1.01	0.878	-0.89	1	0.38	0.99	0.03	0.984	-0.22	0.892	0.46	0.694
TENM3	0.36	0.465	0.53	0.945	0.28	1	-0.27	0.993	0.19	0.872	-0.26	0.858	-0.54	0.61
plpp	0.36	0.421	0.03	0.994	0.01	1	-0.24	0.979	0.41	0.345	-0.07	0.929	0.08	0.889
tba	0.36	0.236	-0.24	0.921	-0.39	1	-0.05	0.999	0.21	0.485	-0.16	0.694	-0.2	0.547
arh	0.36	0.129	-0.13	0.98	-0.24	1	-0.17	0.993	-0.78	0.104	-0.93	0.0969	-1.11	0.0255
errfi	0.36	0.23	-1.08	0.711	-0.58	1	-0.32	0.984	-0.17	0.843	-0.49	0.588	-0.62	0.399
CRLS1	0.35	0.383	0.59	0.965	0.27	1	0.28	0.961	0.57	0.756	2.32	0.179	0.4	0.285
ACSL1	0.34	0.18	0.37	0.943	-0.7	1	0.63	0.915	-1.76	0.26	-2.64	0.139	-1.96	0.227
pdli3	0.34	0.81	1.12	0.852	-0.52	1	0.25	0.993	1.27	0.162	-0.4	0.781	0.17	0.898

trm61	0.34	0.569	1.04	0.956	-0.23	1	-1.22	0.977	1.19	0.599	-0.11	0.979	-1.01	0.689
ZCRB1	0.34	0.167	-0.06	0.982	0.13	1	0.12	0.981	0.23	0.338	0.01	0.979	-0.02	0.964
FAM65B	0.33	0.835	0.02	0.998	0.47	1	-0.09	0.999	0.91	0.394	1.29	0.295	0.62	0.617
MGAT1	0.33	0.192	0.79	0.88	0.89	1	0.53	0.97	0.95	0.187	0.83	0.36	1.07	0.155
dnja1	0.33	0.269	-0.11	0.963	-0.15	1	-0.05	0.999	0.3	0.269	0.21	0.573	0.22	0.485
rl23	0.33	0.23	-0.03	0.988	0.13	1	0.1	0.983	0.22	0.25	0.27	0.235	-0.02	0.944
CTRL	0.33	0.947	-1.18	0.965	-1.28	1	1.38	0.986	-0.66	0.87	-1.9	0.648	0.81	0.842
OGG1	0.32	0.35	-0.22	0.936	-0.13	1	-0.06	0.998	0.3	0.337	0.14	0.764	0.36	0.256
DUS3L	0.32	0.206	-0.2	0.989	-0.15	1	-1.04	0.963	-0.46	0.783	-0.64	0.751	0.01	0.996
WDR4	0.32	0.631	0.56	0.972	-1.65	1	1.05	0.974	0.58	0.78	0.35	0.903	1.43	0.443
pp14b	0.32	0.417	-0.35	0.893	-0.17	1	0.35	0.921	0.11	0.803	0.25	0.583	0.47	0.179
ivns1abpa	0.32	0.643	-0.08	0.989	-0.37	1	0.04	0.999	0.38	0.513	-0.08	0.936	0	0.999
trp-iii	0.32	0.77	0.03	0.996	-0.11	1	0.29	0.992	0.06	0.955	0.01	0.996	0.35	0.71
ALG3	0.31	0.341	-0.3	0.895	0.14	1	0.14	0.988	0.29	0.324	0.32	0.373	0.32	0.296
NUP54	0.31	0.136	-0.99	0.924	-0.15	1	-0.42	0.992	1.56	0.168	1.92	0.15	0.31	0.844
HBEGF	0.31	0.573	-0.09	0.987	-0.03	1	-0.18	0.993	0.11	0.88	-0.11	0.908	0.15	0.838
aste1	0.3	0.324	-0.03	0.99	0.1	1	0.86	0.978	-0.15	0.618	0.84	0.694	0.29	0.325
fgf1a	0.3	0.842	1.18	0.855	0.07	1	0.27	0.993	0.61	0.579	0.66	0.628	-0.13	0.929
FGF1	0.3	0.842	1.18	0.855	0.07	1	0.27	0.993	0.61	0.579	0.66	0.628	-0.13	0.929
IFRD1	0.29	0.312	0.37	0.797	0.22	1	0.19	0.986	0.63	0.0119	0.62	0.0311	0.54	0.0394
nabp1a	0.29	0.529	0.1	0.99	-0.18	1	-0.06	0.999	0.02	0.983	-0.65	0.491	-1.41	0.0562
ZBTB24	0.29	0.859	1.09	0.887	0.02	1	0.78	0.966	0.25	0.858	0.41	0.803	0.64	0.613
rergla	0.28	0.755	0	1	-0.36	1	0.37	0.988	1.07	0.202	-0.54	0.647	0	1
APOF	0.27	0.69	-0.02	0.996	-0.19	1	-1.1	0.277	0.33	0.543	0.44	0.492	0.53	0.315
SAT2	0.27	0.824	-0.45	0.897	-0.37	1	-0.12	0.995	-0.25	0.624	-0.16	0.819	-0.43	0.383
ARF1	0.27	0.167	-0.05	0.995	0.19	1	-0.12	0.999	-1.09	0.112	-1.43	0.0702	-2.29	0.00127
AQP8	0.27	0.444	1.11	0.882	0.62	1	0.51	0.983	-0.28	0.835	-0.13	0.946	1.57	0.14
abhd2a	0.26	0.586	-0.61	0.909	-0.36	1	-0.66	0.933	-0.88	0.169	-0.31	0.758	-0.39	0.621

mypc1	0.26	0.658	0.39	0.919	0.29	1	0.04	0.999	0.04	0.945	0.41	0.472	0.3	0.565
hem2	0.25	0.578	-0.17	0.96	-0.38	1	0.08	0.997	0.01	0.984	-0.14	0.805	0.4	0.307
LOC798500	0.25	0.909	0.92	0.933	0.26	1	0.14	0.999	-0.53	0.727	0.38	0.855	-1.22	0.371
API5	0.25	0.299	-0.05	0.983	0	1	0.03	0.999	0.23	0.303	0.15	0.614	0.04	0.897
LIN9	0.25	0.79	-0.75	0.855	-0.85	1	-0.75	0.872	-0.4	0.567	-0.05	0.966	0.18	0.838
SMAD5	0.24	0.482	0.97	0.929	0.87	1	0.68	0.98	1.67	0.17	0.74	0.674	0.11	0.953
pycard	0.24	0.436	0.54	0.802	-0.06	1	-0.02	0.999	0.25	0.561	0.22	0.686	0.03	0.955
hpbp1	0.24	0.32	-0.62	0.975	2.24	1	1.71	0.95	0.47	0.856	1.95	0.426	1.89	0.372
C1D	0.22	0.942	-0.06	0.982	-0.08	1	0.06	0.995	-0.02	0.962	-0.14	0.66	0	0.995
LOC106562066	0.22	0.37	-0.4	0.947	-0.31	1	-0.59	0.953	0.97	0.149	1.02	0.206	0.33	0.707
tpc2l	0.22	0.37	-0.4	0.947	-0.31	1	-0.59	0.953	0.97	0.149	1.02	0.206	0.33	0.707
fsta	0.22	0.827	-0.24	0.969	-0.09	1	-0.41	0.973	0.45	0.52	0.58	0.488	1.08	0.1
SNX14	0.22	0.306	0.01	0.997	0	1	0.31	0.89	0.18	0.53	0.06	0.89	0.31	0.267
SMEK1	0.21	0.834	-0.14	0.983	-0.72	1	-0.79	0.856	0.39	0.583	-0.06	0.962	1.06	0.102
prva	0.2	0.852	0.3	0.961	-0.45	1	-0.55	0.96	0.41	0.586	0.25	0.814	0.13	0.891
AAED1	0.18	0.727	0.26	0.976	1.05	1	0.3	0.992	0.47	0.608	1.38	0.141	0.65	0.488
EMP3	0.17	0.28	0.07	0.957	0.05	1	0.17	0.869	0.3	0.0359	0.19	0.281	0.18	0.243
tcof	0.16	0.927	0.26	0.981	1.13	1	0.46	0.988	1.23	0.219	1.41	0.235	0.48	0.703
i20ra	0.16	0.819	0.71	0.741	0.11	1	0.61	0.815	-0.15	0.801	0.09	0.91	0.45	0.365
f131b	0.16	0.96	-1.38	0.938	2.94	1	0.85	0.989	0.42	0.875	3.24	0.155	0.92	0.711
GAMT	0.15	0.927	-0.67	0.938	0.05	1	2.21	0.23	0.4	0.727	-0.69	0.591	1	0.329
NOP14	0.14	0.482	-0.14	0.924	-0.06	1	-0.03	0.999	-0.14	0.398	0	0.996	0.15	0.393
pcat1	0.14	0.849	0.06	0.993	0.17	1	1.05	0.67	0.08	0.933	-0.36	0.699	0.43	0.58
METTL10	0.14	0.935	0	1	0.22	1	0.17	0.999	0.49	0.689	0.96	0.471	-1.52	0.155
FABP7	0.13	0.955	0.97	0.938	0.35	1	-0.14	0.999	0.69	0.669	1.2	0.506	-0.64	0.714
zgc:101663	0.13	0.945	1.25	0.875	0.03	1	-1.17	0.921	-0.29	0.847	0.35	0.85	-0.06	0.974
ptprub	0.09	0.93	0.35	0.946	0.47	1	0.16	0.993	1.11	0.0496	0.89	0.191	0.04	0.962
lyam1	0.09	0.953	-0.33	0.971	-0.51	1	-0.33	0.993	-0.61	0.561	0	0.998	-1.3	0.181

apol3	0.08	0.938	0.24	0.965	-0.66	1	-0.62	0.921	-0.24	0.749	-1.42	0.0338	-0.41	0.568
TIPIN	0.07	0.955	-0.3	0.966	-0.24	1	-0.23	0.993	-0.29	0.754	-0.84	0.377	-1.11	0.158
MTFR2	0.06	0.933	-0.6	0.943	0.05	1	-0.44	0.988	-1.18	0.212	-0.89	0.468	-0.2	0.721
tnr6	0.06	0.949	3.34	0.588	3.95	0.783	1.23	0.97	3.29	0.0494	4.05	0.0347	1.11	0.591
HOOK1	0.06	0.929	1.33	0.93	1.33	1	1.04	0.977	0.94	0.643	1.25	0.598	0.88	0.69
SEC23A	0.05	0.981	0.32	0.979	-0.8	1	0.06	0.999	0.75	0.564	-1	0.508	-0.03	0.987
TLL1	0.03	0.97	0.33	0.94	0.72	1	0.15	0.993	1.05	0.0272	0.91	0.103	0.5	0.351
hsc70	0.01	0.995	0.55	0.929	-0.18	1	-0.47	0.97	1.26	0.0637	0.73	0.407	0.1	0.922
EXO5	-0.01	0.995	-1.34	0.906	0.04	1	-2.26	0.67	-1	0.505	-0.66	0.749	-1.68	0.248
RSPH10B	-0.02	0.987	0.28	0.953	-1.01	1	-1.25	0.921	1.52	0.00331	1.41	0.326	0.41	0.492
mfsd2aa	-0.03	0.991	-0.11	0.994	0.08	1	-0.31	0.995	-1.59	0.201	0.06	0.981	-1.14	0.411
AACS	-0.03	0.982	-0.25	0.985	-0.01	1	0.53	0.99	-1.24	0.354	-0.9	0.607	-1.58	0.241
pepe	-0.03	0.995	-2.46	0.859	0.01	1	-0.68	0.993	-2.29	0.299	-1.16	0.712	-0.86	0.756
cbpa2	-0.04	0.995	-0.88	0.98	-1.41	1	1.6	0.984	-1.34	0.735	-1.47	0.767	1.13	0.79
MRPS7	-0.04	0.963	0.02	0.997	0.09	1	0.38	0.97	-0.37	0.52	-0.09	0.921	-0.13	0.854
si:dkey-32m20.1	-0.05	0.979	-0.21	0.982	-0.14	1	-1.04	0.896	0.37	0.74	-0.25	0.873	0.2	0.876
elob	-0.06	0.811	-0.36	0.957	-1.28	1	-0.91	0.827	0.19	0.832	-0.02	0.989	-0.33	0.709
calc1	-0.06	0.979	1.68	0.829	-0.23	1	-0.01	0.999	-0.34	0.845	-0.37	0.865	0.2	0.915
BOP1	-0.06	0.914	-0.52	0.895	-0.08	1	-1.31	0.97	-0.52	0.331	-0.57	0.374	-0.76	0.151
ZPLD1	-0.06	0.983	-1.36	0.913	-0.3	1	0.33	0.997	0.5	0.785	-0.74	0.737	-0.44	0.825
RPL36	-0.07	0.822	-0.04	0.986	0.12	1	0.06	0.993	0.12	0.604	0.18	0.487	1.07	0.183
intu	-0.08	0.93	-0.3	0.953	-0.22	1	-0.14	0.995	-1.01	0.0591	0.12	0.895	0.49	0.425
NAA15	-0.08	0.796	0.25	0.988	-1.86	1	-0.49	0.993	0.16	0.947	-1.17	0.588	-0.68	0.738
rla2	-0.08	0.76	-0.03	0.987	0.15	1	0.16	0.956	0.12	0.577	0.22	0.355	-0.06	0.823
UBE2K	-0.09	0.613	0	0.998	-0.03	1	0.02	0.999	0.01	0.954	-0.09	0.624	0.02	0.94
FUT10	-0.1	0.938	-0.65	0.925	-0.16	1	-0.19	0.995	-1.13	0.137	-0.09	0.949	-0.23	0.828
mvda	-0.1	0.948	-0.16	0.986	-0.01	1	0.46	0.983	-0.57	0.585	-0.15	0.928	-1.04	0.291

tnr14	-0.1	0.779	0.03	0.99	-0.03	1	-0.03	0.999	0.03	0.932	-0.22	0.519	0.13	0.695
erbeta	-0.11	0.954	-0.46	0.965	-1.98	1	-1.32	0.894	-1.56	0.171	-0.56	0.747	0.22	0.895
sal	-0.11	0.94	-0.17	0.985	-0.96	1	-0.43	0.983	-0.48	0.629	-1.08	0.309	-0.14	0.911
rs11	-0.12	0.561	-0.03	0.984	0.12	1	0.11	0.97	0.09	0.634	0.17	0.412	-0.02	0.932
PPP2R2D	-0.14	0.524	0.08	0.977	-0.01	1	0.15	0.977	0.25	0.334	-0.29	0.353	-0.03	0.939
rl19	-0.14	0.424	0.07	0.991	0.26	1	-0.41	0.97	-0.55	0.374	0.1	0.928	-0.45	0.508
tppc3	-0.14	0.453	0.11	0.989	-0.15	1	-0.21	0.993	-0.5	0.536	-0.72	0.435	-1.05	0.161
ATP8	-0.15	0.939	0.39	0.974	1	1	0.47	0.992	0.51	0.727	0.8	0.63	0.15	0.932
SLC50A1	-0.15	0.83	-0.44	0.905	-0.49	1	-0.12	0.993	-1	0.0248	-0.45	0.439	-0.23	0.692
dnjc7	-0.15	0.546	0.1	0.968	0.27	1	0.25	0.95	-0.2	0.511	-0.02	0.972	-0.2	0.52
SEPSECS	-0.15	0.525	-0.43	0.97	-0.13	1	1.32	0.897	-0.54	0.697	-1.39	0.333	0.46	0.76
SERP1	-0.15	0.557	-0.2	0.925	0.03	1	0.11	0.988	0.35	0.14	0.08	0.833	0.37	0.146
enoph	-0.15	0.52	0.43	0.95	0.55	1	-0.47	0.977	1.08	0.147	0.59	0.562	-1	0.208
CYB5R2	-0.15	0.91	-0.23	0.977	-0.13	1	0.21	0.993	-0.59	0.451	-0.2	0.869	-1.07	0.16
COX3	-0.15	0.59	-0.52	0.941	-0.33	1	0.46	0.978	-0.28	0.995	-0.69	0.507	-1.11	0.27
FEZ1	-0.15	0.929	0.69	0.933	0.96	1	0.12	0.999	0.52	0.629	0.3	0.844	-1.21	0.214
AKTIP	-0.16	0.232	-0.15	0.85	0.06	1	0.03	0.996	-0.14	0.275	-0.14	0.367	-0.01	0.963
at1b1	-0.16	0.895	1.36	0.588	0.94	1	0.02	0.999	2.04	0.0033 1	1.48	0.0584	0.2	0.833
ARL4C	-0.16	0.801	0.47	0.88	0.15	1	-0.04	0.999	-0.27	0.572	0.08	0.917	0.27	0.607
pdli1	-0.17	0.715	0.8	0.894	0.17	1	-0.08	0.999	1.03	0.18	0.65	0.531	-0.32	0.747
nkiras1	-0.17	0.825	-0.4	0.929	-0.02	1	0.28	0.981	-1.19	0.0182	-0.51	0.44	0.08	0.912
tryp	-0.17	0.776	-0.23	0.953	0.22	1	0.5	0.871	-0.39	0.366	-0.51	0.322	0.1	0.861
MED17	-0.18	0.418	0.39	0.933	-0.12	1	-1.36	0.449	0.57	0.44	0.1	0.94	0.31	0.631
ERGIC2	-0.18	0.618	0.49	0.946	-0.28	1	0.13	0.999	-0.37	0.704	-1.09	0.268	-0.18	0.876
ergi2	-0.18	0.618	0.49	0.946	-0.28	1	0.13	0.999	-0.37	0.704	-1.09	0.268	-0.18	0.876
CNBP	-0.18	0.587	-0.09	0.993	-0.18	1	-0.59	0.977	-0.74	0.479	-0.42	0.778	-1.21	0.234
lkha4	-0.19	0.91	-0.04	0.996	-1.09	1	-0.64	0.973	0.88	0.389	0.34	0.831	0.8	0.472
lgals3bpa	-0.19	0.95	0.55	0.977	0.29	1	-1.12	0.977	-0.28	0.917	0.15	0.967	-0.82	0.722

HVCN1	-0.19	0.692	-0.18	0.989	-0.35	1	0.96	0.964	0.9	0.512	1.02	0.538	0.2	0.912
zgc:110063	-0.19	0.851	-0.15	0.938	-0.16	1	-0.36	0.598	-0.2	0.362	-0.56	0.0167	-0.33	0.125
LOC100174881	-0.19	0.9	0.16	0.986	-0.9	1	0.65	0.97	-0.56	0.578	-1.17	0.28	-0.44	0.697
XIRP1	-0.19	0.935	2.69	0.596	1.99	1	1.44	0.921	0.02	0.994	0.4	0.864	0.08	0.97
rl10	-0.19	0.273	-0.2	0.894	0.09	1	-0.18	0.95	0.2	0.303	0.23	0.33	-0.09	0.711
CPTP	-0.19	0.41	-0.25	0.855	-0.01	1	0.16	0.961	-0.31	0.135	-0.31	0.212	-0.12	0.627
CNIH4	-0.2	0.566	-0.05	0.996	-1.56	1	0.44	0.993	0.72	0.628	0.12	0.964	0.65	0.69
catA	-0.2	0.695	-0.28	0.982	-0.17	1	-0.4	0.992	0.1	0.953	-0.24	0.904	0	0.999
SAMHD1	-0.2	0.933	-0.25	0.986	-0.18	1	-0.57	0.991	-1.36	0.348	-0.02	0.994	-0.33	0.866
MX2	-0.2	0.945	0.49	0.979	0.51	1	-0.72	0.99	-0.35	0.882	-0.91	0.723	-1.42	0.474
LRP11	-0.21	0.803	-0.21	0.969	0.36	1	0.22	0.992	-1.15	0.0373	-0.06	0.957	0.46	0.478
rs8	-0.21	0.257	-0.49	0.953	0.08	1	-1.27	0.752	0.54	0.585	0.41	0.762	0.29	0.805
FUBP3	-0.21	0.5	-0.07	0.985	0.18	1	-0.07	0.999	-0.3	0.453	-0.07	0.92	0.23	0.607
TAF11	-0.22	0.894	-0.11	0.936	-0.41	1	0.04	0.993	-1.76	0.0683	-0.07	0.753	-0.89	0.419
PHF8	-0.22	0.54	0.15	0.963	0.18	1	-0.14	0.992	-0.17	0.695	0.11	0.859	-0.13	0.793
MED24	-0.22	0.358	0.05	0.982	0.07	1	0.1	0.988	-0.16	0.495	-0.14	0.641	-0.21	0.384
MYOD1	-0.22	0.69	-0.26	0.943	-0.35	1	0.13	0.993	-0.6	0.14	-0.37	0.496	-0.17	0.742
trp-ii	-0.22	0.965	-0.06	0.998	-0.1	1	1.55	0.981	0.24	0.955	-0.05	0.993	1.34	0.717
dhr11	-0.22	0.331	0.02	0.993	0.04	1	-0.04	0.998	-0.04	0.873	-0.13	0.636	-0.12	0.637
rnh2c	-0.22	0.816	-0.46	0.934	-0.43	1	-0.28	0.988	-0.69	0.276	-0.62	0.433	-1.25	0.0471
rfa1	-0.22	0.514	-0.04	0.988	-0.02	1	0.15	0.981	-0.46	0.0969	-0.23	0.554	-0.27	0.387
rl10a	-0.23	0.18	-0.05	0.977	0.1	1	0.05	0.993	0.01	0.952	0.1	0.675	-0.14	0.433
galt2	-0.24	0.667	-0.19	0.945	0.02	1	-0.04	0.999	0.17	0.632	0.38	0.308	0.25	0.471
ssrd	-0.24	0.478	-0.33	0.908	-0.19	1	0.16	0.986	-0.14	0.73	-0.17	0.734	-0.15	0.723
LOC100335039	-0.25	0.161	-0.77	0.94	-0.71	1	0.07	0.999	1.39	0.231	2.16	0.102	1.3	0.29
RDH12	-0.25	0.413	-1.19	0.884	0.22	1	1.29	0.95	-1.23	0.4	0.51	0.761	1.16	0.459
CELF4	-0.26	0.713	0.45	0.919	0.75	1	-0.1	0.999	0.1	0.887	1.12	0.0524	0.18	0.789

UCHL3	-0.26	0.757	-0.49	0.926	-0.31	1	0.08	0.999	-0.4	0.543	-0.32	0.713	-1.16	0.0558
ddb2	-0.26	0.853	0.72	0.9	0.5	1	0.33	0.988	0.07	0.945	0.21	0.864	-0.24	0.8
SREBF2	-0.26	0.76	-0.35	0.948	-0.1	1	0.27	0.988	0.08	0.924	0	0.998	-0.48	0.483
ERLIN2	-0.27	0.0722	-0.04	0.982	0.09	1	0.06	0.988	-0.34	0.0164	-0.26	0.113	-0.24	0.113
erln2	-0.27	0.0722	-0.04	0.982	0.09	1	0.06	0.988	-0.34	0.0164	-0.26	0.113	-0.24	0.113
zgc:194281	-0.27	0.91	0.17	0.99	0.73	1	-1.19	0.95	0.74	0.635	0.13	0.959	-1.14	0.455
MYNN	-0.27	0.604	-0.03	0.994	-0.42	1	-0.73	0.959	-0.66	0.496	-0.52	0.681	-1.15	0.216
HSPB7	-0.27	0.888	1.45	0.85	0.07	1	0.95	0.96	-0.46	0.751	-0.52	0.769	-0.27	0.869
cgl	-0.28	0.197	0.02	0.997	0.27	1	-0.58	0.964	0.05	0.961	0.01	0.993	0.56	0.526
WWOX	-0.28	0.501	-0.12	0.972	-0.25	1	-0.05	0.999	-0.43	0.217	-0.18	0.732	-0.37	0.332
VWA8	-0.28	0.824	-0.21	0.913	-0.08	1	0.2	0.953	-0.4	0.0676	-0.44	0.0911	-0.23	0.34
COPS8	-0.28	0.854	0.13	0.96	0.17	1	-0.01	0.999	0.35	0.222	0.19	0.643	0.09	0.823
csn8	-0.28	0.854	0.13	0.96	0.17	1	-0.01	0.999	0.35	0.222	0.19	0.643	0.09	0.823
cgnb	-0.28	0.447	-1.01	0.797	-0.8	1	-0.11	0.999	-0.14	0.884	-0.08	0.955	0.86	0.253
myct1a	-0.29	0.334	0.14	0.994	-1.99	1	-0.87	0.988	-1.99	0.31	-1.97	0.416	-0.14	0.963
MYCT1	-0.29	0.334	0.14	0.994	-1.99	1	-0.87	0.988	-1.99	0.31	-1.97	0.416	-0.14	0.963
RAB3B	-0.29	0.905	0.41	0.979	-2.36	1	-1.51	0.921	-0.18	0.931	-2.05	0.219	0.07	0.976
pimt	-0.29	0.133	0.23	0.939	0.25	1	-0.14	0.989	0.53	0.101	0.18	0.7	0.18	0.667
ppce	-0.29	0.374	-0.04	0.996	-0.4	1	0.43	0.988	-0.52	0.643	-0.98	0.429	-1.96	0.0474
MYRIP	-0.29	0.727	1.07	0.861	-0.24	1	-1.05	0.575	1.06	0.269	1.52	0.168	0.67	0.543
PINK1	-0.29	0.246	0.03	0.99	0.18	1	0.1	0.989	-0.36	0.107	-0.26	0.355	0.15	0.592
PPIA	-0.29	0.28	-0.01	0.997	0.22	1	-0.01	0.999	0.44	0.12	0.36	0.313	0.2	0.554
CANT1	-0.3	0.339	-0.77	0.9	0	1	0.09	0.999	-1.65	0.0285	-0.81	0.402	-0.63	0.476
lap4a	-0.3	0.259	-0.06	0.982	0.11	1	-0.09	0.992	-0.04	0.911	0.08	0.842	-0.04	0.906
EPHB1	-0.3	0.811	-0.33	0.966	-0.29	1	-0.46	0.981	-0.89	0.293	-1.19	0.228	-0.39	0.715
al3a2	-0.31	0.0954	-0.45	0.965	-0.03	1	-0.98	0.952	-0.13	0.939	-0.87	0.566	-0.66	0.631
myod	-0.31	0.275	-0.07	0.979	-0.2	1	-0.83	0.972	1.49	0.235	1.37	0.38	-0.3	0.276
OGFR	-0.31	0.625	0.05	0.994	0.4	1	0.18	0.993	-0.06	0.937	-0.05	0.966	0.32	0.634

AP1S2	-0.31	0.822	0.08	0.993	-0.19	1	0.64	0.97	-0.84	0.368	-1.47	0.156	-1.01	0.289
mstn1	-0.32	0.491	0.27	0.939	0.02	1	-0.21	0.981	-0.15	0.76	-0.25	0.641	-0.19	0.696
angl1	-0.32	0.609	-0.6	0.951	-0.41	1	0.62	0.979	0.63	0.601	0.33	0.849	1.21	0.283
CCND1	-0.32	0.334	0.18	0.982	-0.44	1	0.01	0.999	-0.01	0.991	-0.26	0.827	-1.01	0.189
USP20	-0.33	0.602	-0.02	0.996	0.19	1	0.13	0.982	-0.27	0.316	0.06	0.902	0.09	0.789
EXOC7	-0.33	0.374	-0.7	0.939	-1.18	1	-0.09	0.999	-0.63	0.578	-1.19	0.34	0.3	0.828
gcg1	-0.33	0.881	-0.6	0.971	-0.84	1	0.48	0.993	-0.3	0.9	-0.73	0.784	1.24	0.539
THRB	-0.33	0.0508	0.53	0.939	0.56	1	-0.08	0.999	-1.11	0.144	-0.43	0.704	-1.05	0.192
PER2	-0.34	0.613	0.07	0.992	-0.17	1	0.05	0.999	-0.18	0.824	-0.03	0.98	-0.15	0.864
NCOA2	-0.34	0.163	0.1	0.982	0.2	1	-0.05	0.999	0.56	0.161	0.74	0.11	0.69	0.0934
FXYD6	-0.34	0.431	1.56	0.871	0.41	1	1.47	0.921	-0.23	0.909	-0.88	0.655	-0.49	0.204
GNPTG	-0.34	0.236	-0.31	0.855	0.32	1	-0.19	0.97	-0.42	0.0981	-0.04	0.937	-0.43	0.11
alat2	-0.35	0.0968	-0.01	0.997	-0.05	1	0.04	0.999	-0.3	0.558	0.17	0.815	0.22	0.71
anx11	-0.35	0.78	0.61	0.938	0.76	1	0.9	0.921	0.88	0.316	1.26	0.211	0.12	0.921
FUT7	-0.35	0.819	-0.42	0.957	-0.46	1	-0.28	0.993	-0.99	0.227	-0.63	0.57	-1.19	0.16
EHD3	-0.35	0.447	-0.14	0.971	-0.3	1	0.22	0.979	-0.95	0.287	-0.56	0.218	-1.28	0.0863
enoa	-0.35	0.309	-0.01	0.998	0.18	1	0.14	0.988	-0.25	0.438	-0.09	0.853	-0.54	0.0852
zn706	-0.35	0.223	-1.63	0.826	0.28	1	0.28	0.997	-0.07	0.969	0.34	0.874	0.07	0.973
rng2a	-0.36	0.252	0.38	0.953	-0.21	1	0.53	0.964	1.32	0.051	0.9	0.279	0.91	0.205
NPHP3	-0.36	0.365	-0.5	0.934	-0.19	1	-0.18	0.993	-1.16	0.000907	-0.3	0.512	-0.52	0.15
HPX	-0.36	0.118	1.48	0.697	1.29	1	-0.19	0.998	0.87	0.336	0.96	0.38	0.37	0.739
addb	-0.36	0.445	0.07	0.986	0.46	1	-0.07	0.999	0.41	0.324	0.45	0.367	-0.26	0.589
APOH	-0.36	0.0228	-0.06	0.98	0.24	1	0.19	0.961	0.41	0.0958	-0.27	0.373	-0.25	0.337
SDHA	-0.37	0.25	0.29	0.939	-0.11	1	0.25	0.998	0.32	0.821	0.19	0.761	0.2	0.696
KANSL3	-0.37	0.122	-0.37	0.962	0.1	1	0.03	0.999	-1.2	0.147	-0.63	0.584	0.06	0.962
zn235	-0.37	0.286	0.23	0.962	0.31	1	-0.25	0.984	0.38	0.497	0.14	0.868	0.35	0.568
DIO2	-0.37	0.849	0.1	0.994	-0.37	1	-0.31	0.995	-2.51	0.0362	-0.91	0.582	-1.43	0.274
NDC80	-0.37	0.299	-0.23	0.938	0.04	1	-0.33	0.93	-1.43	0.155	-1.19	0.347	-1.82	0.0807

MYH7	-0.37	0.76	1.77	0.873	-0.47	1	-1.93	0.257	-1.13	0.181	0.56	0.829	-0.67	0.496
ndka	-0.37	0.418	-0.18	0.959	0.1	1	0.28	0.97	-0.29	0.502	-0.16	0.794	-0.55	0.181
SND1	-0.37	0.186	-0.26	0.969	0.16	1	-0.36	0.981	-0.12	0.904	-0.06	0.965	-0.03	0.98
tcp4	-0.37	0.356	-1.04	0.803	-0.67	1	0.42	0.98	-0.59	0.464	-0.37	0.739	0.6	0.481
faah2a	-0.38	0.294	0.06	0.993	-0.04	1	0.05	0.999	-0.14	0.862	-0.22	0.82	-0.31	0.676
xrcc6bp1	-0.38	0.678	-0.12	0.971	-0.09	1	0.25	0.963	-0.48	0.138	-0.32	0.44	-0.25	0.503
PIF1	-0.38	0.877	-0.72	0.959	-0.28	1	-0.81	0.981	-1.57	0.304	-0.94	0.657	-0.23	0.917
TMEM160	-0.38	0.217	-0.63	0.977	0.13	1	0.03	0.999	-2.56	0.205	-2.43	0.328	-0.44	0.876
olfml3b	-0.38	0.217	-1.14	0.915	-0.97	1	1.48	0.887	-0.24	0.889	-0.06	0.981	1.56	0.236
lipia	-0.39	0.258	0.03	0.994	0.11	1	-0.36	0.892	-0.29	0.375	0.06	0.92	-0.26	0.471
LIPH	-0.39	0.258	0.03	0.994	0.11	1	-0.36	0.892	-0.29	0.375	0.06	0.92	-0.26	0.471
zgc:123244	-0.39	0.606	0.11	0.986	-0.09	1	-0.43	0.97	-0.44	0.499	-1.07	0.115	-0.87	0.157
sms1a	-0.39	0.894	-0.24	0.99	-0.23	1	0.99	0.979	0.66	0.755	-0.11	0.975	1.74	0.349
thas	-0.39	0.0645	0	0.998	0.23	1	0.17	0.956	-0.27	0.178	-0.11	0.717	-0.05	0.859
rpc7l	-0.4	0.206	-0.14	0.959	0.03	1	0.31	0.921	-0.45	0.122	-0.45	0.19	-0.24	0.469
si:ch211-235o23.1	-0.4	0.666	0.55	0.929	0.07	1	-1.42	0.424	-0.22	0.804	0.28	0.798	-0.84	0.259
fabp10a	-0.4	0.659	-0.01	0.999	-0.32	1	-0.32	0.986	-0.58	0.421	-1.52	0.0488	-0.65	0.39
mcp	-0.4	0.331	0	1	-0.11	1	-0.07	0.998	-0.52	0.143	-0.22	0.663	-0.03	0.95
APH1B	-0.41	0.0379	-0.35	0.672	-0.08	1	-0.1	0.983	-0.48	0.0104	-0.41	0.0533	-0.49	0.0106
rb39b	-0.41	0.559	0.21	0.927	0.01	1	-0.01	0.999	0.44	0.0768	0.43	0.148	0.21	0.466
PARPBP	-0.41	0.759	-0.78	0.927	-0.73	1	-0.39	0.989	-1.64	0.0724	-0.92	0.442	-0.19	0.888
m4a4a	-0.41	0.106	-0.37	0.965	0.92	1	-0.71	0.963	-1.06	0.248	-0.13	0.937	-1.4	0.134
nipblb	-0.41	0.119	1.13	0.77	1.04	1	-0.66	0.95	1.11	0.125	0.95	0.291	0.7	0.393
leg3	-0.42	0.505	-0.43	0.737	-0.11	1	0.07	0.995	0.08	0.827	0.12	0.763	0.01	0.984
PRR11	-0.42	0.768	-0.59	0.946	-0.53	1	-0.39	0.992	-1.31	0.179	-1.08	0.381	-0.93	0.395
zyg11	-0.42	0.145	-0.31	0.986	0.89	1	-2.2	0.815	1.97	0.23	1.03	0.658	0.56	0.795
LOC100194623	-0.42	0.352	0.47	0.855	0.36	1	0.45	0.896	0.05	0.936	-0.06	0.932	-0.59	0.151

ALDOA	-0.42	0.352	0.47	0.855	0.36	1	0.45	0.896	0.05	0.936	-0.06	0.932	-0.59	0.151
pm20d1.2	-0.43	0.152	-0.31	0.882	0.02	1	-0.01	0.999	-1.04	0.000358	-0.48	0.15	-0.35	0.243
gbp	-0.43	0.57	-0.52	0.925	-0.47	1	-0.2	0.993	-1.04	0.0811	-0.72	0.338	-0.3	0.702
ATP7A	-0.43	0.111	0.01	0.999	0.5	1	-1.05	0.961	0.12	0.773	-0.81	0.665	0.08	0.86
hig1a	-0.43	0.44	0.01	0.998	0.04	1	0.22	0.977	0.14	0.758	-0.03	0.968	-0.23	0.605
MEMO1	-0.44	0.0317	0.62	0.91	1.04	1	0.32	0.985	0.7	0.31	0.84	0.298	0.16	0.859
i13r2	-0.44	0.801	-0.25	0.977	-0.42	1	-0.23	0.993	-1.35	0.0845	-0.41	0.734	0.74	0.406
if2a	-0.44	0.0895	-0.1	0.991	-0.49	1	-0.25	0.993	-0.27	0.787	-0.16	0.915	-1.76	0.0307
PUS7	-0.44	0.828	0.15	0.914	0.15	1	0.1	0.977	-0.02	0.94	0.02	0.947	0.09	0.66
SCRIB	-0.45	0.139	0.3	0.897	0.27	1	0.09	0.993	-0.09	0.804	-0.06	0.909	-0.19	0.574
MXRA8	-0.45	0.091	0.01	0.999	-0.31	1	-0.2	0.993	0.84	0.198	0.7	0.399	1.08	0.109
spit2	-0.45	0.108	0.26	0.943	-0.02	1	0.23	0.98	-0.83	0.04	-0.86	0.0662	-1.01	0.0152
STK35	-0.45	0.339	-0.81	0.927	-0.62	1	-0.39	0.992	-1.48	0.122	-1.22	0.302	-1.51	0.134
ACVR1	-0.46	0.0772	-0.21	0.925	0.04	1	0.17	0.972	-0.3	0.238	-0.23	0.5	-0.22	0.449
ILF2	-0.46	0.374	-0.22	0.958	-0.28	1	0.02	0.999	-0.51	0.267	-0.79	0.128	-1.18	0.00934
ITPA	-0.46	0.2	-0.28	0.946	-0.24	1	0.25	0.981	-0.4	0.419	-0.35	0.57	-0.07	0.924
FMR1	-0.47	0.177	-0.2	0.958	0.07	1	-0.2	0.984	-1.02	0.0114	-0.53	0.286	-0.57	0.0824
CENPH	-0.47	0.274	0.05	0.994	-0.14	1	-0.02	0.999	1.23	0.0522	1.02	0.181	0.8	0.243
MGLL	-0.47	0.615	0.04	0.996	-0.17	1	0.36	0.984	-1.2	0.114	-0.77	0.407	0.07	0.951
ALDH4A1	-0.47	0.71	-0.16	0.984	0.08	1	0.25	0.993	-0.64	0.451	-1.13	0.233	-0.25	0.818
CHID1	-0.48	0.0187	-0.17	0.925	-0.11	1	-0.13	0.973	-0.43	0.0267	-0.51	0.021	-0.44	0.0294
SNX9	-0.48	0.0175	-0.55	0.869	-1	1	0.2	0.992	-0.85	0.0736	-0.77	0.178	0.25	0.678
rasf2	-0.48	0.263	0.08	0.984	-0.25	1	-0.24	0.977	-0.4	0.33	-0.41	0.415	-0.57	0.163
mfsd2ab	-0.48	0.466	0.28	0.957	0.29	1	-0.15	0.993	-1.08	0.0461	-0.22	0.799	-0.49	0.431
UBALD2	-0.48	0.478	0.08	0.99	0.09	1	-0.52	0.95	-1.07	0.0541	-1.02	0.123	0.05	0.955
wdr26b	-0.49	0.0568	-0.11	0.963	-0.01	1	-0.17	0.973	-0.31	0.25	-0.34	0.282	-0.42	0.121
foxo1a	-0.49	0.345	-0.16	0.971	0.2	1	-0.24	0.981	-0.63	0.162	-0.47	0.416	-0.44	0.386
GMDS	-0.49	0.857	-0.12	0.994	-0.05	1	1.73	0.933	-0.7	0.737	-0.66	0.805	-0.52	0.819

dok2	-0.49	0.431	-2.46	0.6	-0.08	1	-0.02	0.999	-1.76	0.161	-2.01	0.179	-0.43	0.481
ACOX3	-0.5	0.0132	0.89	0.872	0.82	1	-0.06	0.999	-0.13	0.912	-0.98	0.315	-0.69	0.442
cep83	-0.5	0.57	-0.82	0.859	-1.2	1	-0.39	0.981	-1.8	0.0102	-1.2	0.144	-0.75	0.33
RFX6	-0.51	0.833	2.44	0.753	0.04	1	3.14	0.43	2.7	0.0775	3.14	0.0778	2.18	0.182
WRB	-0.52	0.119	-0.13	0.962	-0.04	1	-0.05	0.999	-0.59	0.0558	-0.73	0.0396	-0.82	0.0113
CENPA	-0.52	0.706	-0.81	0.927	-0.27	1	-0.58	0.979	-1.16	0.256	-0.71	0.607	-1.07	0.326
zgc:153916	-0.53	0.0903	0.65	0.909	-0.08	1	-0.05	0.999	0.51	0.5	0.7	0.423	-0.16	0.872
RIMKLA	-0.53	0.553	0.07	0.993	0.36	1	-0.08	0.999	-1.39	0.0501	-0.77	0.403	-0.19	0.85
LRAT	-0.53	0.384	-0.4	0.932	-0.37	1	-0.48	0.95	-1.05	0.0435	-0.79	0.205	-1.14	0.0345
pgdh	-0.54	0.0722	-0.89	0.894	-1.53	1	-0.67	0.972	-0.8	0.462	-0.62	0.666	-0.62	0.611
act	-0.54	0.426	-0.42	0.935	-0.13	1	-1.15	0.443	-0.06	0.948	-0.54	0.47	-0.05	0.959
PGM1	-0.54	0.056	-0.72	0.95	-2.14	1	-1.98	0.685	-0.08	0.967	1.04	0.535	0.3	0.866
idhp	-0.54	0.282	0.13	0.99	-0.11	1	0.66	0.97	0.07	0.959	1.03	0.388	1.15	0.257
mald2	-0.54	0.0365	-0.33	0.826	0.1	1	-0.02	0.999	-0.79	0.00181	-0.66	0.018	-0.53	0.0377
MFSD5	-0.55	0.521	-0.1	0.959	0.06	1	0.12	0.977	-0.12	0.581	-0.28	0.246	0.14	0.555
folr1	-0.55	0.839	0.18	0.993	0.58	1	2.42	0.779	0.75	0.72	-0.61	0.827	0.25	0.923
CLCN3	-0.55	0.0292	-0.16	0.96	-0.15	1	-0.35	0.963	-0.81	0.856	-0.82	0.03	-0.23	0.561
DHRS1	-0.55	0.0109	-0.14	0.94	0.36	1	-1.01	0.856	-0.51	0.0118	-0.49	0.034	-0.31	0.148
kpyk	-0.55	0.382	-0.32	0.959	0.08	1	0.46	0.97	-0.19	0.83	-0.23	0.835	-1.34	0.0517
SC5D	-0.55	0.454	-0.37	0.945	-0.19	1	0.17	0.993	-0.77	0.212	-0.71	0.356	-1.93	0.00216
TMEM39B	-0.55	0.0032	-1.77	0.918	1.38	1	0.68	0.993	-2.02	0.319	-1.29	0.639	-0.62	0.814
moes	-0.56	0.218	0.64	0.867	1.12	1	0.53	0.95	0.75	0.18	0.01	0.992	-0.01	0.991
BAT1	-0.56	0.343	-0.38	0.938	0.05	1	-1.11	0.97	-0.69	0.891	-0.67	0.294	-0.92	0.0851
DHCR7	-0.56	0.458	-0.34	0.956	0.03	1	0.11	0.992	-0.82	0.194	-0.55	0.517	-1.24	0.0561
TNNI1	-0.56	0.82	1.46	0.915	-0.61	1	-0.1	0.999	-0.46	0.825	-0.4	0.885	-0.29	0.901
PGAM1	-0.57	0.142	-0.08	0.996	0.17	1	0.83	0.814	0.33	0.856	-1.58	0.343	-0.73	0.263
rl2	-0.57	0.113	0.57	0.885	0.24	1	0.52	0.948	0.29	0.643	0.63	0.346	0.41	0.519
kidins220b	-0.57	0.0586	0	1	0.1	1	-0.05	0.999	-0.07	0.891	-0.07	0.918	0.34	0.436

MCM3	-0.57	0.746	-0.27	0.983	-0.23	1	0.5	0.991	-1.32	0.3	0.22	0.542	-0.31	0.284
TTC17	-0.57	0.853	0.31	0.968	0.04	1	-1.3	0.682	0.5	0.594	-0.19	0.891	-1.99	0.344
slc44a5b	-0.58	0.26	-0.34	0.934	-0.05	1	-0.31	0.973	-1.09	0.0174	-0.59	0.292	-0.3	0.587
npm	-0.58	0.496	0.14	0.962	-0.1	1	-0.17	0.981	-0.25	0.471	-0.36	0.367	-0.44	0.192
PCBP2	-0.58	0.25	-0.28	0.965	0.16	1	-0.07	0.999	-0.65	0.368	-0.45	0.644	-1.1	0.122
tssp	-0.58	0.0358	0.14	0.986	-1.66	1	-0.18	0.996	-0.49	0.585	0.04	0.98	-1.18	0.157
COG2	-0.58	0.119	-0.18	0.959	0.19	1	0.33	0.981	0.65	0.303	0.43	0.606	1	0.112
TNNI2	-0.59	0.77	0.84	0.96	1.43	1	0.38	0.998	0.24	0.927	-0.37	0.906	0.2	0.941
ALDOB	-0.6	0.105	-0.31	0.919	0.15	1	0.14	0.961	-0.74	0.0309	-0.45	0.297	-0.55	0.126
mald3	-0.6	0.262	-0.05	0.993	0.07	1	0.15	0.993	-0.53	0.288	-0.43	0.499	-1.04	0.0351
NXF1	-0.6	0.19	0.25	0.971	0.42	1	-0.52	0.97	0.15	0.88	-0.19	0.873	-1.26	0.0841
GATM	-0.61	0.715	-0.24	0.985	0.51	1	1.48	0.864	-0.42	0.782	-0.86	0.606	0.8	0.583
RUFY2	-0.61	0.635	-0.02	0.998	-0.38	1	0.23	0.997	-1.05	0.305	-0.02	0.992	-0.05	0.977
mtus1a	-0.61	0.202	-0.39	0.972	-0.74	1	0.57	0.983	-0.53	0.698	-0.31	0.875	1.57	0.184
RRAGC	-0.61	0.0267	-0.23	0.974	-0.33	1	0.98	0.769	0.66	0.365	0.46	0.63	1.24	0.0796
atrap	-0.61	0.393	-0.31	0.957	0.28	1	-0.16	0.995	-0.59	0.36	-0.47	0.581	-0.2	0.812
STAT1	-0.61	0.291	0.15	0.993	0.11	1	-0.71	0.986	0.27	0.898	0.61	0.798	-0.47	0.815
TTPA	-0.62	0.315	0.12	0.987	0.86	1	-0.08	0.999	-0.21	0.815	0.01	0.993	-0.33	0.708
natd1	-0.62	0.428	-0.33	0.775	-0.12	1	-0.16	0.97	-0.4	0.0686	-0.21	0.454	0.03	0.915
TTK	-0.62	0.638	-0.54	0.954	-0.22	1	1.71	0.95	-1.35	0.18	-2.27	0.328	-0.73	0.547
TICRR	-0.62	0.573	-0.61	0.939	0.01	1	-0.61	0.95	-1.22	0.163	0.7	0.424	-1.09	0.243
glo2	-0.62	0.0102	1.31	0.898	-0.23	1	-0.01	0.999	0.72	0.632	-0.11	0.966	0.2	0.915
TMEM218	-0.62	0.0921	1.07	0.69	1.18	1	0.04	0.999	0.35	0.614	0.36	0.681	-0.27	0.723
med13b	-0.63	0.0254	0.3	0.898	0.14	1	-0.32	0.915	-0.15	0.659	0	0.996	-0.15	0.687
plcd	-0.63	0.403	0.34	0.956	-0.07	1	-0.17	0.995	-0.56	0.419	-0.6	0.478	-0.71	0.307
ASPDH	-0.63	0.603	0.14	0.989	-0.28	1	-1.74	0.542	-0.81	0.421	-0.29	0.857	-1.83	0.0579
ikaros	-0.64	0.375	0.7	0.869	0.26	1	-0.15	0.995	-0.01	0.992	-0.04	0.973	-1.01	0.116
IKZF1	-0.64	0.375	0.7	0.869	0.26	1	-0.15	0.995	-0.01	0.992	-0.04	0.973	-1.01	0.116

NUDT18	-0.64	0.426	-0.23	0.971	-0.14	1	0.01	0.999	-0.79	0.254	-0.72	0.402	-1.21	0.0842
LAMB1	-0.64	0.091	0.17	0.977	0.04	1	-0.31	0.907	-0.14	0.67	0.11	0.912	-0.21	0.524
MELK	-0.65	0.343	-0.38	0.967	-0.22	1	0.62	0.976	-1.6	0.0996	-0.85	0.513	-0.01	0.992
NAPRT	-0.65	0.166	-0.03	0.994	-0.05	1	0.3	0.971	-0.8	0.0655	-1.06	0.0338	-0.6	0.194
LOC100136751	-0.65	0.824	-0.17	0.993	-0.27	1	0.75	0.992	0.82	0.721	-0.83	0.777	1.36	0.543
UAP1L1	-0.65	0.261	-0.25	0.959	0.1	1	-0.04	0.999	-1.74	0.000923	-1.37	0.0192	-0.68	0.216
HEG1	-0.65	0.376	0.86	0.82	-0.15	1	2.24	0.779	0.5	0.652	-0.5	0.852	-0.55	0.447
HLF	-0.66	0.0488	0.33	0.957	0.02	1	0.27	0.975	-1.02	0.1	-0.39	0.483	-0.26	0.61
MCM10	-0.66	0.282	-0.32	0.949	-0.06	1	0.12	0.998	-0.62	0.266	-0.79	0.227	-0.44	0.491
trp-i	-0.66	0.897	-0.77	0.982	-1.17	1	1.01	0.993	-1.51	0.674	-1.36	0.772	0.3	0.95
ct054	-0.66	0.159	0.1	0.983	0.25	1	0.07	0.999	-0.56	0.196	-0.32	0.597	-0.17	0.756
boka	-0.66	0.235	-0.24	0.98	-0.15	1	-0.32	0.992	-0.61	0.536	-1.01	0.36	-1.16	0.212
TPM1	-0.66	0.349	0.24	0.966	0.19	1	0.06	0.999	-0.36	0.607	0.18	0.862	-0.31	0.687
HABP4	-0.67	0.0815	-0.47	0.959	0.13	1	0.32	0.993	-0.2	0.884	-0.22	0.9	0.6	0.616
RAD51	-0.67	0.502	-0.66	0.929	-0.48	1	-0.03	0.999	-1.05	0.202	-1.26	0.197	-1.54	0.0691
LDHA	-0.67	0.739	1.28	0.919	0.26	1	-0.45	0.993	-0.63	0.717	-1.57	0.382	-0.71	0.695
MSMO1	-0.68	0.26	-0.18	0.973	-0.03	1	0.2	0.992	-0.41	0.492	-0.25	0.765	-1.3	0.0192
urah	-0.68	0.626	0.04	0.992	-0.18	1	0.11	0.993	0.25	0.472	-0.32	0.445	0	0.999
glyg	-0.69	0.0219	-0.12	0.965	-0.13	1	-0.11	0.992	0.37	0.212	0.13	0.775	0.03	0.943
SRPK3	-0.69	0.127	-0.08	0.985	0.25	1	-0.18	0.989	-0.99	0.018	-0.63	0.212	-0.98	0.0232
PDIA3	-0.69	0.152	0.17	0.979	0.12	1	0.26	0.989	-0.74	0.306	-1.32	0.101	0.38	0.624
UBE2C	-0.7	0.693	-0.3	0.96	-0.37	1	-0.22	0.992	-0.73	0.257	-0.31	0.742	-0.18	0.837
prvb	-0.7	0.695	1.19	0.943	0.14	1	-1.3	0.892	-0.72	0.998	0.11	0.96	-0.56	0.69
DCTN2	-0.71	0.29	0.22	0.925	0.13	1	0.19	0.97	0.22	0.454	0.04	0.932	-0.21	0.509
CDC20	-0.71	0.512	-0.42	0.959	-0.26	1	-0.29	0.993	-1.09	0.222	-0.8	0.486	-0.62	0.554
slc25a25b	-0.71	0.205	-0.25	0.962	-0.32	1	0.24	0.991	0.2	0.785	0.24	0.787	-0.02	0.985
FKBP5	-0.71	0.021	0.9	0.959	-0.81	1	0.08	0.994	-0.42	0.147	-0.49	0.156	-0.77	0.0106

PRDX6	-0.71	0.146	-0.23	0.957	-0.03	1	-0.03	0.999	-0.48	0.312	-0.41	0.493	-1.07	0.0218
CRK	-0.71	0.246	-0.16	0.977	0.12	1	0.76	0.981	-2.03	0.154	-0.28	0.722	-0.1	0.896
pkha1	-0.72	0.508	-0.12	0.976	0.34	1	0.01	0.999	-0.02	0.972	0.13	0.836	-0.13	0.795
SCRN2	-0.72	0.162	-0.07	0.979	0.03	1	-0.05	0.999	-0.48	0.0492	-0.44	0.128	-0.4	0.122
rilpl2	-0.72	0.414	-1.86	0.295	-0.16	1	-0.49	0.973	-1.56	0.0332	-0.62	0.537	-1.02	0.194
RDH8	-0.73	0.053	0.14	0.967	0.09	1	0.09	0.995	-0.62	0.0793	-0.4	0.381	-0.04	0.947
ox2g	-0.73	0.637	-0.77	0.943	-0.64	1	-0.93	0.963	-1.71	0.145	-0.97	0.542	-1.1	0.408
DTX3L	-0.73	0.265	0.49	0.927	0.28	1	-0.36	0.977	-0.47	0.468	1.44	0.177	-1.21	0.0468
htra1b	-0.73	0.422	0.8	0.892	0.44	1	-0.42	0.981	-0.43	0.628	-0.44	0.693	-1.29	0.107
alcama	-0.74	0.0307	-0.03	0.997	0.27	1	0.03	0.999	0.94	0.29	1.08	0.308	0.61	0.557
NR1H4	-0.74	0.123	1.44	0.855	0.63	1	0.95	0.963	-0.92	0.853	-0.85	0.102	-0.84	0.0702
syfb	-0.74	0.0921	-0.29	0.936	-0.3	1	0.13	0.993	-0.23	0.617	-0.24	0.683	-0.22	0.662
aplnra	-0.75	0.0327	0.93	0.569	0.74	1	0.01	0.999	-0.63	0.158	-0.02	0.981	-0.8	0.0839
apja	-0.75	0.0327	0.93	0.569	0.74	1	0.01	0.999	-0.63	0.158	-0.02	0.981	-0.8	0.0839
PAR2	-0.75	0.0292	-0.05	0.988	0.05	1	-0.09	0.993	-1.21	0.000358	-0.77	0.0387	-0.71	0.0337
DACT2	-0.75	0.104	-0.19	0.962	-0.21	1	-0.16	0.992	-0.32	0.495	-1.18	0.0153	-0.44	0.362
phS	-0.75	0.384	-0.06	0.988	-0.05	1	0.24	0.971	-0.09	0.845	-0.05	0.941	-0.45	0.235
ctrb	-0.75	0.899	-1.2	0.971	-0.63	1	0.83	0.995	-0.67	0.888	-1.3	0.811	0.04	0.994
p2ry1	-0.76	0.4	-0.85	0.881	-0.38	1	-0.6	0.964	-1.24	0.106	-0.73	0.469	-0.36	0.718
dpod1	-0.76	0.463	-0.5	0.952	-0.47	1	0.57	0.973	-0.94	0.297	-1.26	0.224	-1.07	0.245
si:dkey-46a10.3	-0.76	0.0192	-0.09	0.979	-0.23	1	-0.03	0.999	-0.52	0.0895	-0.49	0.18	-0.66	0.0375
ASCC3	-0.77	0.236	-0.17	0.979	0.29	1	0.25	0.856	-0.56	0.375	-0.08	0.799	-0.4	0.572
CDK2	-0.77	0.373	-0.19	0.982	-0.53	1	0.2	0.994	-1.02	0.168	-1.2	0.171	-0.9	0.259
CTC1	-0.77	0.191	0.62	0.91	0.85	1	2.3	0.953	0.62	0.374	0.54	0.539	0.98	0.773
ercc6l	-0.77	0.47	-1.01	0.869	-0.61	1	-0.62	0.97	-1.48	0.0943	-1.11	0.312	-1.3	0.164
amotl2a	-0.78	0.0654	-0.34	0.915	-0.22	1	0.4	0.921	0.22	0.602	0.17	0.761	0.3	0.49
HEXA	-0.78	0.0789	-0.12	0.979	0.08	1	-0.2	0.985	-0.85	0.0414	-0.57	0.26	-1.1	0.0105

TMEM200A	-0.78	0.429	-0.28	0.972	-0.4	1	-0.23	0.993	-0.38	0.706	0	0.998	-1.29	0.138
mgt5b	-0.78	0.25	0.61	0.902	-0.03	1	-0.11	0.999	0.03	0.977	-0.27	0.78	-0.78	0.234
tdo2a	-0.79	0.052	-0.64	0.855	-0.41	1	-0.83	0.689	-1.34	0.0102	-1.08	0.0704	-0.96	0.0761
VDAC2	-0.79	0.0652	0.07	0.966	0.17	1	-0.32	0.961	-0.2	0.3	-0.22	0.719	-0.64	0.132
s12a1	-0.79	0.58	1.94	0.695	0.98	1	1.35	0.886	0.34	0.817	0.15	0.946	-1.41	0.241
olm2a	-0.8	0.397	0.25	0.977	0.09	1	-0.16	0.999	-1.25	0.116	-0.8	0.443	-0.54	0.578
asic1	-0.81	0.429	1.02	0.855	0.8	1	0.22	0.995	0.33	0.762	1.06	0.322	-0.55	0.604
PPIL2	-0.81	0.365	-0.14	0.939	0.05	1	0.04	0.998	-0.11	0.643	-0.01	0.978	0.09	0.74
CPLX1	-0.82	0.299	-0.04	0.996	0.25	1	0.35	0.984	-1.04	0.136	-0.96	0.259	-0.83	0.267
PYY	-0.82	0.725	-0.36	0.984	-1	1	0.4	0.997	-0.51	0.828	-1.35	0.58	1.52	0.454
P4HA1	-0.82	0.0234	0.35	0.957	0.51	1	0.16	0.971	1.1	0.107	0.43	0.122	1.1	0.124
AZIN1	-0.83	0.462	0.67	0.939	-1.07	1	-0.18	0.999	0.45	0.695	0.4	0.791	-0.56	0.632
hmdh	-0.83	0.544	-0.09	0.976	-0.01	1	0.11	0.992	0.11	0.746	-0.25	0.492	-0.03	0.946
MMS22L	-0.83	0.28	-0.23	0.973	-0.35	1	-0.33	0.986	-1.23	0.0682	-0.98	0.232	-1.54	0.028
RAD21	-0.84	0.234	-0.57	0.919	-0.21	1	-0.42	0.973	-1.14	0.0721	-0.7	0.389	-0.32	0.694
eif4ebp3l	-0.84	0.00685	-0.14	0.979	0.16	1	0.26	0.981	-0.41	0.415	-0.24	0.738	-0.32	0.568
SLC40A1	-0.85	0.057	-0.42	0.902	0.04	1	-0.43	0.941	-1.12	0.0087	-0.66	0.193	-0.82	0.0626
pptc7a	-0.85	0.0192	-0.06	0.989	0.02	1	0.17	0.992	-1.06	0.0114	-1.13	0.0183	-0.97	0.0252
CISH	-0.85	0.218	-0.09	0.988	-0.04	1	-0.08	0.999	0.77	0.178	0.51	0.507	0.65	0.301
5ntc	-0.85	0.0558	-0.35	0.841	-0.31	1	-0.12	0.989	-0.63	0.0199	-0.46	0.152	-0.17	0.602
rtkn2a	-0.85	0.559	-0.63	0.954	-0.45	1	-0.42	0.992	-1.81	0.117	-1.38	0.339	-1.14	0.381
RTKN2	-0.85	0.559	-0.63	0.954	-0.45	1	-0.42	0.992	-1.81	0.117	-1.38	0.339	-1.14	0.381
ZNF326	-0.85	0.452	0.21	0.983	0.45	1	-0.49	0.982	0.56	0.61	-0.49	0.73	-1.69	0.0836
si:ch73-141c7.1	-0.86	0.053	-0.21	0.949	0	1	-0.12	0.993	-0.23	0.57	-0.31	0.513	-0.41	0.293
tm186	-0.86	0.166	0.24	0.962	0.48	1	-0.25	0.989	-1.3	0.022	-0.33	0.691	-0.68	0.271
abhd2b	-0.86	0.257	-0.56	0.9	-0.48	1	-0.41	0.97	-0.2	0.779	0.42	0.583	0.37	0.594
znrf2b	-0.86	0.0843	-0.38	0.929	-0.07	1	-0.22	0.988	-0.67	0.161	-0.41	0.515	-0.07	0.922

dcam	-0.86	0.0843	0.17	0.97	-0.43	1	-0.16	0.993	-1.02	0.028	-1.12	0.0351	-1.35	0.00465
ef2	-0.86	0.000205	0.81	0.588	0.29	1	-0.38	0.95	0.6	0.146	0.2	0.749	0.1	0.863
nrxn1a	-0.87	0.155	-0.78	0.813	0.11	1	-0.29	0.983	-0.77	0.172	-0.49	0.514	-1.28	0.0279
tm56b	-0.88	0.0748	-0.37	0.932	-0.18	1	-0.08	0.999	-0.94	0.0504	-0.16	0.843	0.23	0.712
KLHL26	-0.88	0.0699	-0.26	0.953	0.1	1	-0.5	0.919	-1.11	0.0149	-0.85	0.115	-0.94	0.0482
CDCA8	-0.88	0.445	-0.52	0.955	-0.39	1	-0.46	0.985	-1	0.321	-0.9	0.476	-1.32	0.197
CCDC61	-0.89	0.289	-0.3	0.966	-0.34	1	-0.31	0.991	-0.29	0.754	0.04	0.979	-1.31	0.0908
CEP72	-0.89	0.496	-0.53	0.958	-0.21	1	-0.61	0.98	-1.79	0.0923	-0.65	0.674	-1.44	0.207
CALCA	-0.9	0.413	0.71	0.932	-1.17	1	-0.48	0.983	0.52	0.636	-0.35	0.816	-0.45	0.704
THYN1	-0.9	0.198	-0.34	0.956	-0.17	1	0.2	0.993	-1.16	0.068	-0.77	0.336	-0.78	0.257
tmx2b	-0.9	0.00502	0.02	0.997	0.35	1	0.13	0.997	-1.26	0.0309	-1.34	0.0473	-0.78	0.216
PPP1R35	-0.9	0.386	-0.53	0.947	-0.37	1	-0.53	0.977	-1.23	0.166	-0.7	0.563	-1.24	0.186
SGK1	-0.9	0.166	-0.47	0.93	-0.3	1	-0.27	0.988	-1.02	0.0876	-0.82	0.26	-1.54	0.013
NUSAP1	-0.9	0.453	-0.38	0.982	-0.25	1	-1.17	0.961	-1.51	0.324	-1.07	0.597	-1.55	0.335
ZWILCH	-0.91	0.496	-0.04	0.997	-0.01	1	-0.27	0.995	-1.09	0.344	-1.26	0.364	-1.8	0.116
MVP	-0.92	0.0241	0.04	0.996	-0.12	1	-0.61	0.95	1.39	0.0317	0.81	0.315	0.16	0.86
TLR3	-0.92	0.289	-0.07	0.994	0.41	1	-0.11	0.999	-0.52	0.552	-0.23	0.86	-0.29	0.774
glpc	-0.92	0.244	1.1	0.953	-0.85	1	-0.71	0.992	2.01	0.0283	2.12	0.0444	-0.76	0.767
TAL1	-0.93	0.0847	0.97	0.902	0.37	1	0.53	0.981	0.56	0.623	0.03	0.99	-0.29	0.828
pe2r4	-0.93	0.246	0.66	0.943	0.91	1	1.23	0.892	0.88	0.441	1.44	0.263	1.33	0.237
ki67	-0.93	0.349	-0.45	0.957	-0.07	1	-0.6	0.97	-1.15	0.184	-0.78	0.496	-1.12	0.222
P2RX4	-0.94	0.0315	-0.31	0.934	0.13	1	-0.2	0.985	-1.11	0.00781	-0.99	0.0369	-0.67	0.123
md2l1	-0.95	0.445	-0.55	0.956	-0.42	1	-0.25	0.996	-1.37	0.193	-0.95	0.491	-1.45	0.189
tmem182a	-0.95	0.318	-0.12	0.989	0.17	1	-0.35	0.99	-1.39	0.0924	-0.05	0.975	-1.88	0.0282
tm182	-0.95	0.318	-0.12	0.989	0.17	1	-0.35	0.99	-1.39	0.0924	-0.05	0.975	-1.88	0.0282
SLC25A34	-0.96	0.266	0.25	0.953	0.14	1	0.23	0.982	0.08	0.902	0.23	0.723	-0.15	0.803

CDKN3	-0.96	0.376	-0.44	0.96	-0.31	1	-0.61	0.973	-1.06	0.271	-0.94	0.435	-1.11	0.27
cry-dash	-0.97	0.206	-0.16	0.983	-0.29	1	0	1	-1.28	0.0653	-0.89	0.299	-0.48	0.573
gsh1	-0.97	0.0938	-0.27	0.967	0.08	1	0.72	0.921	0.39	0.621	-0.32	0.765	-0.63	0.42
nAChRa9	-0.98	0.148	0.78	0.852	0.08	1	-0.04	0.999	0.61	0.357	0.14	0.902	-1.01	0.124
phospho1	-0.99	0.0146	0.07	0.986	0.16	1	0.32	0.958	-1.21	0.00214	-0.88	0.0458	-0.8	0.0442
phop1	-0.99	0.0146	0.07	0.986	0.16	1	0.32	0.958	-1.21	0.00214	-0.88	0.0458	-0.8	0.0442
PPIB	-0.99	0.0953	0.58	0.956	0.7	1	0.85	0.963	0.79	0.508	1.14	0.409	0.97	0.429
PARP3	-0.99	0.758	0.76	0.949	0.92	1	-0.22	0.999	0.44	0.792	-0.82	0.661	1.37	0.337
ctl2	-0.99	0.00174	0.2	0.949	0.15	1	-0.04	0.999	-0.07	0.878	-0.07	0.904	-0.01	0.984
rir1	-0.99	0.148	-0.12	0.988	-0.17	1	-0.27	0.992	-0.23	0.819	-0.07	0.962	-0.24	0.816
RRM1	-0.99	0.148	-0.4	0.943	-0.5	1	-0.13	0.998	-1.33	0.0346	-1.08	0.148	-1.41	0.0301
LONP2	-0.99	0.291	-0.38	0.961	-0.7	1	-0.83	0.941	-0.17	0.882	-0.15	0.923	-0.4	0.711
hgfa	-1	0.122	0.58	0.909	0.3	1	-0.14	0.997	-0.3	0.683	-0.14	0.897	-0.41	0.575
slc20a1a	-1	0.11	-0.15	0.983	0.07	1	0.17	0.995	-0.12	0.9	-0.47	0.614	-0.77	0.291
s20aa	-1	0.11	-0.15	0.983	0.07	1	0.17	0.995	-0.12	0.9	-0.47	0.614	-0.77	0.291
COBL	-1	0.0072	0.12	0.971	0.38	1	0.28	0.957	-0.83	0.0108	-0.33	0.433	-0.53	0.117
rs17	-1	0.131	-0.01	0.994	0.1	1	0.09	0.983	0.17	0.352	0.22	0.287	-0.03	0.901
TTLL3	-1.01	0.171	0.29	0.963	0.03	1	0.23	0.993	-0.08	0.942	-0.09	0.946	-0.36	0.675
tmem106bb	-1.01	0.105	0.12	0.98	0.17	1	-0.21	0.988	0.32	0.521	0.38	0.528	0.31	0.566
KLHL6	-1.01	0.411	0.4	0.94	0.5	1	0.07	0.999	0.54	0.4	0.35	0.687	0.24	0.76
s38a3	-1.01	0.0293	0.63	0.897	0.37	1	-0.47	0.964	-0.35	0.616	0.14	0.896	0.19	0.818
GLE1	-1.01	0.539	1.42	0.885	-0.86	1	0.21	0.993	1.27	0.367	1.52	0.363	0.31	0.687
CXCR4	-1.02	0.12	0.44	0.921	0.36	1	-0.09	0.999	-0.9	0.142	-0.2	0.841	-0.35	0.65
sh21a	-1.02	0.0216	0.24	0.952	-0.06	1	-0.33	0.964	-0.31	0.503	-0.52	0.322	-0.73	0.0952
thbs3a	-1.03	0.0708	0.91	0.707	0.64	1	-0.39	0.97	-0.23	0.727	-0.26	0.751	0.19	0.784
adb4c	-1.03	0.166	-0.06	0.994	-0.4	1	-0.32	0.988	0.18	0.846	-0.3	0.778	-0.91	0.212

rir2	-1.03	0.184	-0.04	0.991	-0.08	1	-0.28	0.956	0.25	0.497	0.18	0.714	0.01	0.986
shrprbck1r	-1.03	0.175	-0.2	0.979	0.02	1	-0.7	0.941	-0.33	0.698	-0.33	0.761	-1.05	0.154
OBFC1	-1.03	0.242	-0.37	0.96	-0.66	1	-0.13	0.999	-1.49	0.0568	-1.4	0.131	-1.13	0.178
BUB1B	-1.03	0.384	-0.79	0.93	-0.58	1	-0.51	0.983	-1.64	0.104	-1.41	0.25	-1.29	0.231
SASS6	-1.03	0.377	-0.86	0.925	-0.47	1	-0.27	0.993	-1.21	0.235	-1.13	0.366	-1.44	0.171
RRM2	-1.03	0.184	-0.35	0.959	-0.35	1	-0.32	0.988	-1.19	0.0937	-1.07	0.21	-1.5	0.0405
DENND5B	-1.03	0.0988	-0.04	0.993	0.93	1	0.09	0.999	-0.36	0.591	-0.23	0.809	-0.19	0.809
LGI1	-1.03	0.298	-1.16	0.826	-0.34	1	0.07	0.999	-0.2	0.871	-0.95	0.399	-0.37	0.743
rs15	-1.04	0.115	0.53	0.919	0.11	1	0	1	0.3	0.669	0.23	0.804	-0.6	0.354
gnai3	-1.05	0.0742	-0.07	0.963	0.19	1	-0.4	0.97	-0.15	0.773	-0.18	0.361	-0.42	0.522
RNF13	-1.05	0.111	0.04	0.987	0.26	1	-0.07	0.993	-0.23	0.33	0.02	0.966	0.1	0.733
cahz	-1.05	0.112	0.36	0.95	-0.37	1	-0.19	0.993	-0.01	0.991	-0.54	0.517	-0.87	0.183
PHYHD1	-1.05	0.251	-0.05	0.99	0.19	1	0	0.999	-0.4	0.297	-0.24	0.64	-0.51	0.196
BORA	-1.05	0.504	-0.78	0.945	-0.83	1	-0.61	0.986	-1.48	0.261	-1.58	0.32	-1.45	0.297
djc17	-1.06	0.337	-0.28	0.781	-0.15	1	0.06	0.993	0.03	0.92	0.1	0.712	0.01	0.97
ATP5E	-1.06	0.0168	0.39	0.917	0.38	1	-0.16	0.995	-0.18	0.735	-0.67	0.426	-0.46	0.312
nar5	-1.06	0.561	0.41	0.979	-1.37	1	-1.08	0.97	0.19	0.928	-1.02	0.61	-0.67	0.717
CRIP2	-1.06	0.0336	-0.24	0.958	0.15	1	0.09	0.999	-1.38	0.00427	-0.87	0.117	-0.86	0.0827
mic1	-1.07	0.549	0.23	0.932	-0.03	1	-0.03	0.999	-0.05	0.905	-0.02	0.97	-0.03	0.945
RPL32	-1.07	0.126	0	0.998	0.02	1	0.1	0.987	0.06	0.838	0.13	0.683	-0.04	0.883
KATNAL1	-1.08	0.116	0.1	0.988	0.01	1	-0.3	0.988	-0.08	0.932	-0.16	0.885	-0.07	0.945
BCL6	-1.08	0.191	0.54	0.672	0.4	1	-1.05	0.837	-0.23	0.471	0.06	0.914	-0.43	0.161
phyhiplb	-1.08	0.181	0.69	0.915	-0.51	1	-0.51	0.971	-0.06	0.955	-0.6	0.551	-0.71	0.394
ud2a2	-1.08	0.078	-0.04	0.989	0.24	1	0	1	-0.48	0.0717	-0.11	0.796	-0.11	0.759
FHL1	-1.08	0.0876	-1.12	0.69	0.73	1	1.03	0.977	-2.95	0.0768	-0.6	0.285	-0.66	0.177
rn151	-1.09	0.166	0.28	0.968	-0.5	1	-0.43	0.979	-0.39	0.648	-0.84	0.359	-0.99	0.199
nkd2b	-1.09	0.357	0.26	0.982	0.62	1	-0.04	0.999	-0.59	0.617	0.34	0.843	-1.08	0.336
RPL35A	-1.11	0.0395	0.48	0.915	0.77	1	0.28	0.981	-0.4	0.485	-0.34	0.639	0.03	0.972

DNAL4	-1.11	0.113	0.2	0.979	0.3	1	-0.01	0.999	0.06	0.955	-0.31	0.772	0.09	0.933
SUPT6H	-1.11	0.563	-0.55	0.971	0.1	1	0.25	0.97	0.3	0.412	-0.06	0.919	0.28	0.474
hoxb5a	-1.12	0.185	0.79	0.898	0.3	1	0.1	0.999	0.23	0.825	0.17	0.903	-0.36	0.719
pi15a	-1.12	0.0382	0.6	0.859	-0.29	1	-0.53	0.935	0.03	0.966	-0.51	0.444	-1.38	0.00934
AGTR2	-1.13	0.00041	0.36	0.851	0.14	1	-0.21	0.97	-0.26	0.402	-0.28	0.465	-0.86	0.00413
CDKL1	-1.13	0.13	-0.05	0.995	0.43	1	-0.18	0.995	-1.32	0.0545	-0.88	0.306	-0.63	0.426
acy3.1	-1.13	0.208	0.1	0.991	0.26	1	-0.2	0.995	-1.81	0.0262	-1.51	0.115	-1.01	0.249
mical2b	-1.13	0.0187	-0.27	0.947	-0.15	1	-0.21	0.987	-0.86	0.056	-0.55	0.33	-1.07	0.022
LOC100136153	-1.14	0.134	0.3	0.969	0.36	1	0.31	0.992	-0.54	0.553	-0.89	0.384	-0.66	0.479
p4k2a	-1.14	0.095	0.38	0.948	0.57	1	-0.21	0.993	0.14	0.877	-0.14	0.903	-0.95	0.159
fa60a	-1.14	0.242	0	0.999	-0.17	1	-0.15	0.993	-0.37	0.448	-0.25	0.709	-0.08	0.91
ZSWIM7	-1.14	0.206	-0.31	0.969	-0.2	1	-0.7	0.956	-0.12	0.922	-0.16	0.916	-1.09	0.214
DDIT4	-1.14	0.244	0.14	0.973	0	1	-0.06	0.999	-0.48	0.249	0.08	0.918	-0.35	0.446
PON2	-1.15	0.362	-0.04	0.988	0.11	1	0.19	0.966	-0.24	0.357	-0.03	0.95	0.03	0.931
CREM	-1.15	0.408	-0.2	0.953	-0.19	1	-0.15	0.99	-0.21	0.611	-1.64	0.359	-2.5	0.088
STX11	-1.16	0.0192	-0.32	0.939	-0.28	1	-0.3	0.976	-0.66	0.159	-0.68	0.227	-0.41	0.44
PEBP1	-1.16	0.0394	0.09	0.987	0.33	1	-0.31	0.98	-0.59	0.291	-0.66	0.324	-0.62	0.281
PMF1	-1.16	0.173	-0.52	0.941	-0.53	1	-0.06	0.999	-1.2	0.122	-0.77	0.453	-0.81	0.351
DALRD3	-1.17	0.0647	0.56	0.914	0.32	1	-0.29	0.985	-0.53	0.41	-0.11	0.918	-0.21	0.786
fa10	-1.17	0.0127	-0.1	0.958	0.18	1	-0.24	0.871	-0.25	0.219	-0.08	0.793	-0.01	0.963
tf2l1	-1.17	0.128	0.71	0.902	0.61	1	0.84	0.894	-0.66	0.393	-0.46	0.655	-1.02	0.179
LOC100136778	-1.18	0.17	-0.02	0.993	0.1	1	-0.01	0.999	0.06	0.761	0.03	0.919	0.13	0.5
k1c18	-1.18	0.17	-0.02	0.993	0.1	1	-0.01	0.999	0.06	0.761	0.03	0.919	0.13	0.5
HAUS2	-1.18	0.0806	-0.08	0.991	-0.1	1	-0.19	0.993	-0.3	0.696	-0.58	0.494	-0.59	0.412
RPL37	-1.18	0.284	-0.26	0.98	-0.24	1	-1.53	0.695	-0.04	0.979	0.18	0.92	-0.39	0.759
rl37	-1.18	0.284	-0.26	0.98	-0.24	1	-1.53	0.695	-0.04	0.979	0.18	0.92	-0.39	0.759

REEP2	-1.19	0.221	-0.27	0.956	-0.23	1	-0.02	0.999	-0.35	0.537	-0.27	0.724	0.04	0.96
CCNB3	-1.19	0.4	-0.86	0.938	-0.75	1	-1.27	0.921	-1.37	0.268	-1.1	0.486	-1.73	0.173
SSNA1	-1.2	0.0605	-0.11	0.987	0.05	1	-0.18	0.993	-0.36	0.608	-0.46	0.578	-0.64	0.337
CENPP	-1.2	0.293	-0.47	0.96	-0.12	1	-0.23	0.996	-1.02	0.336	-1.04	0.428	-1.33	0.215
ZNF704	-1.21	0.239	0.07	0.994	-0.26	1	-0.14	0.999	-0.48	0.665	-0.51	0.709	-1.14	0.25
MIS12	-1.21	0.362	-0.44	0.968	-0.37	1	-0.52	0.988	-1.87	0.101	-1.03	0.499	-1.62	0.18
LSM7	-1.22	0.175	0.11	0.965	0.07	1	0.23	0.956	-0.1	0.757	-0.07	0.869	-0.23	0.455
RAB14	-1.22	0.112	-0.05	0.988	-0.02	1	0.08	0.995	-0.33	0.344	-0.33	0.43	0.11	0.806
alb1	-1.22	0.0843	-0.28	0.963	0.24	1	-0.26	0.99	-0.92	0.166	-0.81	0.329	-0.52	0.507
CENPN	-1.22	0.308	-0.62	0.949	-0.54	1	-0.37	0.993	-1.65	0.117	-1.09	0.422	-1.54	0.166
ptmaa	-1.23	0.366	0	0.999	0.1	1	0.12	0.98	-0.37	0.0653	-0.02	0.971	-0.01	0.97
MEOX1	-1.23	0.0482	-0.1	0.987	-0.3	1	-0.01	0.999	-1.34	0.0222	-1.8	0.00663	-0.35	0.636
CENPL	-1.23	0.237	-0.62	0.942	-0.05	1	-0.95	0.938	-1.48	0.112	-0.8	0.521	-1.12	0.267
upp	-1.23	0.111	-0.06	0.985	-0.1	1	0.11	0.992	0.41	0.169	0.21	0.607	0.19	0.614
CEP135	-1.24	0.407	-0.72	0.953	-0.76	1	-0.85	0.973	-1.56	0.227	-1.27	0.171	-1.69	0.0341
erp27	-1.24	0.502	-1.52	0.9	0.98	1	-0.05	0.999	-0.03	0.99	1.09	0.604	0.52	0.798
ndub4	-1.25	0.388	-0.01	0.999	0.87	1	0.03	0.999	-0.57	0.707	-0.42	0.835	-0.93	0.525
lce	-1.25	0.801	-0.68	0.984	-0.8	1	-0.06	0.999	-1.25	0.761	-1.03	0.849	-0.91	0.841
cnd3	-1.25	0.263	-0.62	0.943	-0.42	1	-0.57	0.98	-1.58	0.114	-1.14	0.367	-1.38	0.194
HSPB1	-1.25	0.387	0.28	0.945	-0.11	1	-1.38	0.909	-1.31	0.306	0.08	0.927	-1.03	0.467
hbb1	-1.27	0.0655	0.45	0.939	-0.37	1	-0.32	0.984	-0.01	0.995	-0.62	0.464	-1.02	0.134
rnasel3	-1.28	0.201	-0.22	0.982	0.21	1	0.26	0.993	-0.11	0.935	0.01	0.997	0.45	0.701
rl14	-1.28	0.0586	-0.04	0.996	0.15	1	0.44	0.97	-0.11	0.891	-0.38	0.65	-0.4	0.576
ODF3B	-1.28	0.447	-0.49	0.971	-0.29	1	-0.48	0.993	-0.56	0.751	-1.23	0.515	-1.41	0.368
UBE2T	-1.28	0.0843	-0.31	0.962	-0.27	1	0.35	0.977	-1.2	0.0823	-1.01	0.228	-1.69	0.0185
GAPDH	-1.28	0.469	-3.62	0.781	2.07	1	1.36	0.98	-1.79	0.229	-1.8	0.324	-0.87	0.633
LOC100136769	-1.29	0.0727	0.52	0.931	-0.21	1	-0.22	0.993	0	0.998	-0.53	0.567	-0.9	0.215

ELK3	-1.29	0.0279	-0.2	0.915	0.11	1	-0.08	0.992	-0.18	0.448	-0.22	0.435	-0.08	0.78
VMP1	-1.29	0.00458	-0.4	0.925	-0.18	1	0.24	0.982	-0.49	0.315	-0.44	0.465	-0.76	0.116
sphm	-1.29	0.382	-0.03	0.993	0.34	1	0.01	0.999	-0.31	0.32	0.01	0.983	0.36	0.25
hyep	-1.29	0.28	-0.04	0.988	0.01	1	0.03	0.999	-0.1	0.778	-0.2	0.606	-0.28	0.367
PCID2	-1.3	0.383	0.03	0.991	-0.41	1	-0.16	0.97	0.05	0.86	-0.41	0.124	-0.22	0.38
dcup	-1.3	0.0497	-0.02	0.997	0.66	1	-0.05	0.999	-0.16	0.85	-0.64	0.429	-1.07	0.101
tr-alpha	-1.31	0.0718	-0.18	0.977	0.2	1	-0.22	0.992	-1.03	0.0732	-0.71	0.327	-0.53	0.42
cdn2b	-1.31	0.101	0.55	0.936	-0.27	1	-0.28	0.992	-0.13	0.901	-1.04	0.251	-1.07	0.177
tbx6l	-1.31	0.107	0.34	0.962	-0.05	1	-0.56	0.97	0.47	0.593	-0.17	0.901	-1.44	0.0656
ADRA2C	-1.33	0.785	0.83	0.982	-0.11	1	-0.74	0.997	0.97	0.822	-0.01	0.999	-0.72	0.879
NUF2	-1.33	0.212	-0.73	0.933	-0.35	1	-0.34	0.993	-1.42	0.143	-1.04	0.401	-1.44	0.16
SLC25A24	-1.34	0.00552	0.41	0.916	0.07	1	0.03	0.999	-0.28	0.582	-0.5	0.382	-0.37	0.479
rasgrf2b	-1.35	0.106	0.01	0.997	-0.17	1	0.06	0.999	-0.81	0.0459	-0.18	0.779	-0.1	0.867
tm45b	-1.35	0.19	0.22	0.982	0.29	1	0.12	0.999	0.04	0.978	-0.69	0.594	-1.09	0.285
TMOD4	-1.35	0.0386	0.38	0.945	-0.3	1	-0.3	0.985	-0.14	0.872	-0.7	0.379	-1.11	0.0867
AURKB	-1.36	0.385	-0.87	0.929	-0.75	1	-0.44	0.992	-1.67	0.127	-1.42	0.294	-1.51	0.195
CCNA2	-1.37	0.273	-0.63	0.953	-0.49	1	-0.63	0.98	-1.51	0.176	-1.19	0.406	-1.25	0.303
GATC	-1.38	0.0685	-0.42	0.81	-0.02	1	-0.26	0.953	-0.13	0.902	-0.33	0.768	0.37	0.691
ap2a	-1.38	0.275	0.31	0.936	0.09	1	0.24	0.981	-0.14	0.795	0.07	0.93	-0.08	0.905
SWI5	-1.38	0.0107	-0.17	0.973	-0.22	1	-0.5	0.941	-0.43	0.431	-0.27	0.725	-1.35	0.00985
vig1	-1.38	0.383	-0.07	0.996	0.22	1	-1.1	0.961	-0.25	0.895	-0.15	0.956	-1.42	0.342
RSAD2	-1.38	0.383	-0.07	0.996	0.22	1	-1.1	0.961	-0.25	0.895	-0.15	0.956	-1.42	0.342
kith	-1.39	0.162	-0.13	0.982	-0.01	1	-0.41	0.963	-0.24	0.704	0.09	0.927	-0.5	0.384
CCNE2	-1.4	0.161	-0.53	0.952	-0.64	1	-0.56	0.977	-1.51	0.0992	-1.55	0.155	-2.05	0.0306
AMPD3	-1.41	0.0701	0.76	0.897	0.11	1	-0.67	0.95	0.05	0.953	0.21	0.693	-0.77	0.34
panE1	-1.41	0.225	-0.75	0.938	-0.69	1	-0.47	0.988	-1.84	0.0768	-1.39	0.278	-1.87	0.0857
CENPM	-1.41	0.225	-0.75	0.938	-0.69	1	-0.47	0.988	-1.84	0.0768	-1.39	0.278	-1.87	0.0857

IVD	-1.43	0.486	0.82	0.959	-0.67	1	1.3	0.964	-0.85	0.675	-1.42	0.534	-1.97	0.281
prs27	-1.45	0.0993	0.14	0.975	-0.13	1	0.31	0.97	-0.64	0.122	-0.5	0.34	-0.75	0.0821
RNF34	-1.45	0.0241	0.82	0.826	0.52	1	0.28	0.977	0.33	0.568	-1.13	0.117	1.3	0.00672
depdc1a	-1.45	0.357	-0.89	0.943	-0.76	1	-0.64	0.986	-1.63	0.241	-1.09	0.557	-1.35	0.372
RHBG	-1.46	0.0365	0.43	0.943	-0.11	1	0.07	0.999	0.91	0.941	0.57	0.305	0.92	0.0486
PNPO	-1.46	0.114	0.29	0.973	0.39	1	0.54	0.977	-0.19	0.873	0.03	0.989	-0.41	0.715
LOC100136922	-1.46	0.0422	-0.02	0.991	0.09	1	0.13	0.974	0.14	0.524	0.31	0.185	-0.01	0.976
IRF2	-1.46	0.195	-0.56	0.954	-0.01	1	-0.31	0.993	-1.82	0.0741	-0.36	0.163	-1.21	0.276
CCNF	-1.46	0.294	-1.05	0.925	-0.35	1	-0.74	0.977	-2.92	0.0167	-1.04	0.53	-1.6	0.222
PRC1	-1.47	0.356	-0.94	0.94	-0.51	1	-0.72	0.981	-1.71	0.222	-1.2	0.513	-0.99	0.55
plcd3a	-1.48	0.0382	0.08	0.986	0.42	1	0.05	0.999	-0.73	0.107	-0.4	0.506	-0.32	0.563
HAUS6	-1.48	0.16	-0.55	0.903	0.18	1	0.05	0.999	-0.4	0.519	-0.2	0.824	-0.13	0.867
KLF4	-1.48	0.0397	0.49	0.929	-0.37	1	-0.17	0.993	-0.11	0.902	-0.87	0.253	-0.85	0.196
atad3	-1.49	0.0271	0.51	0.929	0.32	1	-0.26	0.992	-0.12	0.894	-0.48	0.59	-1.17	0.0773
sepp1a	-1.5	0.0386	-0.09	0.976	0.18	1	0.03	0.999	-0.05	0.899	0.11	0.809	0.24	0.455
PBK	-1.5	0.231	-0.63	0.953	-0.43	1	-0.49	0.989	-1.73	0.126	-1.38	0.33	-1.28	0.3
topk	-1.5	0.231	-0.63	0.953	-0.43	1	-0.49	0.989	-1.73	0.126	-1.38	0.33	-1.28	0.3
selo	-1.51	0.0992	0.04	0.99	-0.15	1	0.12	0.992	-0.48	0.142	-0.62	0.103	-0.31	0.398
CHAF1A	-1.51	0.0628	-0.4	0.957	-0.29	1	-0.47	0.977	-1.54	0.0433	-1.15	0.209	-1.78	0.024
caco1	-1.52	0.07	0.11	0.986	0.02	1	-0.32	0.981	0.45	0.5	0.64	0.407	-0.15	0.864
hba4	-1.52	0.0226	0.76	0.909	0.13	1	0.16	0.999	0.53	0.547	0.12	0.935	0.17	0.883
NCAPH2	-1.52	0.148	-0.58	0.947	-0.51	1	-0.73	0.969	-1.79	0.0638	-1.44	0.219	-2.05	0.0414
psf2	-1.53	0.331	0.02	0.998	-0.3	1	-0.1	0.999	-1.32	0.363	-1.5	0.39	-2.27	0.113
SEC13	-1.54	0.18	-0.1	0.984	-0.25	1	0.31	0.974	-0.24	0.687	-0.33	0.639	-0.71	0.167
ESCO2	-1.55	0.207	-0.25	0.983	-0.17	1	-0.43	0.992	-1.56	0.163	-1.21	0.396	-1.76	0.134
HIC2	-1.57	0.162	-0.26	0.971	0.18	1	0.35	0.985	0.4	0.633	0.46	0.658	0.71	0.376
TGM2	-1.57	0.355	1.79	0.855	1.8	1	0.7	0.986	-0.16	0.944	-0.49	0.841	-1.13	0.514
UCP2	-1.58	0.0949	0.62	0.939	0.51	1	0.36	0.992	0.03	0.991	-1.15	0.291	-1.8	0.0495

UCHL1	-1.59	0.0479	1.45	0.781	-0.19	1	1.14	0.892	-1.37	0.0699	1.3	0.267	-1.08	0.182
serph	-1.6	0.524	-0.36	0.987	-1.25	1	-0.85	0.99	-1.1	0.646	-2.77	0.262	-2.39	0.275
5nt1a	-1.6	0.496	-2.1	0.884	3.3	1	-0.68	0.993	-0.65	0.794	0.96	0.744	-0.92	0.709
HBAD	-1.61	0.0367	0.3	0.966	-0.34	1	-0.28	0.992	-0.41	0.632	-0.94	0.305	-1.23	0.109
CENPQ	-1.61	0.176	-0.42	0.968	-0.51	1	-1.19	0.921	-1.93	0.0748	-1.8	0.168	-1.56	0.179
FAM83D	-1.64	0.0554	-0.48	0.882	-0.18	1	-0.57	0.845	-0.52	0.254	-0.81	0.118	-0.97	0.0331
MCM2	-1.64	0.0117	-0.08	0.99	-0.33	1	-0.49	0.956	-0.44	0.488	-1.06	0.112	-1.55	0.0082
ppp1r3cb	-1.65	0.168	0.79	0.939	0.23	1	-0.67	0.977	0.56	0.669	-0.09	0.97	-1.06	0.392
CYGB	-1.68	0.0193	0.58	0.925	0.88	1	-0.86	0.856	0.44	0.568	0.28	0.795	-1.19	0.0907
SLC25A37	-1.69	0.0131	0.3	0.953	-0.36	1	-0.16	0.993	-0.26	0.693	-0.66	0.331	-0.89	0.119
hpda	-1.69	0.146	0.31	0.979	0.13	1	0.46	0.989	-1.38	0.208	-1.83	0.151	-0.87	0.489
sld5	-1.69	0.0464	-0.37	0.96	-0.04	1	0.07	0.999	-1.15	0.154	-0.88	0.387	-1.71	0.0396
c1ql2	-1.7	0.0192	0.12	0.967	0.36	1	0.09	0.993	0.06	0.883	0.54	0.124	0.5	0.108
shlb2	-1.72	0.00971	0.2	0.977	-0.07	1	-0.39	0.977	-0.62	0.347	-0.67	0.401	-0.92	0.157
pgk	-1.75	0.0843	-0.1	0.984	0.17	1	-0.14	0.993	0.4	0.441	0.32	0.64	0.34	0.553
OTC	-1.77	0.0755	-0.23	0.975	-0.64	1	-0.88	0.863	-0.8	0.273	-1.48	0.0692	-0.9	0.237
trf	-1.79	0.625	-0.15	0.981	-0.21	1	0.37	0.973	0.01	0.993	0.07	0.944	-0.24	0.737
INSIG1	-1.81	0.263	-0.59	0.94	-0.36	1	0.39	0.988	-0.79	0.396	-0.32	0.82	-0.58	0.587
ABCG2	-1.88	0.0126	0.68	0.938	0.04	1	0.49	0.97	-1.28	0.0669	-0.75	0.407	-0.7	0.375
mical3a	-1.9	0.0653	0.5	0.957	-0.24	1	-0.85	0.954	0.39	0.747	-0.29	0.861	-1.06	0.321
ERO1A	-1.91	0.176	1.41	0.884	1.2	1	1.65	0.856	0.02	0.99	0.2	0.936	-0.77	0.639
GRHPR	-1.93	0.115	0.41	0.971	0.58	1	0.27	0.961	-0.48	0.864	-0.53	0.185	-0.41	0.246
LARP7	-1.94	0.0068	0.05	0.982	0.07	1	-0.19	0.956	-0.42	0.821	-0.83	0.257	-0.66	0.312
MTMR12	-2.04	0.0275	1.05	0.855	0.92	1	0.13	0.999	-1.52	0.0822	-1.43	0.171	-1.73	0.0578
MASTL	-2.13	0.0989	-0.54	0.962	-0.43	1	-0.65	0.981	-1.99	0.0976	-1.67	0.256	-1.62	0.207
adhx	-2.23	0.0152	0.22	0.982	0.3	1	-1.18	0.815	-2.19	0.0112	-2.33	0.018	-1.95	0.0288
htra1a	-2.23	0.0479	-0.48	0.962	-0.37	1	-0.07	0.999	-1.52	0.155	-3.26	0.00657	-2.22	0.0438

hba1	-2.44	0.0428	0.53	0.932	-0.27	1	-0.03	0.999	0.04	0.968	-0.6	0.516	-0.88	0.236
TM2D2	-2.49	0.456	2.06	0.898	1.36	1	2.4	0.892	-0.18	0.954	1.02	0.742	-0.07	0.984
FIS1	-2.84	0.254	-0.1	0.939	-0.03	1	0.08	0.981	-2.44	0.293	-2.56	0.363	-0.1	0.567
BOLA1	-2.96	0.121	0.09	0.959	0.09	1	0.11	0.977	0.03	0.924	0.02	0.966	0.18	0.393
CNOT1	-3.26	0.245	0.14	0.979	0.25	1	0.28	0.978	-0.18	0.18	-0.24	0.124	-0.37	0.00798
nr2f1a	-3.28	0.00611	-1.51	0.82	0.75	1	-1.22	0.915	-0.96	0.416	-1.24	0.374	-2.35	0.0381

Figures

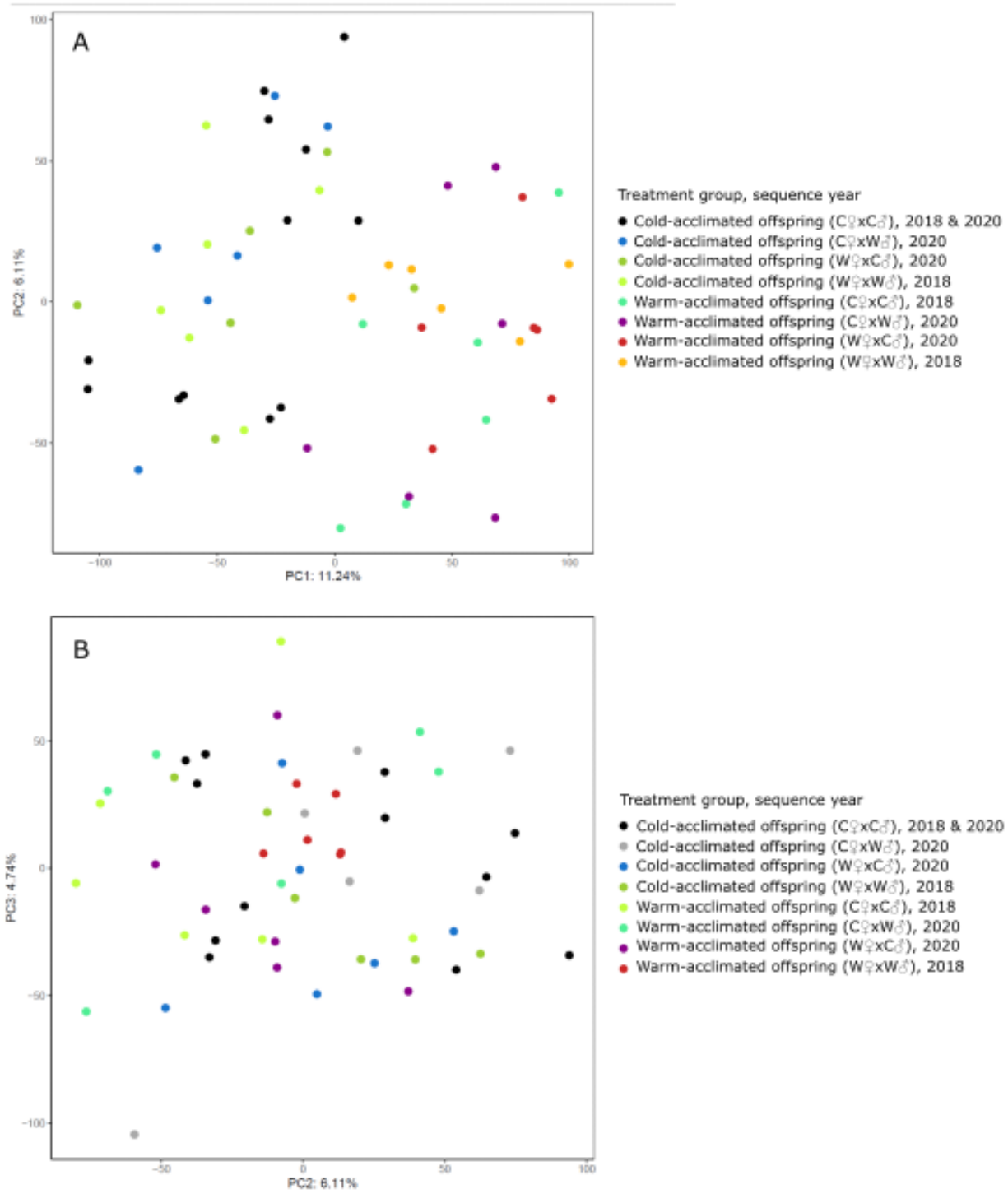
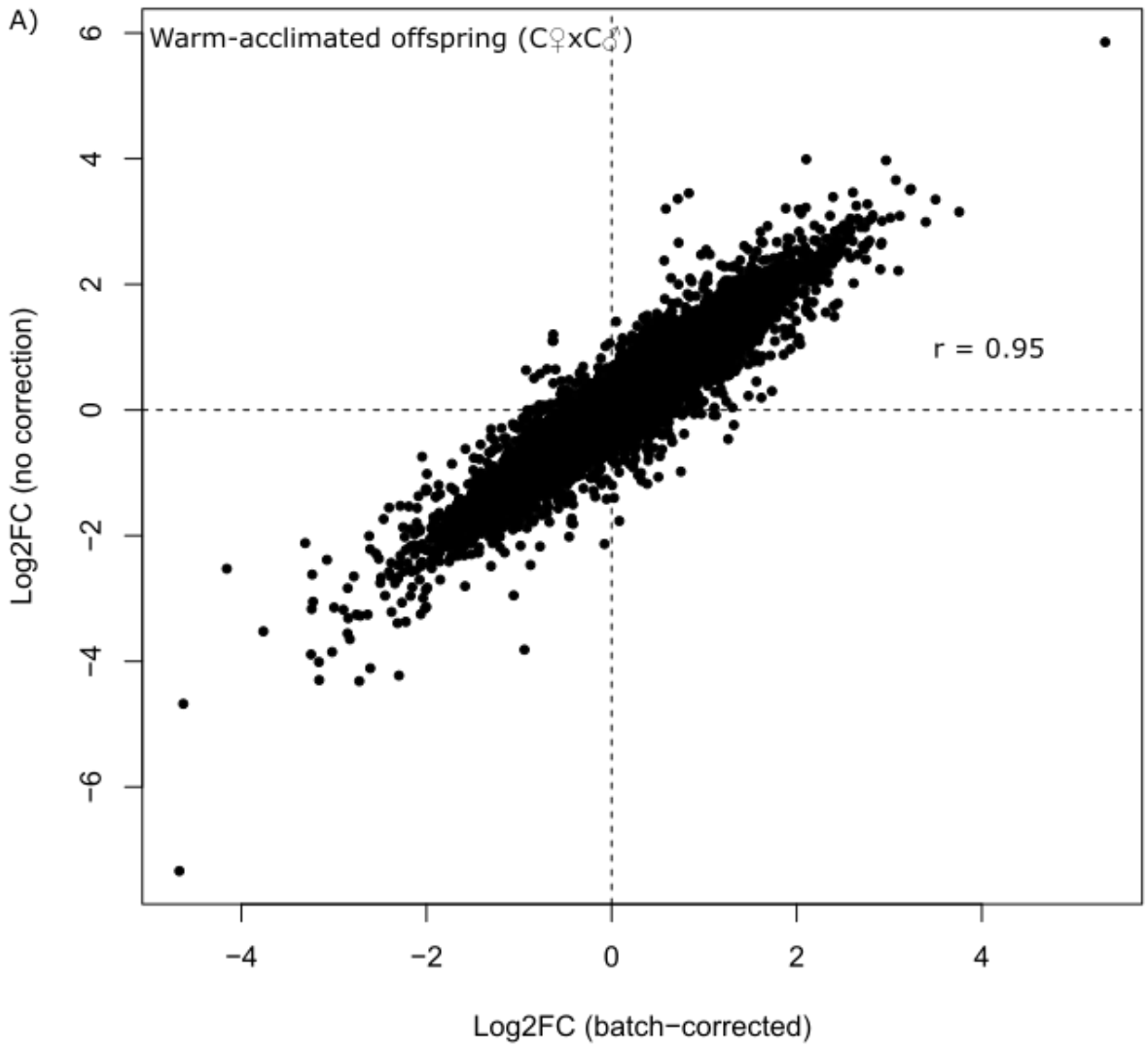
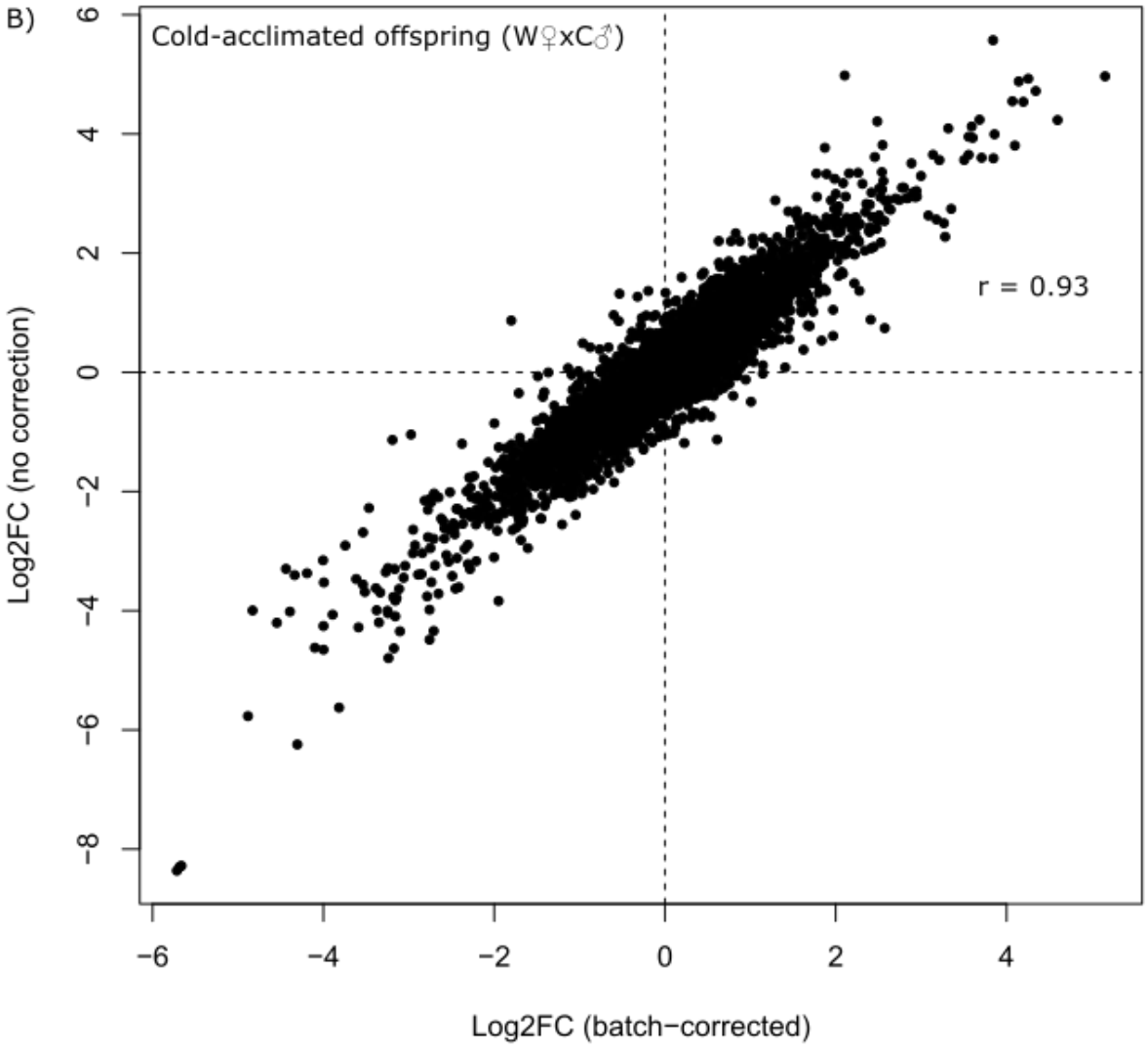
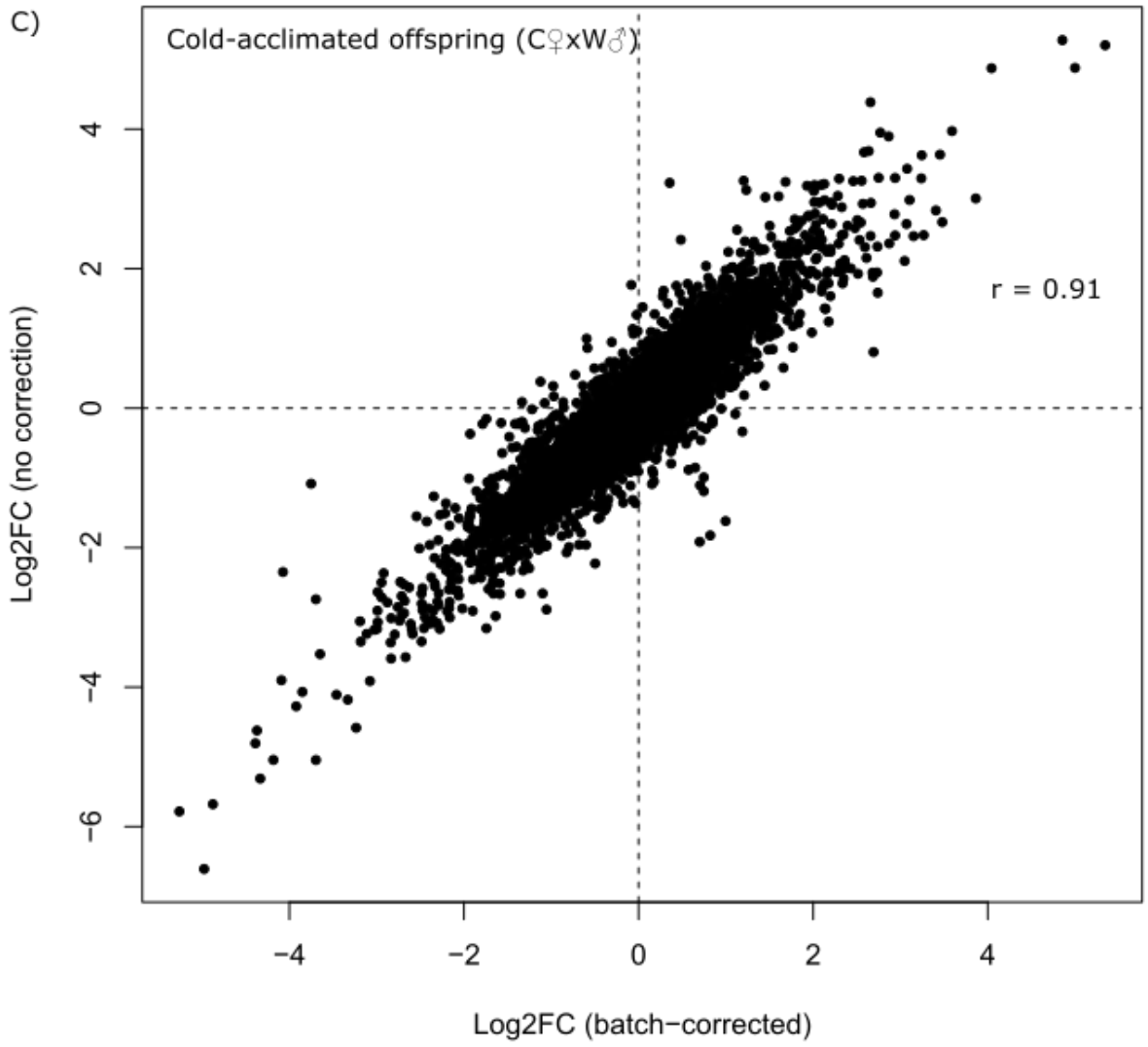
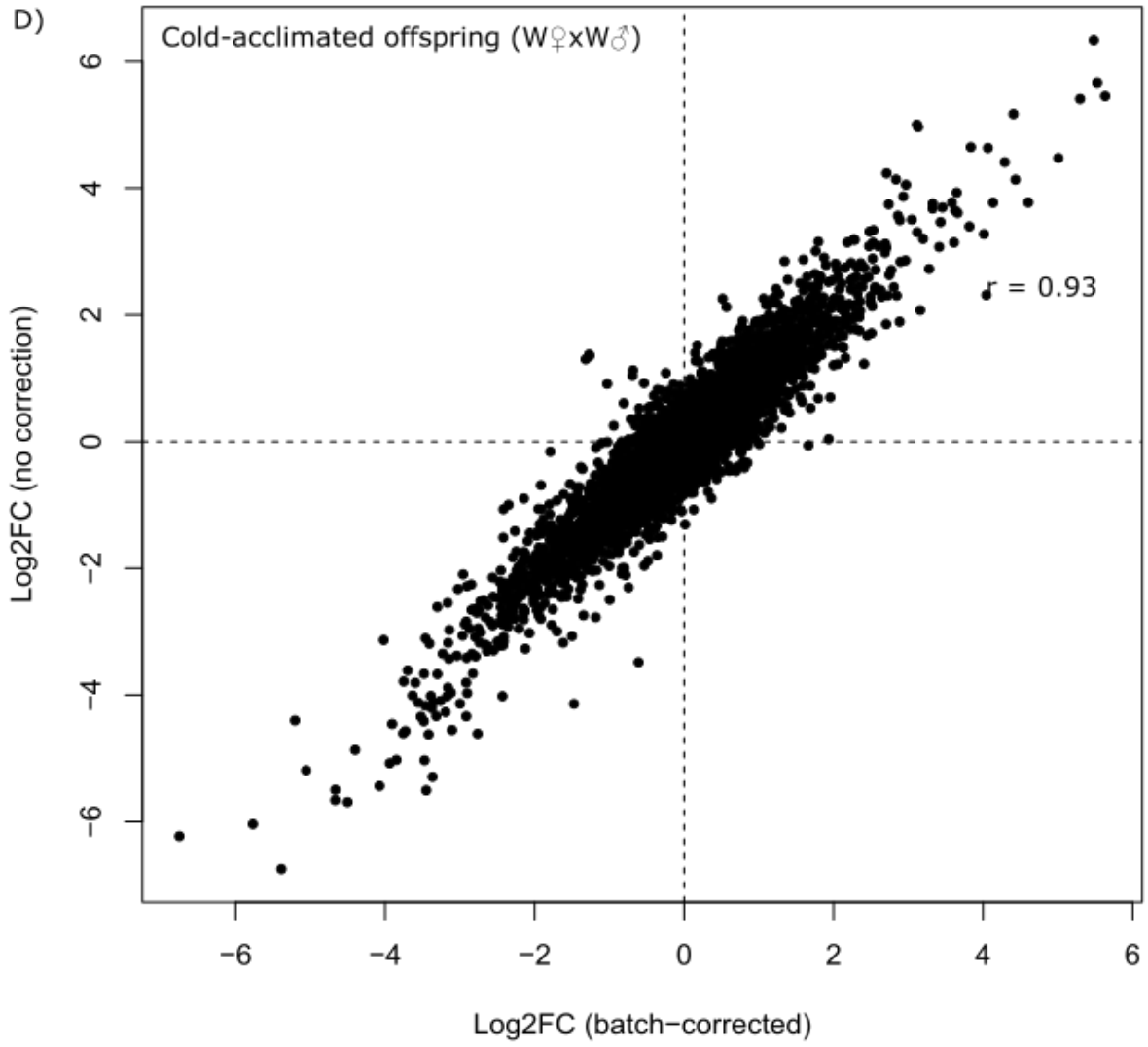

A
PC2: 6.11%
PC1: 11.24%
Treatment group, sequence year
Cold-acclimated offspring (C♀xC♂), 2018 & 2020
Cold-acclimated offspring (C♀xW♂), 2020
Cold-acclimated offspring (W♀xC♂), 2020
Cold-acclimated offspring (W♀xW♂), 2018
Warm-acclimated offspring (C♀xC♂), 2018
Warm-acclimated offspring (C♀xW♂), 2020
Warm-acclimated offspring (W♀xC♂), 2020
Warm-acclimated offspring (W♀xW♂), 2018
B
PC3: 4.74%
PC2: 6.11%
Treatment group, sequence year
Cold-acclimated offspring (C♀xC♂), 2018 & 2020
Cold-acclimated offspring (C♀xW♂), 2020
Cold-acclimated offspring (W♀xC♂), 2020
Cold-acclimated offspring (W♀xW♂), 2018
Warm-acclimated offspring (C♀xC♂), 2018
Warm-acclimated offspring (C♀xW♂), 2020
Warm-acclimated offspring (W♀xC♂), 2020
Warm-acclimated offspring (W♀xW♂), 2018

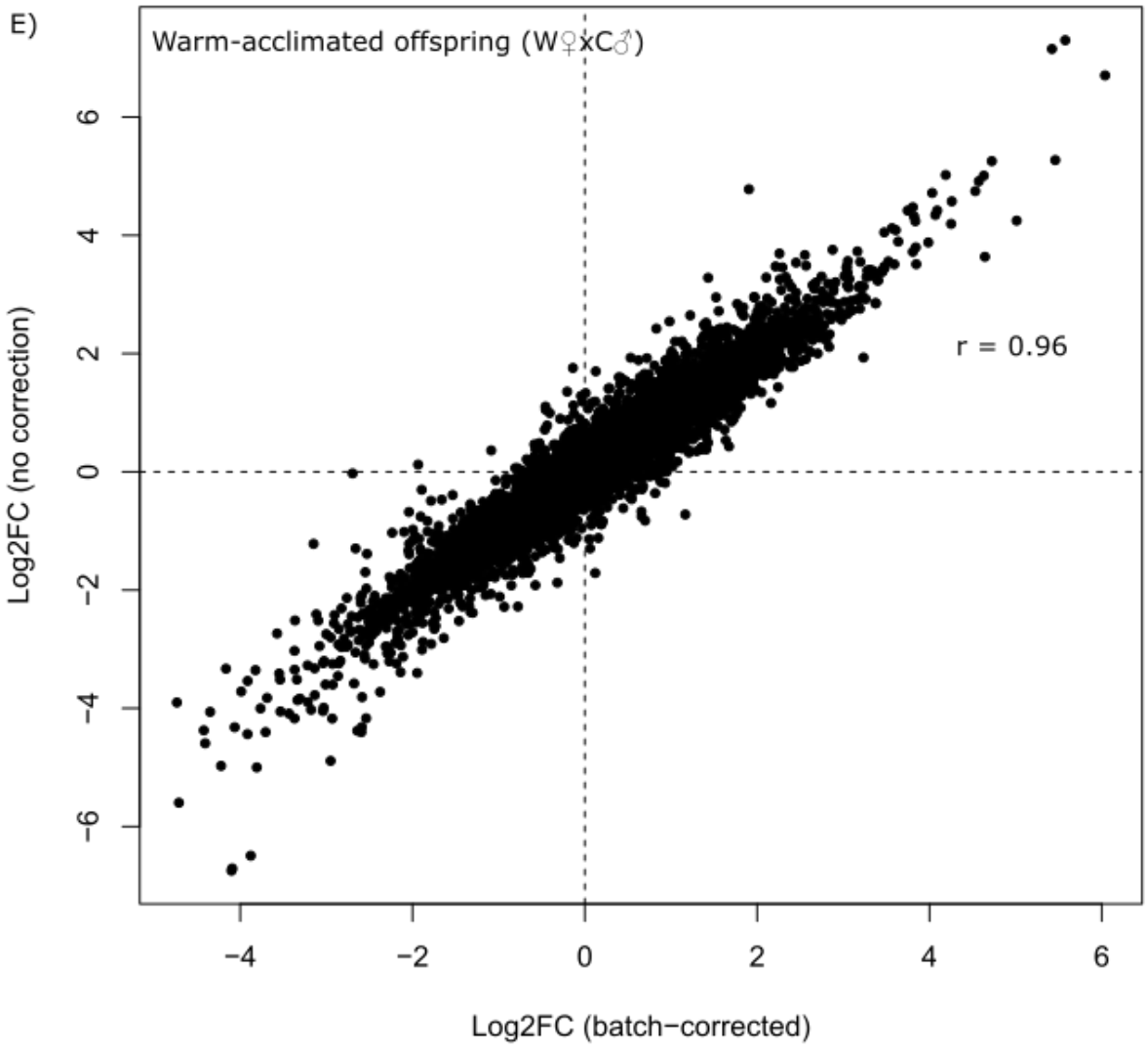
Figure A4.1: Principal component analysis for the number of differentially expressed genes per group (n=6 individuals per group) sequenced in either 2018 or 2020, except for the control group (n=12) which had 6 individuals sequenced in both years.

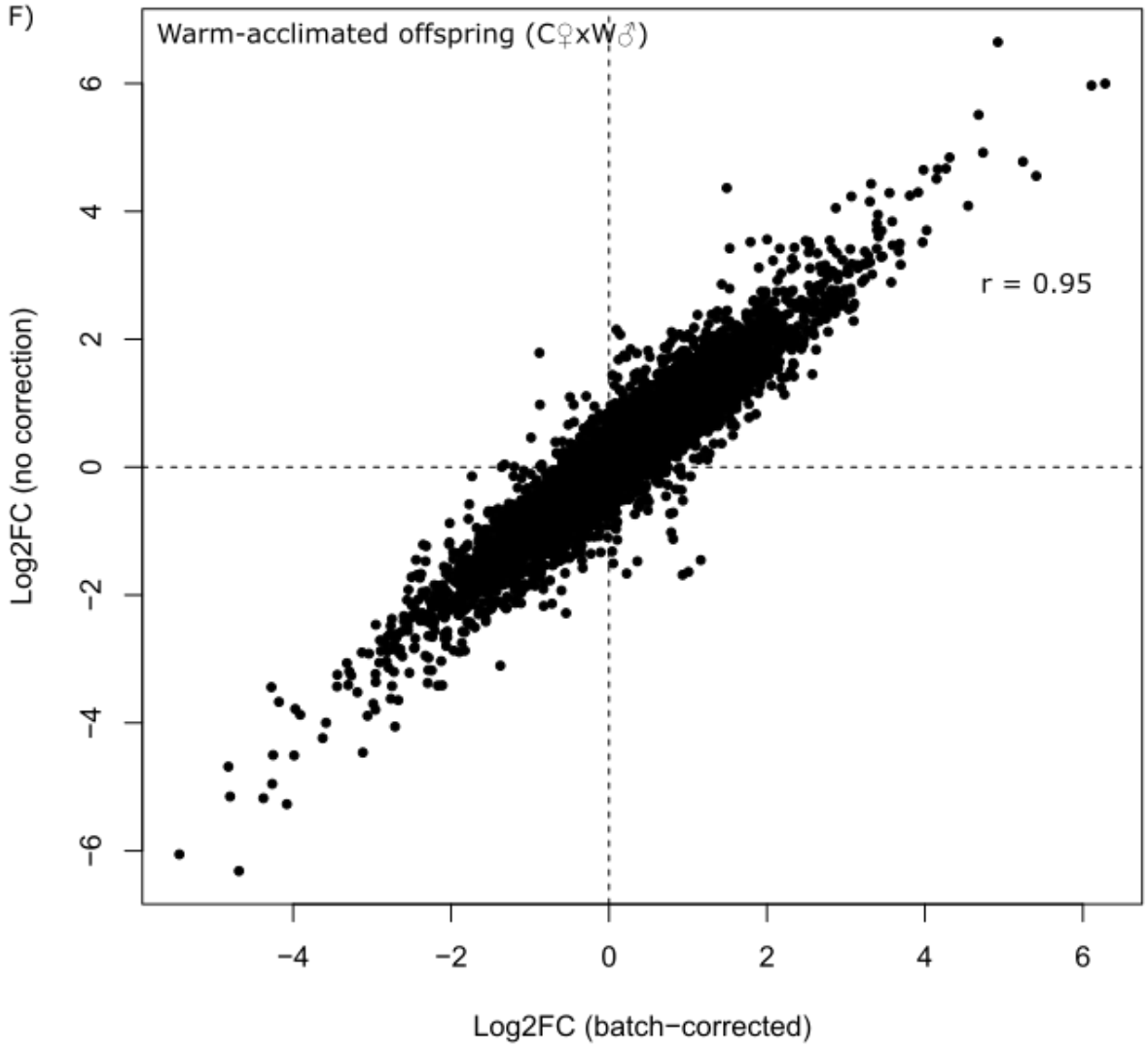

A)
Warm-acclimated offspring (C♀xC♂)
r = 0.95
Log2FC (no correction)
Log2FC (batch−corrected)
6
4
2
0
−2
−4
−6
−4
−2
0
2
4


B)
Cold-acclimated offspring (W♀xC♂)
r = 0.93
Log2FC (no correction)
Log2FC (batch−corrected)
6
4
2
0
−2
−4
−6
−8
−6
−4
−2
0
2
4


C)
Cold-acclimated offspring (C♀xW♂)
r = 0.91
Log2FC (no correction)
Log2FC (batch−corrected)
4
2
0
−2
−4
−6
−4
−2
0
2
4


D)
Cold-acclimated offspring (W♀xW♂)
r = 0.93
Log2FC (no correction)
Log2FC (batch−corrected)
6
4
2
0
−2
−4
−6
−6
−4
−2
0
2
4
6


E)
Warm-acclimated offspring (W♀xC♂)
r = 0.96
Log2FC (no correction)
Log2FC (batch−corrected)
6
4
2
0
−2
−4
−6
−4
−2
0
2
4
6


F)
Warm-acclimated offspring (C♀xW♂)
r = 0.95
Log2FC (no correction)
Log2FC (batch−corrected)
6
4
2
0
−2
−4
−6
−4
−2
0
2
4
6

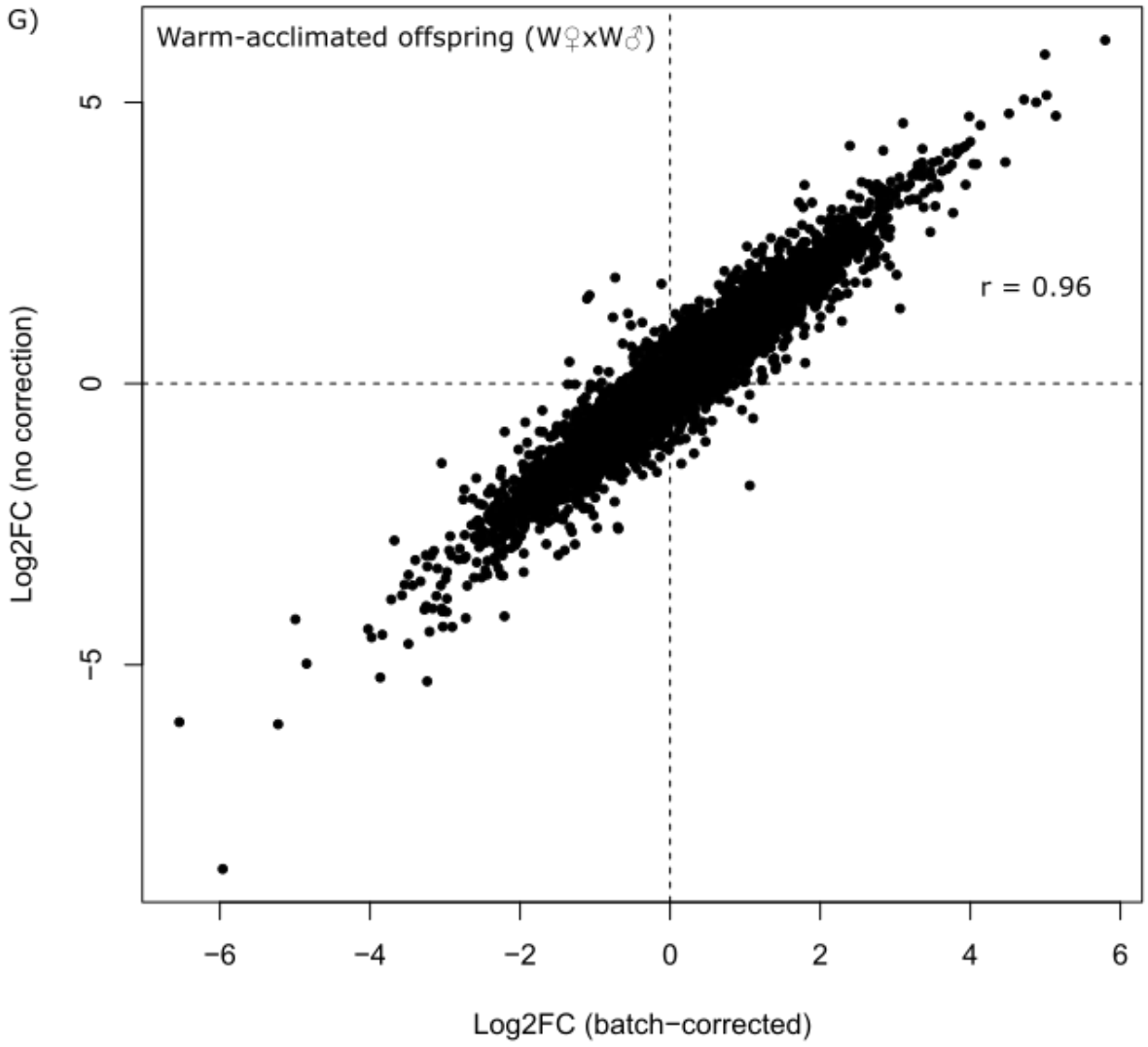


Figure A4.2: Correlation plots to compare the Log2-fold change of differentially expressed genes with and without a batch correction applied. Plots show group comparisons to determine the effect of A) the offspring (within-generation) warm acclimation; B-D) the parental (transgenerational) warm acclimation; and E-G) the combined effect of offspring and parent warm acclimation.

Appendix - References

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