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Research Article

Genetic and environmental basis of transcriptional thermal plasticity of brook charr (*Salvelinus fontinalis*) fry

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Abstract

Variation in transcriptomic responses is recognized as an underlying mechanism driving phenotypic responses to environmental variations. Whether this variation is adaptive or maladaptive can have significant implications for the evolutionary trajectory of populations. Understanding the inheritance of this transcriptional variation across generations, whether it is genetic, non-genetic, or both is limited. To address this knowledge gap, we assessed the expression of targeted genes in brook charr fry (*Salvelinus fontinalis*) reared at two different temperatures (5 and 8 °C) and produced by breeders exposed to either cold or warm thermal regimes during final gonad maturation. Using a high-throughput OpenArray® chip, we measured the relative expression of 10 candidate genes associated with environmental stress. Parental temperature affected the expression of the *SRY-box transcription factor 2*, a gene associated with neurogenesis, and of *Cholecystokinin* and *neuropeptide Y* genes, both associated with appetite regulation, regardless of offspring-rearing temperature. Additive genetic, maternal, and paternal effects were low, with the absence of genotype × environmental interactions indicating that environmental factors may be more important in shaping gene expression.

Key words: salmonids, gene expression, temperature, inheritance, fry

Introduction

Water temperature is a primary environmental factor influencing the distribution, physiology, and behavior of ectothermic species including fishes (Fry 1947; Clarke and Johnston 1999). As climate change progresses, freshwater temperatures are predicted to rise by 1–4 °C by the end of the century (IPCC 2022), creating potential stressful conditions, especially for cold-adapted fish. Investigating whether species have the capacity to cope with climate change can provide insight into population persistence.

Phenotypic plasticity both within and across generations allows organisms to cope with rapid environmental variations (West-Eberhard 2003; Schmid and Guillaume 2017; Bonamour et al. 2019). While within-generation plasticity is widely documented in the literature (Skulason and Smith 1995; Karjalainen et al. 2016; Solberg et al. 2016; Hvas et al. 2017), transgenerational plasticity, where parents influence their subsequent offspring phenotypes, is less explored. Transgenerational plasticity may be particularly relevant under rapid climate change because it is expected to be faster compared with evolutionary responses (Chevin et al. 2010; Shama 2017). Transgenerational plasticity in fishes can also show potentiality to mitigate some negative effects associated with rapid climate change in the progeny (Donelson et al. 2018).

Transmission of phenotypic traits from one generation to the next may arise from both genetic and non-genetic (e.g., environmental) mechanisms (Danchin et al. 2011; Hallsson et al. 2012). The relative importance of genetic versus non-genetic inheritance mechanisms is greatly variable among species and phenotypic traits, and thus determining the balance between these two mechanisms is pivotal to elucidate the evolutionary potential of phenotypic variation. Gene transcription, shaped by both genetics and environmental conditions, offers a promising trait for such evaluation, and unraveling the underlying molecular response of organisms to temperature fluctuations is gaining interest in evolutionary biology and biodiversity conservation (Gibson and Weir 2005; Ouellette 2013).

Temperature increases may be perceived by fish as a stressor. The brain plays a central role in responding to stressors by orchestrating neurobiological and physiological

adjustments through a number of mechanisms related to the stress response, changes to appetite, or neuroplasticity. The stress response axis starts in the forebrain, and involves the brain sympathetic-chromaffin-cell and the hypothalamic-pituitary-interrenal pathways (Wendelaar Bonga 1997). The release of the adrenocorticotropic hormone (ACTH), regulated by the corticotropin-releasing factor (*CRF*), and other neuropeptides, like arginine vasotocin (AVT), regulates cortisol release, cortisol being the primary stress hormone in fish (Wendelaar Bonga 1997; Aluru and Vijayan 2009; Faught et al. 2016; Murugananthkumar and Sudhakumari 2022). Secondary physiological responses to stress will then be mediated through different hormone receptors (mineralocorticoid (MR) and glucocorticoids (GR1 and GR2) receptors). Stress affects food intake regulation, which involves the regulation of several genes that either stimulate or inhibit appetite. Orexigenic signals that stimulate appetite include neuropeptide Y (NPY), orexin, agouti-related protein (AGRP), and ghrelin (Unniappan et al. 2002; Forlano and Cone 2007; Yan et al. 2011; Folkedal et al. 2012). Anorexigenic signals that inhibit appetite include alpha melanocyte-stimulating hormone, which is derived from the hormone precursor proopiomelanocortin (POMC), cholecystokinin (CCK), cocaine- and amphetamine-regulated transcript (CART), leptin (Forlano and Cone 2007; Kehoe and Volkoff 2007; Kang et al. 2010; Yan et al. 2016) and the leptin receptor (LEPR).

The brain's response to stressors also involves modulation in behavioral and cognitive processes that rely on neural plasticity and neurogenesis (Ebbesson and Braithwaite 2012; Sørensen et al. 2013). This modulation is primarily mediated by corticosteroid receptors (MR and GRs) widely expressed in the brain (Lee et al. 1992; Sturm et al. 2005; Sørensen et al. 2013). Temperature effects on neurogenesis have also been previously noted (Dunlap 2016; Topal et al. 2021), and thus genes involved in neurogenesis may serve as important supplementary markers of the stress response. The most well-known markers of neurogenesis in teleosts are the neurogenic differentiation factors 1 and 2 (NEUROD1 and 2) involved in the differentiation of neuronal precursor cells (Wanaka et al. 1997), the brain-derived neurotrophic factor involved in neuron development, transmission, and synaptic plasticity (Binder and Scharfman 2004), and the proliferating cell nuclear antigen involved in the machinery of DNA replication and repair (Kelman 1997; Lai et al. 2017). Thus, the alteration of genes present in the brain may serve to monitor fish health and response to ongoing climate change.

Brook charr (*Salvelinus fontinalis*) is an economically important species in Canada supporting recreational fishing and accounting for 70% of Québec stocked fish (MFFP 2019). This eurythermal salmonid species is native to East and Central North America (Scott and Crossman 1973). While its thermal response may vary among populations, brook charr strains from northern regions usually show a limited thermal tolerance to high water temperatures (Stitt et al. 2014). A predominant intergenerational effect was observed in three related studies using the same brook charr strain, originating from the same experiment as our present study (Venney et al. 2022; Houle et al. 2023; Banousse et al. 2024). In essence, the parental thermal environment during final gonad maturation significantly influenced the post-stocking survival rate and body condition (Houle et al. 2023), liver methylome (Venney et al. 2022), and whole brain transcriptome (Banousse et al. 2024). These effects had both genetic and non-genetic origins for morphological traits and the methylome.

The objective of this study was to investigate the transcriptional basis of both within and transgenerational plasticity in brook charr, focusing on the stress-response, neurogenesis, and food regulation genes. The study further aimed to explore the genetic versus non-genetic (environmental) mechanisms underlying variations in transcriptional responses. We hypothesized that genetic factors (e.g., additive genetic factors) would contribute to the variance in the expression of genes associated with neurogenesis, appetite regulation, cellular stress response, and stress response regulation. Furthermore, we anticipated that maternal effects would play a crucial role in gene expression variance. Additionally, our study used a novel high-throughput qPCR OpenArray chip from the Genomic Network for Fish Identification, Stress and Health (GEN-Fish) (https://gen-fish.ca/) consortium designed for assessing the health of salmonids. Parents were exposed to either cold (from 11.5 °C in September to 3 °C in December) or warm (from 13.5 °C in September to 5 °C in December) thermal regimes during final gonad maturation, differing by 2 °C, reflecting the predicted freshwater warming in Canada by 2100 (Zhang et al. 2019). Progenies from each parental group were then reared at two different thermal regimes (5 and 8 °C) until the yolk sac resorption.

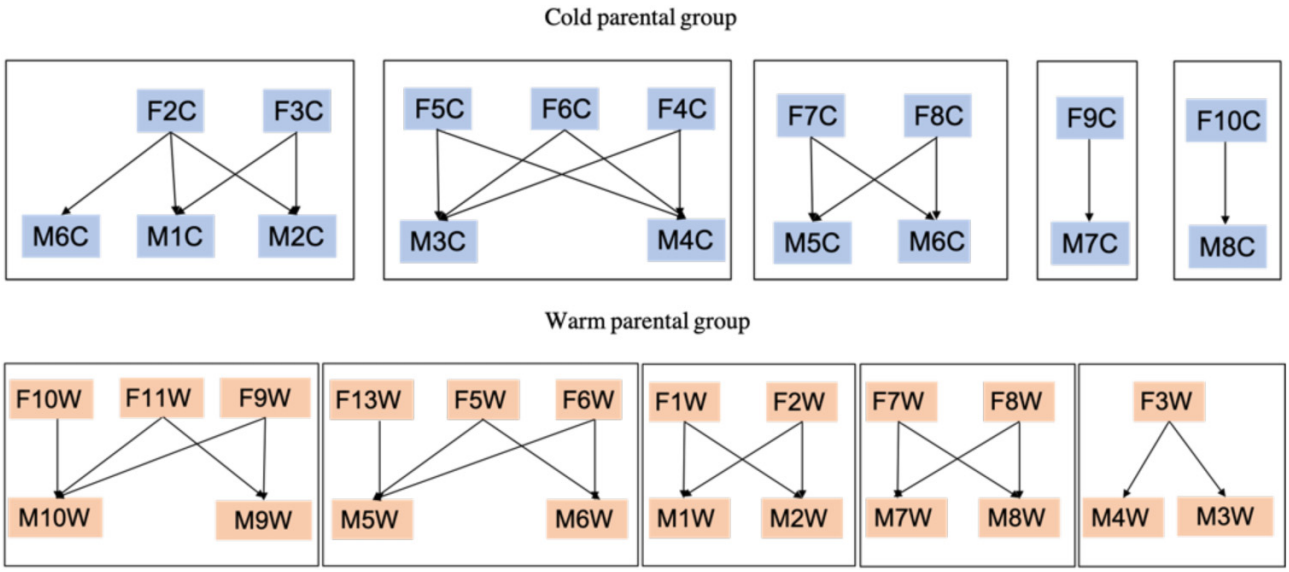
Materials and methods

Breeding design and fish rearing

Animal husbandry, reproduction, and fry rearing were conducted following the recommendations of the Canadian Council on Animal Care. Protocols were performed under the UQAR Animal Care Committee (CPA-76-19-205) for the breeders and by the Laval University Animal Care Committee (VRR-18-111) for the offspring.

Breeders used in this experiment were from the brook charr *Laval* strain, which has been kept in captivity for six generations at the *Station aquicole de Pointe-au-Père* (ISMER–UQAR, Québec, Canada; 48°27 = N, 68°32 = W). The brook charr strain originates from an anadromous wild population in the Laval River, Forestville, Québec (48°44 = N, 69°05 = W) (Bastien et al. 2011). In mid-September 2018, during gonadal maturation, breeders were separated into two parental groups and exposed to two different thermal regimes differing by 2 °C; the "Cold" group experienced a gradual temperature decrease from 11.5 °C in September to 3 °C in December, while the "Warm" group experienced a gradual temperature decrease from 13.5 °C in September to 5 °C in December. At the time of spawning (from mid-November to mid-December), partial factorial breeding was randomly made within each parental group. Initially, we planned to use a factorial breeding design. However, we had to adjust according to the timing of sexual maturation of breeders (Fig. 1). Specifically, when it was possible, the eggs extracted from each female were fertilized by two different males, and sperm from

Fig. 1. Diagram of the breeding design for each parental group (cold and warm) of brook charr. Letters identifying female (F) and male (M) are followed by an individual number and a letter indicating the parental temperature regime (C for cold and W for warm).



each male was used to fertilize two different females, generating full and half siblings within the same family group (Banousse et al. 2024). No crosses were made between siblings to prevent inbreeding. This design resulted in the production of 16 families from the cold group, and 21 from the warm group. Unequal family numbers resulted from the unavailability of sexually mature fish at the same time. The fertilized eggs from each family were split into two batches and then transferred to the LARSEM facilities at Laval University (Québec City, QC., Canada). One batch was reared at 5 °C and the second one at 8 °C. The same temperature regimes were maintained until the yolk sac resorption. Thirteen families from the cold parental group and 19 families from the warm parental group survived to the stage of exogenous feeding. At this stage, 10 fry from each family were anesthetized with MS222, transferred in microtubes, and frozen on dry ice. They were then immediately stored at −80 °C for future analysis.

RNA extraction and cDNA synthesis

The brain was dissected out on ice under a binocular microscope. Total RNA was extracted from the whole brain tissue from a total of 201 fry (n = 3 from each family per parental group per offspring-rearing temperature) using the RNeasy Fibrous Tissue Kit (Qiagen, Inc.) following manufacturer instructions. RNA concentration and RNA purity were measured using a Nanodrop ND-1000 Spectrophotometer version 3.3.0 (NanoDrop Technologies, Inc., Delaware, USA), and RNA integrity was evaluated using electrophoresis. All RNA samples used in the current experiment showed intact 28S and 18S rRNA bands, with 260/280 ratios ranging from 1.8 to 2.1. The RNA samples diluted to a concentration of 200 ng μL^{-1} were then stored at −80 °C. Reverse transcription was performed using the Quantitect Reverse Transcription Kit (Qiagen, Inc.). The cDNA concentration and purity were assessed using the Nanodrop ND-1000 Spectrophotometer version 3.3.0 and the cDNA was then stored at −20 °C until further analysis. Reverse transcription efficiency was verified for a reference gene (*β-actin*) and a target gene (*NPY*) using serial dilutions of a pool of RNA samples representing the different experimental conditions. Corresponding cDNA values were measured with Nanodrop.

Gene expression quantification

For the 201 cDNA samples, 10 µL subsamples (150 ng μL^{-1}) were sent to the Great Lakes Institute for Environmental Research (GLIER) at University of Windsor (Windsor, ON, Canada) for quantitative real-time PCR using TaqMan OpenArray chips (Thermo Scientific, Burlington, ON, Canada). Each chip contained a total of 48 subarrays, each one including 56 wells, allowing simultaneous transcript expression duplicate analysis of 28 genes (24 candidate genes and 4 reference genes). The primers and probes of the selected genes used in this study were from an early version of a transcriptional profiling chip designed by the GEN-FISH team (Table 1). More specifically, gene sequences of four salmonid genera (*Salmo*, *Oncorhynchus*, *Salvelinus*, and *Coregonus*), including brook charr, were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) and aligned using Geneious Prime software v6.1.6. Genes primers and probes were then designed using the conservative regions of the target genes sequences using Primer Express® software (v3.1). The 24 candidate genes represent categories of neuroplasticity (*BDNF*, *PCNA*, *NEUROD1*, *NEUROG1*, *DCX*, *SRY-box transcription factor 2* (*SOX2*), and *NGF*), metabolism/appetite regulation (*CCK.L* (an isoform of *CCK*), *POMC*, *NPY*, *LEPR*, *AGRP-1*, and *CART*), stress response regulation (*MR*, *GR1*, *GR2*, *HSD11B2*, *AVT1*, and *CRFB1*), and cellular stress indicators (*HSP70A*, *HSP90AA*, *HSP90BA*, *CIRBPA*, and *HSF1*). Four reference genes (*EF-1* A, *RPL13A*, *RPL7*,

Table 1. Sequences of primers and probes used on the salmonid Genomic Network for Fish Identification, Stress and Health brain chip.

Name	Sequence (5′–3′)	Length (bp)	Tm_primer express	%GC_primer express	Homology %
	F: ATACTGCTGGACCCTGTGCCT	21	59.6	57	NA*
AGRP	R: CGGGATGGGAGTCGTCTAGA	20	58.7	60	
	P: CCTGATCCAGCTGGCTA	17	69	59	100%
	F: TGACCAACGAAAAGCAACTGC	21	59.9	48	
CART	R: AGGGACTTGGCCGAATTTCT	20	58.6	50	
	P: CTGCAGACCAAGAGA	15	68	53	100%
	F: GGGTCCCAGCCACAAGATAA	20	58.4	55	
CCK.L	R: CTCGTACTCCTCTGCACTGCG	21	59.8	62	
	P: TGGGCTGGATGGAC	14	68	64	100%
	F: CGCTGTATGCACATCAACGG	20	59.7	55	
LEPR	R: GTCAGGAGCTCTGCTGTTGTGA	22	59.2	55	
	P: CCGGGACCTGGAGTGA	16	70	69	100%
	F: AACCTCATCACAAGGCAGAGGT	22	58.7	50	
NPY	R: ACGTGTCTGTGCTCTCCTTCAG	22	58.2	55	
	P: AGGTCCAGCCCTGAC	15	68	67	100%
	F: AGAACAGCATCCTGGAGTGCA	21	59.4	52	NA*
POMC	R: GGGAGTTGGGTTGGAGATGG	20	60.1	60	
	P: ACCTCACCGCCGAAT	15	69	60	100%
	F: TCATTGCTTTCTTACCGCGC	20	59.9	50	NA*
CRFB1	R: AGGAGGGGAAGAGACTGTTGCT	22	59.4	55	
	P: CCGCTCCACATCACGA	16	70	63	100%
	F: CTAGACCCAGACTGCCTAGAGGAC	24	58.6	58	
AVT1	R: GCCAAACCACCCATTAAGGC	20	60.1	55	
	P: ACGTCAGTCACCCAGCGA	18	70	61	100%
	F: GTCTTTGGCCTGTATCCCCC	20	59.2	60	
GR1	R: AGCTCGACATCCCTGATCCA	20	59.2	55	
	P: TGCCCTCGGTCAGTGA	16	68	63	100%
	F: ATGGCAGACCAGTGTGAACAGAT	23	59.5	48	
GR2	R: GAGCAGCAGCAGAACCTTCAT	21	58.3	52	
	P: AGACTGCAGGTGTCTCA	17	70	53	100%
	F: CATAGTCAATGTCAGCTGCTCCC	23	59.7	52	
MR	R: GCTGCTGCTCTGGCTTCTTCT	21	60	57	
	P: CCAGCAGCAACACA	14	68	57	100%
	F: CTGTCTAGCAGCGTACGGAGC	21	58.9	62	
HSD11B2	R: ATGGTGGACACTTTGACCCC	20	58.1	55	
	P: CCTGTTCATCAACACACT	18	68	44	100%
	F: TCCTTCCTCTGTGGTCACCAC	21	58.6	57	
BDNF	R: AGGCACTTGGTTGCTGATCA	20	57.9	50	
	P: TGTCGACCTGTATGCAT	17	68	47	100%
	F: TGGAGGCTCTGAAGGACCTG	20	59	60	
PCNA	R: GAGCTGAACTAGGGAGACGTGG	22	59	59	
	P: CTGTTGGGACGTGAGCT	17	69	59	100%
	F: ATGTCATCAGACCCCCAGGA	20	58.7	55	
NEUROD1	R: GATTCGAGTGCGCCGTTTT	19	59.8	53	
	P: CGACCTGGACTGATGA	17	69	59	95%
	F: GTCTGTGAACGTGAAGGCCTC	21	58.4	57	
DCX	R: TTGGGCCGAACAAACTCCT	19	59.1	53	
	P: AGCCAGAAGAACCTG	15	69	53	100%

Table 1. (*concluded*).

Name	Sequence (5′–3′)	Length (bp)	Tm_primer express	%GC_primer express	Homology %
	F: GGGGTGTACTCTGTGTGTGAGAGT	24	58.2	54	
NGF	R: AATGTTGACGTCAGGGAGCAC	21	58.7	52	
	P: AGAGTGTCAGCAACTG	16	68	50	100%
	F: AGAGCGAGGAATGAAGCCAC	20	58.1	55	
NEUROG1	R: GTTTCTCTCGCGGTCGTTTG	20	59.4	55	
	P: CGTGGTCAAGAAGAA	15	69	47	100%
	F: TCAGACAGGAGACCTACGGGAC	22	59.2	59	
SOX2	R: TTGGGACATGTGGAGTCTGC	20	58.1	55	
	P: TTACCGGGCGCTGAG	15	68	67	100%
	F: CCCAAGTTCAGCAGGCAGTAC	21	58.5	57	
HSF1	R: GCCGTGAAGAGACCGGTACT	20	57.9	60	
	P: TGCAGGGCTCGCCT	14	69	71	95,24%
	F: GAGAACACTGTCCTCCAGCTCC	22	58.9	59	
HSP70A	R: CCCTGAAGAGGTCGGAACAC	20	58.2	60	
	P: ACACCTCCATCACCAGG	17	68	59	100%
	F: AAGATCGAGGTCACCCCTGA	20	58.1	55	
HSP90AA	R: GGTGCCAGACTTTGCAATGG	20	60.1	55	
	P: CGGCATCGGCATGA	14	68	64	100%
	F: GAGGTGGAGGAGGACGAGTACA	22	59.2	59	
HSP90BA	R: GCTGTGAAGTGGATGTGGGA	20	58.1	55	
	P: TCTCCAGGGACACAGAC	17	69	59	100%
	F: CGGGAAGGTCTCGTGGATT	19	58.5	58	
CIRBPA	R: TGGTTCTGCCATCGACAGACT	21	59.3	52	
	P: CATTGGAGGGAATGAA	16	69	44	93,75%
	F: GAAGCTTGAGGACAACCCCA	20	58.7	55	
EF1A	R: GAAGCTCTCCACACACATGGG	21	59.2	57	
	P: CCGCCATCATCGTCAT	16	69	56	94,44%
	F: CACTGGAGAGGCTGAAGGTGT	21	58	57	
RPL13A	R: GTGGGCTTCAGACGGACAAT	20	58.6	55	
	P: CATGGTCGTACCTGCT	16	68	56	100%
	F: TCGTCATCAGGATCAGGGGT	20	60	55	
RPL7	R: GAAGATCTGACGCAGACGCA	20	60.5	55	
	P: CAAGGTGCGCAAGGT	15	68	60	100%
	F: TTCTCCCTGCGTTCACCATAC	21	58.6	52	
RPS9	R: GGCCCTTCTTGGCATTCTTT	20	59.1	50	
	P: CCCGGCCGTGTCAA	14	70	71	100%

Note: For each gene, the homology percentage between the primers sequences and the corresponding brook charr sequence obtained from brain fry transcriptome data (Banousse et al. 2024) was calculated using Blast. F: forward; R: reverse; P: probe; *AGRP*: agouti-related protein; *CART*: cocaine and amphetamine-regulated transcript protein type II; *CCK.L*: cholecystokinin; *LEPR*: leptin receptor; *NPY*: neuropeptide Y; *POMC*: adrenocorticotropic hormone; *CRFB1*: cytokine receptor family member b2; *AVT1*: arginine vasotocin; *GR1*: glucocorticoid receptor 1; *GR2*: glucocorticoid receptor 2; *MR*: mineralocorticoid receptor; *HSD11B2*: 11β-hydroxysteroid dehydrogenase 2; *BDNF*: brain-derived neurotrophic factor; *PCNA*: proliferating cell nuclear antigen; *NEUROD1*: neuronal differentiation 1; *DCX*: neuronal migration protein doublecortin; *NGF*: nerve growth factor; *NEUROG1*: neurogenin-1; *SOX2*: transcription factor SOX-2; *HSF1*: heat shock transcription factor 1; *HSP70A*: heat shock protein 70a/70b; *HSP90AA*: heat shock protein 90 (inducible); *HSPA90BA*: heat shock protein 90 (constitutive); *CIRBPA*: cold inducible RNA binding protein; *EF1A*: elongation factor 1 alpha; *RPL13A*: ribosomal protein L13a; *RPL7*: 60S ribosomal protein L7; *RPS9*: 40S ribosomal protein S9. SOX2, SRY-box transcription factor 2.
*The gene sequences for *AGRP*, *POMC*, and *CRF1* were not detected in the assembled brook charr fry brain transcriptome, likely due to their low expression levels. The homology percentages for these genes with the Brain chip primer sequences were determined using sequences from lake trout (*Salvelinus namaycush*) (*CRF1*), Arctic char (*Salvelinus alpinus*) (*POMC*), and Atlantic salmon (*Salmo salar*) transcriptomes available onNCBI under the accession numbers PRJNA682269, PRJNA678849, and 100286779, respectively.

and *RPS9*) were used to normalize the expression of the 24 targeted genes. To calculate the homology percentage of the primer sequences with the brook charr gene sequences, a de novo assembly of the brook charr brain transcriptome obtained from a sub-sample of fry used in the present study (Banousse et al. 2024) was performed using RNA sequencing data. Specifically, paired-end raw reads from five samples were assembled using Trinity (Grabherr et al. 2011), and the assembled transcripts were aligned to the reference transcriptome of lake trout (*Salvelinus namaycush*), a species of the same genus (SaNama v. 1.0; NCBI RefSeq: GCF_016432855.1) (Smith et al. 2022) to target and retrieve sequences for the

28 genes used in the Brain chip. These sequences were then compared to the primer sequences using BLAST (Altschul et al. 1990) to determine homology percentages. Each subarray of the chip was loaded with a cDNA sample in duplicate for each of the 28 genes using an OpenArray™ Accufill™ System. The primers and probes for each of the 28 genes were preloaded onto the chips. A total of 5 chips were run on a QuantStudio 12K Flex Real-Time PCR system.

The use of the GEN-FISH Brain chip allowed us to measure the expression of ten out of the 24 original candidate genes present on the chip. The raw amplification data were used to calculate the PCR efficiency using LinRegPCR (Ramakers et al. 2003). For each gene, the CrT values (fractional PCR cycle number at which fluorescence crosses the quantification threshold) (Bustin et al. 2009) for duplicate assays within each sample were then averaged, and we calculated the starting cDNA concentration (N_0 value) in each sample using the efficiency values according to Tuomi et al. (2010) utilizing the following equation:

$$N_0 = Nq/E^{(\mathrm{Crt+C\text{-}shift})}$$

where Nq represents the quantification threshold, set at 10^{11}, E is the PCR efficiency, Crt is the threshold value of the gene, and C-shift corrects for different primer efficiencies. The C-shift value was calculated using the formula

$$\text{C-shift} = \mathrm{LOG}\left(1/\left(1-(1/E)\right)\right)/\mathrm{LOG}\left(E\right)$$

The stability of the geometric mean of the N_0 of the four reference genes (the normalization factor) was verified using geNorm (Vandesompele et al. 2002) and Normfinder (Andersen et al. 2004) algorithms implemented in the Ctrgene and the dhammarstrom/generefer packages, respectively. The ΔCrT values were obtained by dividing the N_0 value of each sample by the normalization factor for this sample (Wellband et al. 2018).

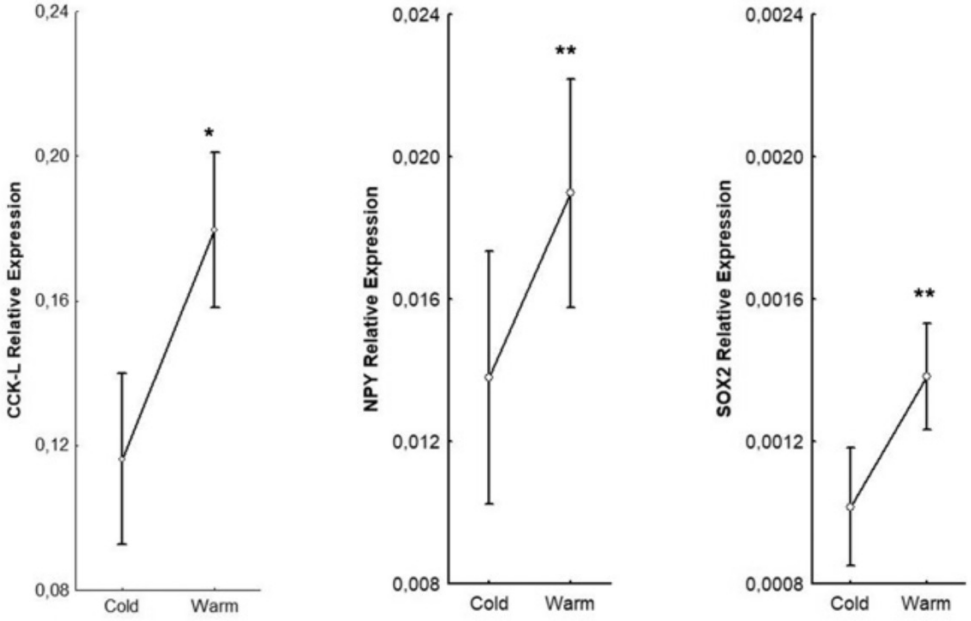
Statistical analysis

We first used linear mixed models (LMMs) in the lme4 package V.1.1-35.3 (Bates et al. 2015) to assess the effects of parental temperature, offspring-rearing temperature, and their interaction on the gene expression of the 10 genes examined. The identity of parents (dam ID, sire ID, dam ID × sire ID) was included as a random effect in the model to assess the contribution of parental effects encompassing both genetic and environmental factors. We included the chip ID as a covariate in the model to control for potential chip effects. BoxCox transformation was applied to the gene expression data to achieve normality. A backward elimination procedure was then employed to simplify the models by eliminating the least significant effects based on their p-values ($p \leq 0.05$, confirmed by likelihood ratio tests—LRTs). For genes *AVT1*, *DCX*, *NEUROD1*, *NPY*, *PCNA*, and *SOX2*, we opted for linear models (LMs) as random effects were not significant (when tested using LRTs). We calculated both conditional and marginal R^2 values using rsquaredGLMM function in the MuMIn package (V.1.47.5; Barton 2009). The p-values were adjusted using the Bonferroni–Holm method to account for multiple testing within the three functional gene groups, categorized into neurogenesis (*BDNF*, *DCX*, *NEUROD1*, *PCNA*, *SOX2*), appetite regulation (*CART*, *CCK.L*, *NPY*), and stress response (*AVT1*, *CIRBPA*).

Using the ASREML package (V4.2 Butler et al. 2023), we then applied a quantitative genetic approach to estimate phenotypic variance components, partitioning total variance (V_P) into additive genetic variance (V_A), dams-related (maternal) variance (V_D), sires-related (paternal) variance (V_S), and residual variance (V_R). Two separate analyses were conducted for each parental group (cold and warm) to evaluate the influence of parental temperature on variance component estimates. Offspring-rearing temperature was included in the model as a fixed effect. Animal identity (linked to the pedigree matrix), dam ID, sire ID, and the dam × sire interaction were included as random effects in the animal model. The animal model uses information from the pedigree matrix to estimate additive genetic variance, thereby capturing the genetic relationships among individuals. This ensures that the additive genetic variance is separated from other sources of variance, including those associated with dam and sire effects. The variance components attributed to dam ID and sire ID represent non-genetic contributions. This approach enables the precise partitioning of phenotypic variance into its genetic and environmental components, ensuring robust estimates of additive genetic variance and other variance components (Vega-Trejo et al. 2018). A total of nine models incorporating all random effects and possible interactions were built for each parental group. Model selection was based on the Akaike information criteria (AIC) weight calculated from the ΔAIC (difference between a model AIC and the lowest AIC obtained among all models), retaining only those with the highest AIC weight, according to Vega-Trejo et al. (2018). For all genes, component estimates from models with high and non-negligeable AIC weight (>10) were averaged to calculate variance components (V_A, V_D, V_S, V_R). Heritability h^2 (V_A/V_P) and the proportion of phenotypic variance attributable to the dam (V_D/V_P), sire (V_S/V_P), and residual (V_R/V_P) were also calculated. All statistical analyses were performed using R software (V. 4.0.5; R Core Team 2021).

To evaluate the differential response of genotypes within each parental group to rearing temperature, a Genotype-by-Environment (G × E) interaction analysis was conducted separately for each parental group (cold and warm) using LMM. For this analysis, only full-sib families were used as the genotypic units, as they are completely independent from one another (seven full-sib families from the cold parental group and eight from the warm parental group). The model included Family ID (genotype), offspring-rearing temperature (environment), and their interaction (G × E) as random factors. The significance of the G × E interaction was assessed using a likelihood ratio test, where the reduced model (excluding the interaction term) was compared to the full model via ANOVA. The variance attributable to the G × E interaction was calculated as the proportion of the G × E variance estimate relative to the total phenotypic variance. Total variance was computed as the sum of the variance components

Fig. 2. Relative expression of *CCK.L* (cholecystokinin), *NPY* (neuropeptide Y), and *SOX2* (SRY-box transcription factor 2) in the brain of brook charr fry produced by parents exposed to cold or warm thermal regimes (PT; parental temperature) during the final gonad maturation. Mean ± SD. Non-transformed data are presented. Linear mixed models were used to assess the effect of PT on the relative abundance of *NPY*, *CCK.L*, and *SOX2*.



for genotype, environment, G × E interaction, and residual variance as described by Lynch and Walsh (1998).

Results

CCK.L, *NPY*, and *SOX2* relative expressions were significantly higher in fry produced by warm parents (Parental temperature (PT): *CCK.L*, $p < 0.05$; *NPY*, $p < 0.01$; *SOX2*, $p < 0.01$) (Figs. 2A–2C, Table 2), without any significant effect of offspring rearing temperature (OT) or their interaction with PT (Table 2). Heritability for *CCK.L* was slightly moderate, decreasing from 15% of the total variance in fry from the cold parental group to 10% in fry from the warm parental group (Fig. 3A; Tables S1 and S2). The sire contribution to the total variance for this gene was 10% in the cold parental group compared to 3% in the warm parental group (Fig. 3C; Tables S1 and S2), while the dam contribution remained very low, accounting for only 0.5% to 1% of the total variance (Fig. 3B; Tables S1 and S2). For *NPY* and *SOX2*, heritability estimates were negligible in fry from both parental groups, with no measurable contributions from either sire or dam (Figs. 3A–3C, Tables S1 and S2).

Relative expressions of *AVT1*, *BDNF*, *CART*, *DCX*, *CIRBPA*, *NEUROD1*, and *PCNA* were not significantly related to temperature conditions of either parents or offspring (Table 2). Heritability estimates for these genes ranged from 0% to 16.8% (Fig. 3A; Tables S1 and S2), with *BDNF* exhibiting the highest heritability. A general decrease was observed in the warm parental group for most genes. Dam contributions ranged from 0% to 18%, generally showing a decline in the warm parental group but increasing considerably for *NEUROD1* and *CART* (Fig. 3B; Tables S1 and S2). Sire contribution was absent for most genes in both parental groups, except for a notable contribution to *CART* expression in fry from the cold parental group (Fig. 3C; Tables S1 and S2). Only one gene (*CCK.L*) showed significant G × E interactions in the cold parental group (Figs. 4 and 5; Tables S3 and S4).

Discussion

In the present study, we evaluated the transcriptional levels of ten genes associated with regulation of the stress response, cellular stress response, appetite regulation, and neurogenesis in the brain of brook charr fry to investigate within generation and transgenerational transcriptional plasticity. The results of this study suggest intergenerational epigenetic inheritance in response to elevated temperatures, supporting those previously obtained with RNA sequencing (Banousse et al. 2024); and underline the absence of genetic variance and parental contributions on the transcript levels of the selected genes.

Transcript levels of three out of the ten measured genes were significantly influenced by parental temperature, specifically genes involved in appetite regulation (*NPY*, *CCK.L*) and neuroplasticity (*SOX2*). None of the genes were influenced by offspring-rearing temperature conditions. This finding may suggest an intergenerational epigenetic inheritance in response to elevated temperature in brook charr as previously suggested by studies on fry from the same experimental design (Venney et al. 2022, whole liver methylome; Banousse et al. 2024, whole brain transcriptome). Furthermore, parental and genetic contributions to the variance

Table 2. Final models retained for each gene analysed.

Genes	Final model	Variable	Estimate	SE	*p*-value	Marg. r^2	Cond. r^2
AVT1 ($\alpha = 0.05$)	AVT1 ~ PT × OT					0.0075	0.0075
		PT	0.254	0.268	0.345		
		OT	0.302	0.279	0.281		
		PT × OT	−0.448	0.375	0.234		
CIRBPA ($\alpha = 0.05$)	CIRBPA ~ PT × OT + (1 | dam)					0.0077	0.1530
		PT	−0.063	0.158	0.691		
		OT	−0.087	0.122	0.478		
		PT × OT	−0.003	0.163	0.984		
DCX ($\alpha = 0.05$)	DCX ~ PT × OT					0.0071	0.0071
		PT	0.040	0.157	0.797		
		OT	0.052	0.163	0.749		
		PT × OT	−0.201	0.219	0.360		
BDNF ($\alpha = 0.025$)	BDNF ~ PT × OT + (1 | dam)					0.0166	0.1595
		PT	−0.325	0.344	0.352		
		OT	0.067	0.267	0.801		
		PT × OT	0.340	0.356	0.340		
PCNA ($\alpha = 0.0125$)	PCNA ~ PT × OT					0.0100	0.0100
		PT	0.023	0.207	0.908		
		OT	−0.363	0.215	0.093		
		PT × OT	0.246	0.289	0.394		
NEUROD1 ($\alpha = 0.016$)	NEUROD1 ~ PT × OT					0.0196	0.0196
		PT	0.089	0.053	0.097		
		OT	0.050	0.055	0.369		
		PT × OT	−0.041	0.074	0.575		
SOX2 ($\alpha = 0.01$)	SOX2 ~ PT + OT					0.0217	0.0217
		PT	0.040	0.012	**0.0017**		
		OT	−0.003	0.012	0.755		
CART ($\alpha = 0.025$)	CART ~ PT × OT + (1 | sire)					0.0261	0.1140
		PT	0.205	0.184	0.273		
		OT	−0.030	0.153	0.844		
		PT × OT	0.077	0.205	0.707		
CCK.L ($\alpha = 0.05$)	CCK.L ~ PT × OT + (1 | dam) + (1 | sire)					0.1112	0.2433
		PT	0.271	0.126	**0.040**		
		OT	0.044	0.094	0.636		
		PT × OT	0.105	0.125	0.401		
NPY ($\alpha = 0.016$)	NPY ~ PT + OT					0.0461	0.0461
		PT	0.168	0.054	**0.0021**		
		OT	0.002	0.053	0.9581		

Note: Estimates, their standard errors, *p*-value, marginal, and conditional r^2 are presented. Linear mixed models (LMMs) were used for *BDNF* (brain derived neurotrophic factor), *CART* (cocaine and amphetamine regulated transcript protein type II), *CCK.L* (cholecystokinin), and *CIRBPA* (cold inducible RNA binding protein). Linear models (LMs) were used for *AVT1* (arginine vasotocin), *DCX* (neuronal migration protein doublecortin), *NEUROD1* (neuronal differentiation 1), *NPY* (neuropeptide Y), *PCNA* (proliferating cell nuclear antigen), and *SOX2* (transcription factor SOX-2). SOX2, SRY-box transcription factor 2. Bold characters indicate significant effect based on the Bonferroni-Holm correction.

Fig. 3. Proportion of expression variance explained by additive genetic (heritability), dam, and sire components for 10 genes grouped by their functional categories (stress response regulation, neuroplasticity, appetite regulation, cellular stress) in brook charr fry produced by cold parental group (blue) and those from warm parental group (orange). (A) Heritability estimates of the gene expression variance in the two parental groups. (B) Dam contribution to the gene expression variance. (C) Sire contribution to the gene expression variance. *AVT1* (arginine vasotocin), *BDNF* (brain-derived neurotrophic factor), *DCX* (neuronal migration protein doublecortin), *NEUROD1* (neuronal differentiation 1), *PCNA* (proliferating cell nuclear antigen), *SOX2* (SRY-box transcription factor 2), *CART* (cocaine and amphetamine-regulated transcript protein type II), *CCK.L* (cholecystokinin), *NPY* (neuropeptide Y), and *CIRBPA* (cold inducible RNA binding protein).

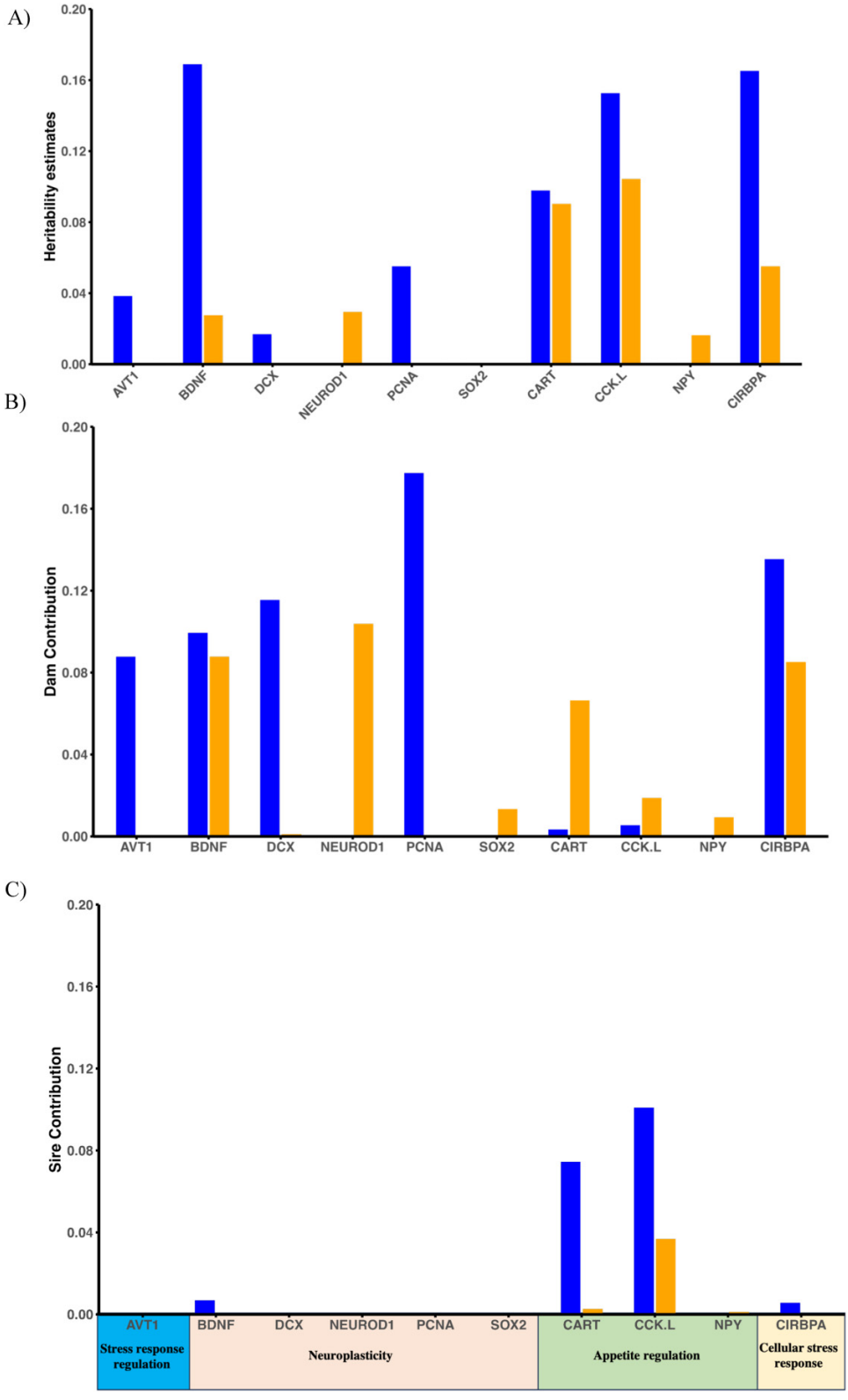
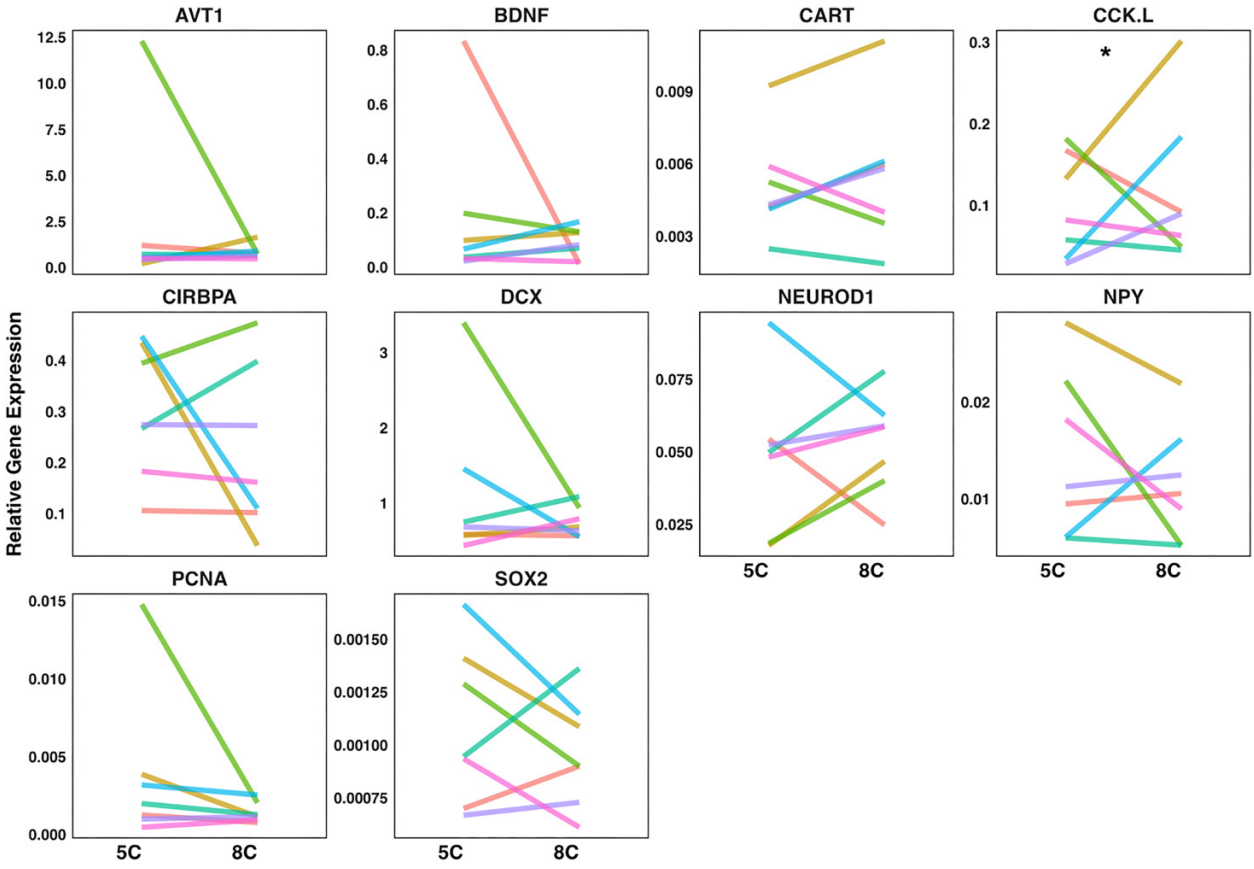


Fig. 4. Reaction norm slopes for the relative gene expression in the brain of brook charr fry from full-sib families within the cold parental group (Genotype, $n = 7$) across two rearing temperature regimes (5 and 8 °C) (environment). Each colored line represents a genotype (full-sib family). Non transformed data are presented. Asterisks indicate significant genotype-by-environment (G × E) interactions based on linear mixed model analysis. *AVT1* (arginine vasotocin), *BDNF* (brain-derived neurotrophic factor), *CART* (cocaine and amphetamine-regulated transcript protein type II), *CCK.L* (cholecystokinin), *CIRBPA* (cold inducible RNA binding protein), *DCX* (neuronal migration protein doublecortin), *NEUROD1* (neuronal differentiation 1), *NPY* (neuropeptide Y), *PCNA* (proliferating cell nuclear antigen), and *SOX2* (SRY-box transcription factor 2).

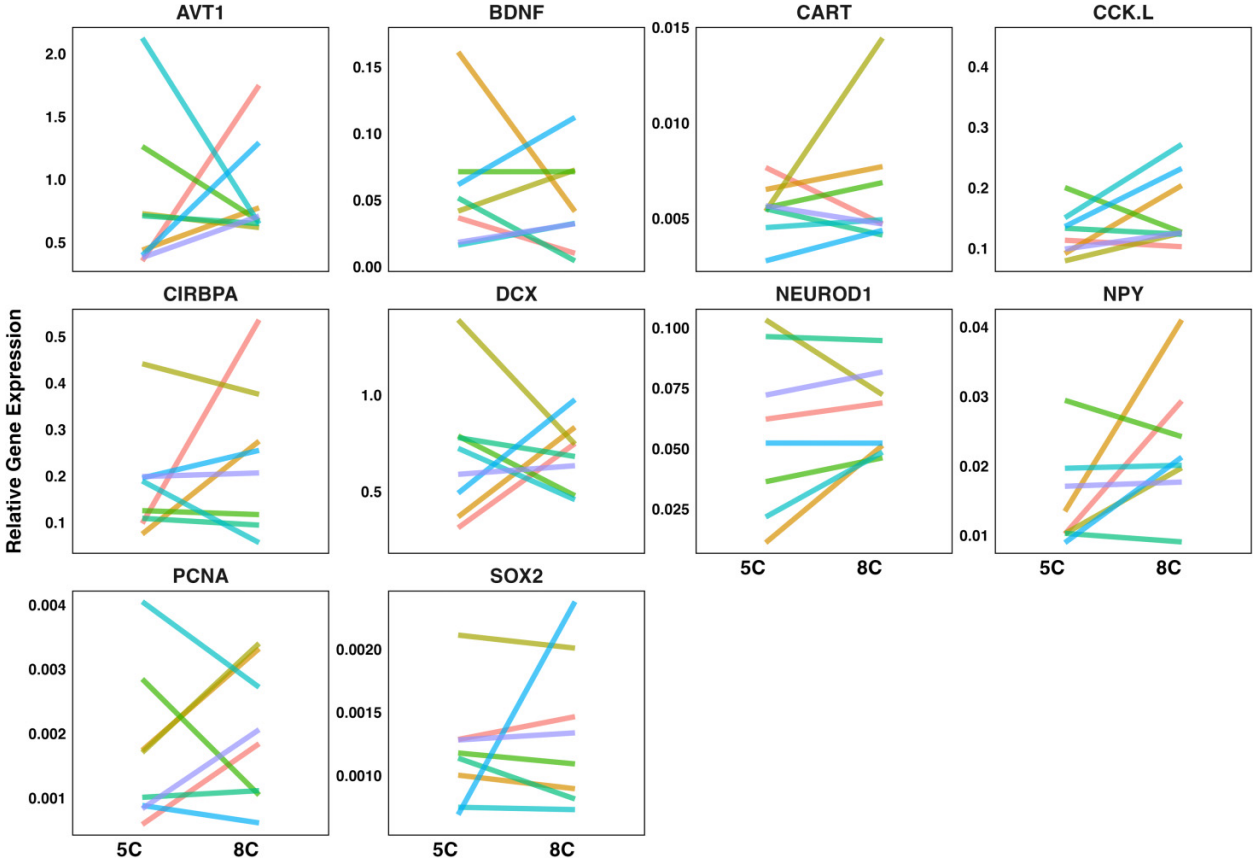


in these transcriptional responses were minimal, indicating that changes were primarily due to environmental factors influencing the parents (epigenetics). Taken together, these results would suggest that environmental pressures may play a more critical role than genetic factors in shaping gene expression in this study. Similar patterns were observed in Chinook salmon *(Oncorhynchus tshawytscha)* exposed to 24 h confinement stress, with minimal genetic effects compared to environmental effects (Wellband et al. 2018). However, this response to environmental variation appears to be species-specific, as such patterns were not evident in sticklebacks (*Gasterosteus aculeatus*) (Leder et al. 2015), and instead, 74%–98% of transcripts exhibited significant additive genetic variance in this species. Studies in humans suggest that the genetic regulation of gene expression can differ substantially between tissue types (Price et al. 2011; GTEx Consortium 2017), and heritability estimates for the same genes can vary depending on the tissue examined. It should be noted that Leder et al. (2015) did not examine the same genes or tissue as ours, which focused on the liver, potentially explaining the observed differences. Bougas et al. (2010) found that 94% of transcripts in whole brook charr fry exhibited significant additive genetic variance, including the *CIRBPA* gene.

Transcript levels of *SOX2* were higher in fry from warm-acclimated parents compared to those from cold parents. *SOX2* is an essential transcription factor for maintaining the properties of neural progenitor stem cells, ensuring they remain in an undifferentiated state until the appropriate time for differentiation. The expression of *SOX2* is typically downregulated just before these progenitor cells differentiate into neural cells, which is a key step in facilitating neurogenesis (Schmidt et al. 2013). Therefore, the observed upregulation of *SOX2* in fry from warm parents may suggest a decrease in neurogenesis. This hypothesis is supported by our previous findings on the whole brain transcriptome of fry from the same experimental design, which showed a significant decrease in numerous biological processes related to neurogenesis in fry from warm-acclimated parents (Banousse et al. 2024).

Fry originating from warm-acclimated parents displayed an upregulation in two appetite-related genes, *NPY* and *CCK.L*. These genes encode for hormones synthesized by neuroendocrine cells in the brain and are involved in food intake reg-

Fig. 5. Reaction norm slopes for the relative gene expression in the brain of brook charr fry from full-sib families within the warm parental group (Genotype, $n = 8$) across two rearing temperature regimes (5–8 °C) (environment). Each colored line represents a genotype (full-sib family). Non transformed data are presented. *AVT1* (arginine vasotocin), *BDNF* (brain-derived neurotrophic factor), *CART* (cocaine and amphetamine-regulated transcript protein type II), *CCK.L* (cholecystokinin), *CIRBPA* (cold inducible RNA binding protein), *DCX* (neuronal migration protein doublecortin), *NEUROD1* (neuronal differentiation 1), *NPY* (neuropeptide Y), *PCNA* (proliferating cell nuclear antigen), and *SOX2* (SRY-box transcription factor 2).



ulation in fish, with *NPY* promoting feeding and *CCK.L* conferring satiety (Volkoff and Rønnestad 2020; Volkoff 2024). Typically, these genes interact to balance food intake, which is especially important for maintaining energy balance with temperature changes (Volkoff 2024). Fish exposed to elevated temperatures generally show upregulation in food intake stimulators and downregulation in food intake inhibitors (e.g., Volkoff and Rønnestad 2020; Volkoff 2024). Thus, while the patterns in the expression level of *NPY* observed in our study aligns with previous studies in fish, the *CCK.L* expression patterns show an opposite trend. For instance, Lustosa et al. (2023) found that *NPY* was significantly upregulated and *CCK* downregulated at higher temperatures in the Amazon fish tambaqui (*Colossoma macropomum*). Similar patterns were observed in red spotted grouper (*Epinephelus akaara*) (Jeon et al. 2020), and cobia (*Rachycentron canadum)* (Nguyen et al. 2023) for *NPY*, and in goldfish (*Carassius auratus)* for *CCK* (Nadermann et al. 2019). Yet, these studies primarily focus on adult fish, unlike ours which examined the first exogenous feeding of fry. Consequently, the observed upregulation of *NPY* could potentially play a role in the brook charr fry ability to acclimate thermally during critical early life stages. However, this hypothesis might be countered by the observed upregulation of *CCK.L* (appetite inhibitor) in fry produced by warm parents, thus affecting their survival. Indeed, survival in brook charr fry produced by the same experimental design showed a decrease of 22% in fry produced by warm parents compared to those originating from cold ones (Gourtay et al. unpublished data).

The evolution of gene expression plasticity in response to environmental changes is shaped by both environmental and genetic factors. In our study, environmental factors, notably temperature, had a clear effect, while genetic variance had a minimal effect on gene expression changes, with the most substantial genetic impact observed on *BDNF* expression, which accounted for only 16.5% of the variance. The average heritability in our study (cold parental group, $h^2 = 57\%$; warm parental group, $h^2 = 3\%$) was lower than that reported in previous studies on Chinook salmon ($h^2 = 20\%$) (Aykanat et al. 2012; Wellband et al. 2018), and Atlantic salmon (*Salmo salar*) ($h^2 = 16.7\%$) (He et al. 2017). The observed low additive genetic variance might indicate insufficient genetic diversity to adapt to the upcoming natural selection pressures. The potential reduction in additive genetic variance may result from

several factors. First, historical natural selection may have differentially affected the genes (Lynch and Walsh 1998). For example, He et al. (2017) found that genes involved in growth had lower additive genetic variance than those involved in energy regulation reflecting differences in selection pressures. Second, genetic variance might also be lost through captive breeding (López et al. 2018). However, the low number of replicates per family could also account for the low additive genetic values obtained. This would need further investigation using wild populations.

Parental thermal environment influenced heritability across most genes, with heritability estimates generally decreasing in fry from cold parental group compared to those from warm parental group. This pattern of lower heritability under unfavorable environments (elevated temperature) has been previously reported in various vertebrates (Charmantier and Garant 2005; Diamond and Martin 2016) including fish (e.g., Atlantic salmon, Garant et al. 2003). The low heritability in traits under unfavorable environments may be due to a decrease in the additive genetic variance and an increase in their environmental components (Falconer 1989). Thus, our results may suggest that the adaptive evolution of transcription variation traits under elevated temperatures in brook charr may be limited, potentially reducing its ability to cope with elevated temperatures over the long term.

Parental effects in brook charr fry were found to be minimal, including both dam and sire influences. Surprisingly, maternal effects were minimal contrasting with our expectations for the significance of maternal contributions during early life stages (Bernardo 1996; Heath et al. 1999; Perry et al. 2004; Wellband et al. 2018). Similar minimal maternal effects were observed in Atlantic salmon at the first feeding stage (He et al. 2017). However, maternal influences might be more significant in fry before yolk sac resorption, as mothers impart various components such as nutrients, hormones, defense elements, maternal mitochondria, and mRNAs to their offspring (Kanaya et al. 1996; Green 2008). In brook charr, maternal effects in early life stages seem to vary depending on the traits studied (Perry et al. 2005; Gourtay et al. 2023).

The genotype (genetic variance) and the environment can interact to influence the evolution of phenotypic plasticity. Different genotypes can respond differently to the same environmental change, resulting in non-parallel reaction norms, a phenomenon known as genotype by environment interaction (G × E) (Falconer and Mackay 1996; Lynch and Walsh 1998). In our study, only one gene (*CCK.L*) out of the 10 genes studied showed a significant G × E interaction in the cold parental group, suggesting that the genetic factors underlying the gene expression variation in response to offspring-rearing temperatures are consistent across genotypes and may evolve in a predictable manner. This could be advantageous for brook charr breeding programs, as it ensures consistent performance of selected genotypes across varying rearing thermal conditions. However, the significant G × E interaction observed in *CCK.L*, involved in appetite regulation, may indicate that specific traits or pathways (e.g., growth) may still exhibit genotype-specific responses to environmental changes. In brook charr, significant G × E interactions were reported in the expression of two genes involved in the physiological pathway of growth (*GHR* and *IGF*) in response to different rearing environments (freshwater vs. saltwater) (Côté et al. 2007). Similarly, brook charr body mass exhibited significant genotype by environment interactions in response to varying temperature rearing (Crespel et al. 2013).

In the present study, the GEN-FISH salmonid Brain chip enabled us to detect the expression of 10 out of the 24 genes initially present on the chip. This study demonstrated that the GEN-FISH salmonid Brain chip can be successfully used for addressing aquaculture and wildlife health-related issues (Semeniuk et al. 2022). The absence of expression in the remaining 14 target genes might be attributed to their low expression levels in brook charr fry, as some genes are expressed only in specific brain regions rather than throughout the whole brain tissue or are activated only at particular developmental life stages (Abdellaoui and Kim 2024). Further, we were unable to retrieve sequences from some genes (*AGRP*, *POMC*, *CRF1*) from a brook charr brain transcriptome. By employing a target gene approach, we were able to partition the variance in gene expression into environmental and genetic factors, which enhanced our predictions on the evolutionary potential of captive-reared populations of brook charr to elevated temperatures. In conclusion, in brook charr, the parental thermal environment affects the plasticity of gene expression related to neuroplasticity and appetite regulation, with minimal genetic influence and genotype by environment interaction.

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Data availability

The data and codes underlying this manuscript are available in Figshare at https://doi.org/10.6084/m9.figshare.26298964.v1.

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Author contributions

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Data curation: GB, CA
Formal analysis: GB, DG, CA
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Supervision: CADS, CA
Validation: CADS, DG, CA
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Competing interests

The authors declare no conflict of interest.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2024-0205.

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