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Exposure to cold temperatures differentially modulates neural plasticity and stress responses in post-smolt Atlantic salmon (*Salmo salar*)

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ABSTRACT

The transfer success of farmed post-smolt Atlantic salmon (*Salmo salar*) to sea-cages rely on neural adaptions to promote stress resilience. As low temperatures impact physiology, this suggests that off-season transfer to cold waters may be challenging. To address this, post-smolts reared at 13 °C seawater were abruptly transferred to 10 °C, 7 °C, and 4 °C, then acclimated to these respective temperatures for 58-days followed by an acute challenge test (ACT) using confinement stress. Plasma and brain samples were collected after i) the abrupt temperature transfer at 1-h and 1-day, ii) 58-days of acclimation, and iii) 1-h post ACT. In tandem to measuring plasma cortisol levels, the expression of key genes involved in telencephalic regulation (*crf, crfbp, mr, gr1, gr2* and *hsd11b2*) and neural plasticity (*neurod, bdnf, pcna,* and *cfos*) were analyzed. Post-smolts exposed to the 7 °C and 4 °C displayed the largest alteration in telencephalic functions, differentially regulating *mr* and *gr1,* to elevate the *mr/g1* ratio for downregulating Gr1, proposing an elevated stress loads. After acclimation, these coincided with blunted stress responses capacities to ACTs for both cortisol and telencephalic neural activity (*cfos*), suggesting a continuation of challenges and reduced the capacity to mount a stress response. Concomitantly, these telencephalic alterations in CRs coincided with a differential modulation in neural plasticity, measured as elevated *bdnf* and *neurod* during the abrupt transfer period (acute) and after acclimation (prolonged), respectively, revealing neural responses are still robustly maintained to retain a degree of stress resilience. However, exposure of post-smolts to 4 °C clearly induced the most adverse and suppressive effects in telencephalic functions, cued by a suppression in *pcna* and stress response capacities, downregulation in the CRF system, and largest elevation in the *mr/g1* ratio. Conversely, acclimating post-smolts to 7 °C elevated *11hsdb2* proposing a greater inhibition of cortisol action that may point to still adequate maintenance of CR and neural processes. Taken together, these findings show that cold temperatures alter key neural processes required for maintaining proper stress management, providing an alternative explanation for reductions in fish stress reactivity commonly observed with declining temperature. Therefore, exposing post-smolts at 13 °C to temperature reductions of 6 °C or greater should be avoided in aquaculture.

1. Introduction

With year-around production, farmed Atlantic salmon (*Salmo salar*) are at increasing risk to experience greater fluctuations in ambient temperature. Abrupt and long-term exposure to temperature variations can have significant physiological consequences, that can modify physiological stress responses and limit capacities to handle additional challenges especially when thermal limits are approached, therefore implicating post-smolt welfare and survival (Claireaux et al., 1995; Crawshaw, 1979; Donaldson et al., 2008; Friedlander et al., 1976; Madaro et al., 2018; Sigholt and Finstad, 1990; Tanck et al., 2000; Van Den Burg et al., 2005, 2006). The first few months after sea cage transfer, known as the early post-smolt phase, is considered the most vulnerable for the salmon lifecycle. Currently, fish are increasingly raised longer in land-based systems at higher temperatures allowing year-round stocking to sea-cages. Therefore, transferring fish during

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"off-season" can facilitate both rapid and prolonged exposures to cold temperatures due to transport, stocking and rearing activities, to which fish may not have the necessary acclimation history. Additionally, fish will also have to simultaneously resist a variety of anthropogenic stressors derived from confinement, handling and transport (Handeland et al., 2003; Jarungsriapisit et al., 2016). Therefore, the transfer success of post-smolts to new rearing conditions will ultimately rely on rapid acclimation, appropriate stress responses, and cognitive functions to promote stress resilience during this critical lifecycle period (Ebbesson and Braithwaite, 2012; Handeland et al., 2014; Jarungsriapisit et al., 2016).

Cortisol, under strict control of the hypothalamus-pituitary-interrenal (HPI)-axis, is critical for mediating an array of cellular functions essential for stressor adaption and re-establishing homeostasis (Mommsen et al., 1999; Wendelaar Bonga, 1997). These actions are sustained through dimerizations with corticosteroid receptors (CRs), including mineralocorticoid receptor (Mr) and glucocorticoid receptors (Gr1 & Gr2) (Mommsen et al., 1999; Wendelaar Bonga, 1997). The Mr and Gr2, having high cortisol affinity, are assumed to regulate baseline functions during low stress levels, while Gr1 with low cortisol affinity is activated during high stress loads (De Kloet et al., 2020; Stolte et al., 2008). However, when cortisol stimulation becomes excessive during periods of allostatic overloads, this can perturb the regulation of CRs as commonly reported for the downregulation of Gr1 functions, that acts as a protective measure against receptor overstimulation (De Kloet et al., 2005; Madaro et al., 2016, 2015). While given the importance of CRs in regulating brain functions, any alterations in their regulation can disturb crucial stress functions such as shift stress response capacities or impact neural plasticity, that may limit the animal's ability to combat additional stressors (Sørensen et al., 2013; Madaro et al., 2015). Therefore, although short-term exposure to stress response activation is critical for promoting stress resilience and re-establishing homeostasis, prolonged activation can detrimentally impact CR regulation, manifesting as changes to brain plasticity and cognition that hamper stress resilience (Sørensen et al., 2013). Furthermore, the importance of 11β-hydroxysteroid dehydrogenase type 2 (Hsd11b2) should not be undermined, as this inactivates cortisol by conversion to cortisone, thereby regulating the availability of cortisol to stimulate Mr and Gr, providing an important layer of feedback for maintaining normal CR functions and the development of neural processes (Alderman and Vijayan, 2012; Mifsud and Reul, 2018). Alternatively, the neural activation of the stress axis is also tightly regulated by the corticotropin-releasing factor (Crf) system which stimulates the release of adrenocorticotropic hormone (Acth) from the pituitary, in turn, activating head kidney cortisol release (Flik et al., 2006; Gorissen and Flik, 2016). As the actions of Crf are inhibited by CRF binding proteins (Crfbp) via direct dimerization, the interplay between these components play important functions in regulating HPI-axis reactivity during stress conditions as demonstrated in goldfish (*Carassius auratus*) (Fryer et al., 1984), trout (*Oncorhynchus mykiss*) (Baker et al., 1996), sea bream (*Sparus auratus*) (Rotllant et al., 2000), and carp (*Cyprinus carpio*) (Manuel et al., 2014b). However, to date most Crf studies in fish have predominately focused on the hypothalamus or pituitary and less so on its role in the telencephalon. Therefore, as elevated stress loads are commonly indicated by altered expression and ratios of *crf*, *crfbp*, *hsd11b2*, *mr*, *gr2* and *gr1* (Herrera et al., 2021; Madaro et al., 2016, 2015; Martos-Sitcha et al., 2014; Samaras et al., 2018), these provide good proxies for shifts in allostatic loads, which may be associated with modulations of vital telencephalic functions (Manuel et al., 2016; Sørensen et al., 2013). Moreover, due to the tetra-ploidy of the Atlantic salmon genome (Lien et al., 2016), additional paralog genes may allow differential regulation to chronic and/or acute stress, promoting elevated plasticity (Macqueen and Johnston, 2014). Hence, paralog specific analysis of these telencephalic regulatory genes was implemented.

Proper cognitive functions of the fish telencephalon rely on modulations to neural plasticity and neurogenesis for learning, memory and decision making, whereby stress can either stimulate or inhibit these processes depending on severity and duration (Ebbesson and Braithwaite, 2012; Grassie et al., 2013; Salvanes et al., 2013; Sørensen et al., 2013). In turn, changes in neuroplasticity and neurogenesis can modulate behavioral flexibility of fish to environmental challenges, therefore impacting stress resilience (Ebbesson and Braithwaite, 2012; Grassie et al., 2013; Salvanes et al., 2013; Shors et al., 2012; Vindas et al., 2017). Neurogenic differentiation factor (Neurod) is a member of pro-neural genes involved in the initiation and regulation of neural differentiation, and is a reliable indicator for neuroplasticity in fishes (Ebbesson and Braithwaite, 2012). Similarly, proliferating cell nuclear antigen (pcna) is commonly used as a marker for cellular proliferation, therefore provides a good proxy for neurogenesis (Sadoul et al., 2018; Sørensen et al., 2011). On the other hand, brain-derived neurotrophic factor (bdnf) is expressed in mature neurons, involved in promoting synapse refinement, neurogenesis and cell survival, allowing for rapid modifications in coping strategies and behavioral phenotypes to manage large stress loads (Manuel et al., 2014a; Mes et al., 2018; Vindas et al., 2017). Conversely, the transcription factor c-fos belongs to a class of immediate early genes (IEG), which are rapidly expressed in neurons following stimuli, providing a marker for neuronal activity (O'Connell and Hofmann, 2011; Pavlidis et al., 2015). Together with plasma cortisol profiles, the use of IEGs such as *cfos*, reveals a refined approach to monitor differences in stress response capacities to different environmental stress loads (Mes et al., 2018). Taken together, these illustrate the importance of neural plasticity for proper management of stressors, therefore as stress is known to alter these functions, their potential as markers for determining changes in fish stress resilience is promising. Furthermore, as evidence in mammals suggests stressors act through CR activity to modify neural plasticity, the analysis of these in conjunction may provide further insight to the regulation of stress over neural plasticity (Chen et al., 2017; Manuel et al., 2016; Sadoul et al., 2018; Sørensen et al., 2013).

Currently, limited knowledge exists on the neural adaptations to stress in post-smolts, and how temperature influences these processes. Although the magnitude of stress is known to modify cortisol concentrations, the thermal difference in HPI-axis activation and circulating levels of cortisol are assumed to originate purely due to altered rates of metabolism (Madaro et al., 2018). In contradiction, others argue that the activation of the HPI-axis proceeds independently of metabolism (Pankhurst, 2011), aligning to a recent study confirming thermal telencephalic modifications, suggesting that temperature itself acts as a stressor in European seabass (*Dicentrarchus labrax*) (Goikoetxea et al., 2021). Therefore, as cold temperatures can be physiologically challenging, this study aims to address the influence of cold exposure on these pathways involved in stress reactivity and telencephalic functions, critical for stress resilience during the post-smolt phase. Finally, as fish may be able to maintain homeostasis in stable conditions compared to exposed ones, differences in the underlying physiology may affect their capacity to mount a stress response, which can be revealed by subjecting fish to an additional stressor known as an acute challenge test (ACT) (Madaro et al., 2016, 2015; Samaras et al., 2018). Therefore, in this study a confinement stressor in a novel environment was implemented as the ACT (Samaras et al., 2018). In this study, we determined the effects of abrupt and prolonged cold exposure on post-smolt circulating plasma cortisol concentrations and telencephalic changes to stress (*crf*, *crfbp*, *mr*, *gr2*, *gr1*, cortisol) and neural (*neurod*, *bdnf*, *pcna*, *cfos*) markers, aiming to observe their contribution to differences in stress resilience.

2. Material and methods

2.1. Fish, experimental procedures and tissue sampling

All experimental procedures were approved by Norwegian Animal Research Authority (Identification number 8017). On May 25th, 496 Atlantic salmon smolts (48.4 ± 1.2 g and 16.6 ± 0.2 cm) were

transported from Vik smolt facility, Øygarden, to the Industry Laboratory at the High Technology Centre in Bergen. Fish were kept in four $1m^2$ square tanks (rearing volume of 500 L) with flow-through freshwater at 12 °C, resulting in biomass of 6.05 kg/m^3. On 1st of June, salinity was increased to 15 ‰, followed by a further increase to 25 ‰ seven days later, and then full-strength seawater (34 ‰) on 14th of June to smoothen SW acclimation (Calabrese et al., 2017). The fish were exposed to continuous light throughout the whole experiment and fed a commercial dry diet (EWOS Microboost 30) continually in excess using automatic feeders. On the 13th of July, post-smolts were randomly distributed equally into eight $1m^2$ square tanks supplied with seawater at 13 °C, resulting in a biomass of 5.1 kg per tank. On August 15th four temperature treatments containing two replicates per treatment were initiated. The four treatments included adjusting water temperature to 10 °C, 7 °C and 4 °C within 30 min, with a control group maintained at 13 °C. Temperature and oxygen saturation (>80%) of water outlet of each tank was measured continuously.

Fish were starved for 15 h before each sampling and 10 fish per treatment (5 fish per duplicate) were sampled for tissues. For samplings, fish were anesthetized using a lethal dose (100 mg l^{-1}) buffered tricaine methanesulphonate (MS222, Sigma-Aldrich, St Loius, MO, USA). Samplings were conducted 1-h (h) and 1-day post temperature reduction and represent the acute temperature "transfer" phase. Following this, post-smolts were kept at the respective temperatures for 58-days before the next sampling to represent the "acclimation" state and ACTs. During this sampling, 20 fish were quickly dip netted out and 10 fish immediately anesthetized for tissues, while the remaining 10 fish were subjected to an Acute Challenge Test (ACT) to observe differences in the capacity to mount a stress response. This applied confinement stress as a novel stressor for 15 min in a new rearing environment using a 15-L tank inside a 150-L holding tank (receiving water from the original treatment). After this, fish were released in the holding tank for 45-min to recover before receiving a lethal dose of MS222 and sampled. Blood from all samplings was collected from the caudal blood vessel using a heparinized syringe and centrifuged (10 min at 4 °C, 3000 rcf), with resultant plasma stored at −80 °C until analysis. At day-1, −58 and 1-h post ACT, whole brain was additionally collected and preserved in RNAlater (Ambion, Foster City, CA, USA) for 24 h at 8 °C prior to being stored at −80° until gene analysis.

2.2. Plasma cortisol analysis

Plasma cortisol concentrations were measured using a custom ELISA analysis, previously validated and published for Atlantic salmon (Madaro et al., 2016, 2015).

2.3. Extraction of RNA, cDNA synthesis and quantitative real-time PCR (qPCR)

Total RNA was extracted from whole telencephalon by the phenol-chloroform method using TRI Reagent® (Sigma, St. Louis, MO, USA) in accordance with Simms et al., 1993. RNA purity was measured using a Nanodrop® ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), while concentration was determined using Qubit™3 Instrument (Thermo Fisher Scientific, Eugene, Oregon). RNA integrity values (RIN) between 8 and 10 on the Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip® kit (Agilent technologies, Palo Alto, CA, USA) indicated sufficient quality. cDNA synthesis was reverse transcribed using 800 ng of total RNA input in concert with oligo (dT) 20 primers and SuperScript III kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer protocol.

RT-qPCR was conducted using gene specific primers (Table. 1) and SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) on the CFX-96 Real-Time PCR detection system platform (Bio-Rad Laboratories, Inc., CA, USA). For each RT-qPCR reaction 3 µl of cDNA, 0.25 µl of forward primer (10 µmol l^{-1}), 0,25 µl of reverse primer (10 µmol l^{-1}), 3.25 µl of DEPC treated dH_2O and 6,25 µl SYBR Green Master Mix was used to reach a final reaction volume of 13 µl. Melt-curve analysis of primer-sets ensured amplification of only a single product with no detectable primer-dimer artefacts. Primer amplification efficiencies (E) were generated by running a 2-fold dilution series (1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640) (pool) in triplicates and ensuring optimal primer efficiency between 1.8 and 2.2. Telencephalon cDNA samples were assayed at a 1:20 dilution. The E were determined by the slope of the regression line a plot of log cDNA dilution versus threshold cycle (Ct) using the formula; $E = 10^{-1/slope}$. Normalized efficiency corrected relative gene expression was calculated according to (Pfaffl, 2004) using the endogenous reference gene elongation factor 1a (*ef1a*). For each qPCR plate a duplicate NTC control was used to ensure no contamination. Plate inter-calibration was achieved using a duplicate pool run on each plate.

2.4. Statistical analysis

For all data, normality of distributions and homogeneity of variance was tested using D'Agostino & Pearson test and Brown-Forsythe test, respectively, followed by One-Way ANOVA tests and Tukey's multiple comparisons to compare differences between treatments within samplings and within a treatment between sampling points. If data did not pass the normality or homogeneity tests, even with data transformations, non-parametric Kruskal-Wallis test followed by Dunn's test were used. Significance level was set at $p < 0.05$. Outlier estimation was identified using Whisker's boxplots (Tukey's) followed by ROUT1% outlier analysis. Statistical analysis was performed using Graphpad

Table 1
List of gene-specific primers and sequences.

Gene name	5′-3′ Forward primer sequence	5′-3′ Reverse primer sequence	Reference
ef1a	CCCCTCCAGGACGTTTACAAA	CACACGGCCCACAGGTACA	(Olsvik et al., 2005)
crfα (crf1a2)	GCACTTGATCCATTCCACAA	ACCGATTGCTGTTACCGACT	(Lai et al., 2021)
crfβ (crf1b1)	TCCATCACTCGTGGAAAAGGA	CAGGGGTTCAACGAGATCTTCA	(Lai et al., 2021)
crfbp	AATGGCCCCGCCCAGAT	ATATAGGAGGTGGAGAGATAGAT	Designed by Authors
hsd11b2α	GTCTAACTACCGTGGCTGTATG	GAACGGCTGCTCTCCTG	Designed by Authors
hsd11b2β	CAAGACAGGTCAGTCTAGTAAC	TCACGGGTGTAGTCCTC	Designed by Authors
mr	AGACTCGACCCCCACCAAG	CGTTAGTGGGACTGGTGCTC	F primer (Kiilerich et al., 2007), R primer (Nilsen et al., 2008)
gr1α	TGCTCAGCACAGTACCAAAG	GAGTTCTCTTCCCGCTTGAC	Designed by Authors
gr1β	TCAGCTTCAGCAGTCCAAC	ACACACCAGGCAGATCTTATG	Designed by Authors
gr2α	AGTGTTCCTGGTTGTTCCTC	TTCATACGGTCCTGGTTGATG	Designed by Authors
gr2β	AAGTCTTTGCCAGGGTTCC	TGTTCCCGTCACACTGTTG	Designed by Authors
neurod	CAATGGACAGCTCCCACATCT	CCAGCGCACTTCCGTATGA	(Mes et al., 2020)
bdnf	ATGTCTGGGCAGACCGTTAC	GTTGTCCTGCATTGGGAGTT	(Mes et al., 2020)
pcna	TGAGCTCGTCGGGTATCTCT	CTCGAAGACTAGGGCGAGTG	(Mes et al., 2020)
cfos	AATGGAACAGCTTTCGCCTGA	TGTCGGTGAGTTCCTTTCGC	(Mes et al., 2018)

Prism 8 (version 8.3.0). All data are given as means ± SEM.

3. Results

3.1. Plasma cortisol

At 1 h post-transfer, plasma cortisol concentrations (Fig. 1) significantly increased in post-smolts transferred to 10 °C, 7 °C and 4 °C compared to the 13 °C control group (Fig. 1). By day 1, cortisol concentrations declined in all the treatment groups with post-smolts at 10 °C ($p < 0.05$) and 4 °C ($p < 0.05$) having lower cortisol concentrations compared to the 13 °C group. Fish exposed to 7 °C maintained similar cortisol levels as to the 13 °C group, however, displayed higher levels than the 4 °C group ($p < 0.05$). Following acclimation, plasma cortisol concentrations were between 0.4 and 8.0 ng/ml containing no differences between experimental groups (Fig. 1). Conversely, all groups responded to ACTs with elevated plasma cortisol concentrations compared to the respective pre-ACT state (13 °C $p < 0.0001$, 10 °C $p < 0.001$, 7 °C $p < 0.0001$, 4 °C $p < 0.05$), while the cortisol response showed to being lower in post-smolts acclimated to 7 °C ($p < 0.05$) and 4 °C ($p < 0.001$) compared to the 13 °C group.

3.2. Telencephalon

3.2.1. Acute temperature transfer (Day 1)

3.2.1.1. Telencephalic regulation. One day after the temperature transfer, no effect was observed for the telencephalic expression of *crf (α, β)*, *crfbp* and *hsd11b2 (α, β)* expression (Fig. 2a, b, c, d, e). Conversely, the telencephalic expression of *mr* and both paralogs of *gr2* (*α, β*) were significantly elevated in post-smolts transferred to 4 °C ($p < 0.05$) compared to the 10 °C and 7 °C groups (Fig. 2f, i, j). Contrastingly, compared to the 13 °C group, the expression of both paralogs of *gr1* (α, β) (Fig. 2g, h) significantly decreased in fish transferred to 7 °C ($p < 0.05$), while the other paralog, *gr1α* (Fig. 2g) also showed lower expression in the 10 °C group. This differential regulation of *mr* and *gr1* increased the *mr/g1* ratio (Fig. 3a, b) for both paralogs of *gr1* (α, β) with increasing magnitude of temperature reduction. Compared to 13 °C, the *mr/gr1* ratio (Fig. 3a, b) was elevated in post-smolts exposed to 4 °C ($p < 0.0001$), 7 °C ($p < 0.0001$) and 10 °C ($p < 0.05$), with the 4 °C group also displaying a significantly higher *mr/gr1* ratio (Fig. 3a, b) compared to the 10 °C group. Simultaneously, the telencephalic *gr1/gr2* ratio (Fig. 3efgh) also significantly decreased in fish at 7 °C and 4 °C compared to the 10 °C and 13 °C groups. Moreover, an increase in telencephalic *mr/gr2β* ratio (Fig. 3d) was observed at 10 °C compared to the 4 °C ($p < 0.01$) and 13 °C group ($p < 0.0001$).

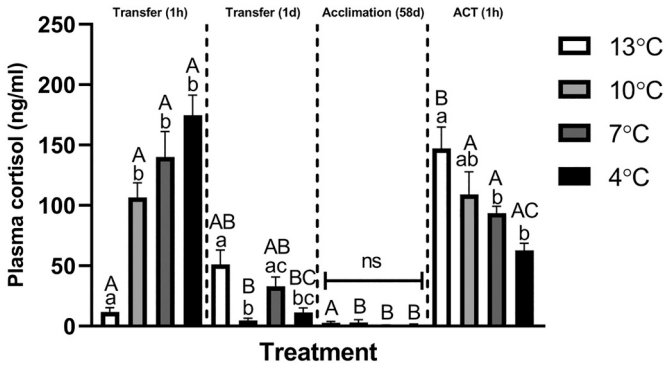

Fig. 1. Changes in plasma cortisol concentrations after 1-h and 1-day post thermal transfer, 58-days of acclimation and 1-h post-ACT confinement stressor. Values are presented as average ± SEM of each treatment ($n = 8$–10 per treatment/sampling). Uppercase letters indicate significant differences within a temperature treatment between samplings, while lowercase letters indicate a significant difference between temperature treatments within a sampling. A significant difference was held at $P < 0.05$.

3.2.1.2. Neural plasticity. The telencephalic expression of *bdnf* (Fig. 4c) was significantly elevated in post-smolts exposed to 7 °C and 4 °C compared to the 13 °C group, while fish exposed to 4 °C also displayed lower *pcna* expression (Fig. 4b). Finally, the expression of *cfos* (Fig. 4c) was significantly lower in post-smolts exposed to 10 °C compared to the rest of the exposure groups.

3.2.2. Acclimation (Day 58)

3.2.2.1. Telencephalic regulation. After acclimation to the respective temperatures (13 °C, 10 °C, 7 °C and 4 °C), the telencephalic expression of both paralogs of *crf* (α, β) was lower in post-smolts acclimated to 4 °C compared to the 13 °C group ($p < 0.05$) (Fig. 2a, b), while the expression of *crfβ* (Fig. 2b) was also lower in post-smolts at 4 °C compared to the 10 °C ($p < 0.05$) and 7 °C ($p < 0.05$) groups. Conversely, the expression of *crfbp* (Fig. 2c) was significantly elevated in post-smolts acclimated to 4 °C ($p < 0.05$) compared to fish at 7 °C, while no differences were observed for fish acclimated to 13 °C and 10 °C. The expression of both paralogs of *hsd11b2* (α, β) (Fig. 2d, e) was higher only for post-smolts acclimated to 7 °C ($p < 0.05$) compared to the 13C group, with no detectable differences for fish at 10 °C or 4 °C. Surprisingly, the expression of CRs at 7 °C and 4 °C inversely mirrored those observed during the abrupt transfer period (Fig. 2f, g, h, i, j), with *mr* expression (Fig. 2f) being significantly elevated for post-smolts acclimated to 7 °C compared to the 4 °C and 10 °C groups, while both *gr1* paralogs (α, β) (Fig. 2g, h) having lower expression in fish acclimated to 4 °C compared to the 7 °C and 13 °C groups. This differential regulation between *mr* and *gr1* increased the *mr/gr1* ratio (Fig. 3a, b) towards the colder temperatures, with post-smolts acclimated to 7 °C and 4 °C having a significantly elevated ratio compared to the 10 °C and 13 °C groups, while the *mr/gr1α* ratio (Fig. 3a) displayed an additional elevation in post-smolts acclimated to 4 °C ($p \langle 0,01$) compared to the 7 °C group. In contrast, post-smolts at 7 °C showed significantly higher *gr2α* expression compared to fish at 4 °C, however, no effect was observed for the *mr/gr2* ratios (Fig. 3c, d). Finally, the *gr1/gr2* ratios (Fig. 3e, f, g, h) significantly decreased in post-smolts acclimated to 10 °C, 7 °C and 4 °C compared to the 13 °C group, while fish at 4 °C had an even lower *gr1/gr2* ratio compared to the 10 °C and 7 °C groups (Fig. 3g, h).

3.2.2.2. Neural plasticity. The expression of *bdnf* (Fig. 4c) and *cfos* (Fig. 4d) were unaffected by acclimation temperature at a steady state. In contrast, coinciding with the elevation in *mr/gr1* ratio (Fig. 3a, b), post-smolts acclimated to 7 °C ($p < 0.001$) and 4 °C ($p < 0.05$) displayed elevated *neurod* expression (Fig. 4a) compared to fish at 13 °C. In contrast, *pcna* expression (Fig. 4b) remained lower in post-smolts acclimated to 4 °C compared to fish at 7 °C ($p < 0,05$), 10 °C ($p < 0.0001$) and 13 °C ($p < 0.05$), reminiscent to that observed during the initial transfer period.

3.2.3. ACTs

3.2.3.1. Telencephalic regulation. The expression profiles of ACT fish in general mirrored those observed during the pre-ACT state, with no significant changes in the expression observed for *crfα*, *crfβ*, *crfbp*, *hsd11b2β* and *gr1β* in response to the ACTs (Fig. 2a, b, c, e). However, although no differences in *crf* expression (Fig. 2a, b) was detected between stressed post-smolts, fish acclimated to 4 °C maintained significantly elevated expression of *crfbp* (Fig. 2c) compared to the 13 °C group. Conversely, post-smolts acclimated 4 °C displayed a down regulation in *hsd11b2α* (Fig. 2d) compared to the respective pre-ACT state ($p < 0.05$), despite the expression profile being otherwise maintained similar to the respective pre-ACT state, with stressed post-smolts acclimated to 7 °C

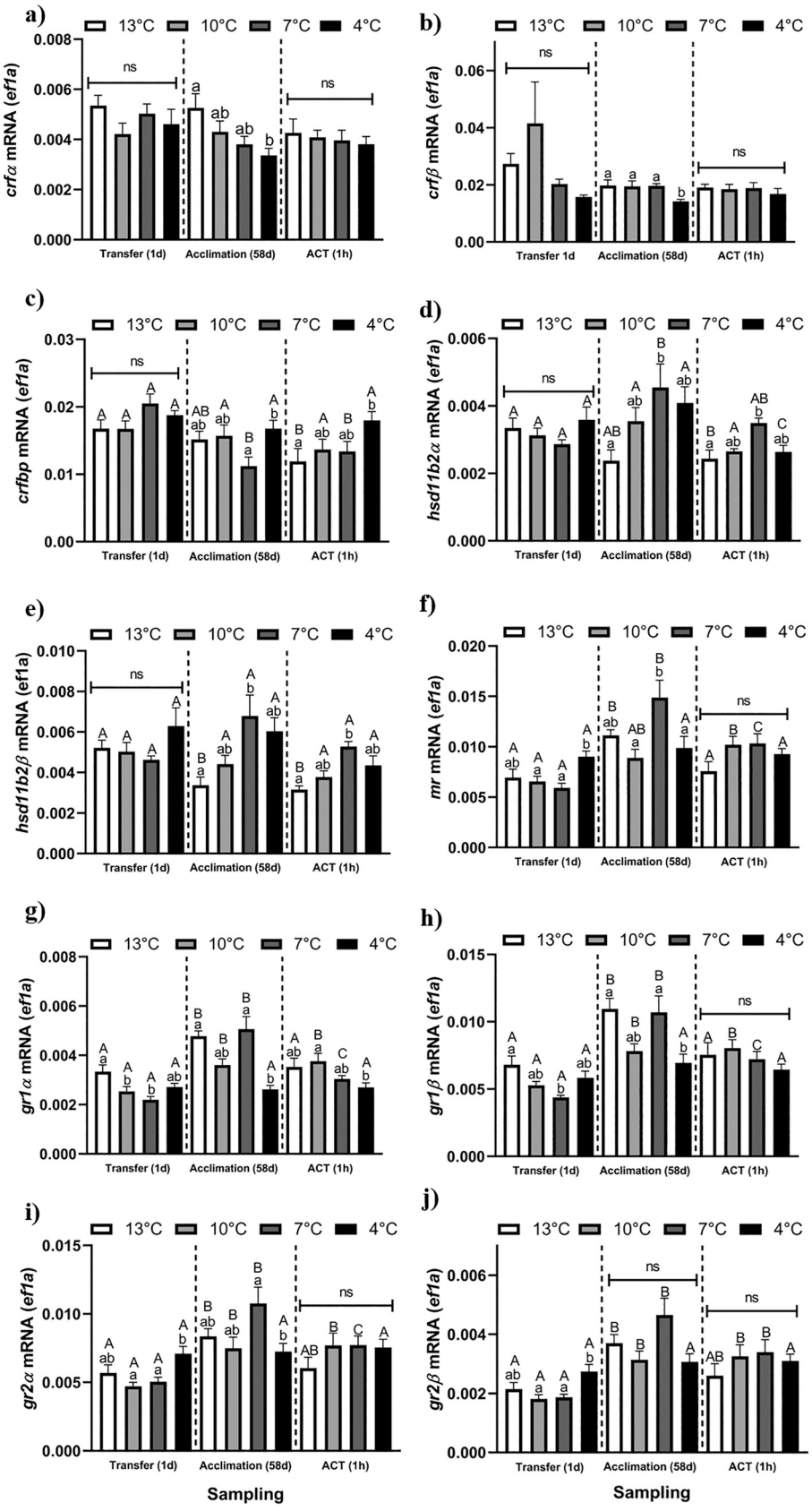

Fig. 2. Changes in whole telencephalon expression of **a)** corticotropin releasing hormone paralog (*crfα*), **b)** corticotropin releasing hormone paralog (*crfβ*), **c)** corticotropin releasing hormone binding protein (*crfbp*), **d)** hydroxysteroid 11-β dehydrogenase 2 paralogα (*hsd11b2α*), **e)** hydroxysteroid 11-β dehydrogenase 2 paralogβ (*hsd11b2β*), **f)** mineralocorticoid receptor (*mr*), **g)** glucocorticoid receptor 1 paralogα (*gr1α*) **h)** glucocorticoid receptor 1 paralogβ (*gr1β*), **i)** glucocorticoid receptor 2 paralogα (*gr2α*) and **j)** glucocorticoid receptor 2 paralogβ (*gr2β*) after 1-day post transfer, 58-days of acclimation and 1-h post-ACT confinement stressor. Values are presented as averages ± SEM of each treatment (n = 8–10 per treatment/sampling). Uppercase letters indicate significant differences within a temperature treatment between samplings, whilst lowercase letters indicate a significant difference between temperature treatments within a sampling. A significant difference was held at $P < 0.05$.

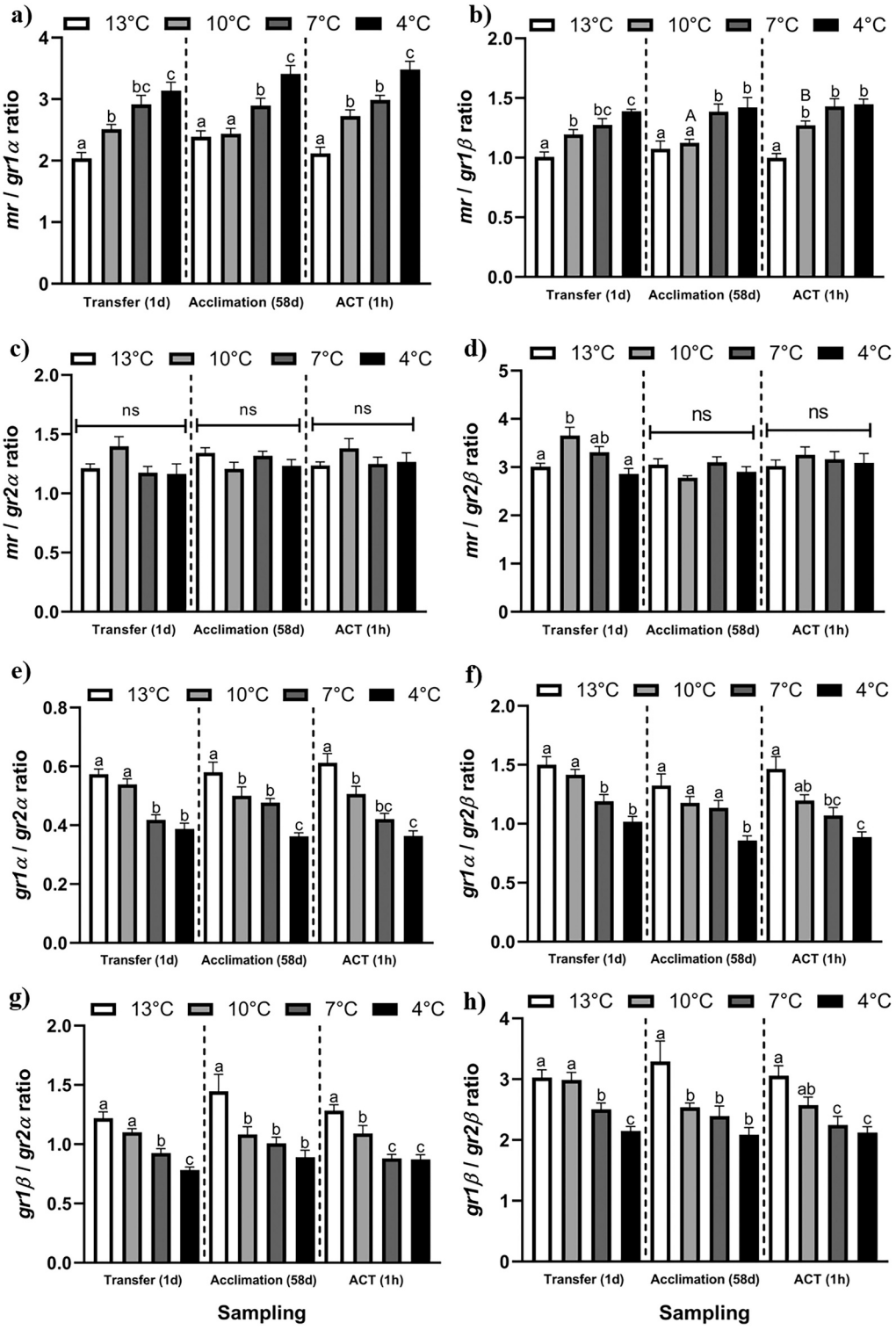

Fig. 3. Whole telencephalic changes in major CR ratio **a)** *mr/gr1α*, **b)** *mr/gr1β*, **c)** *mr/gr2α*, **d)** *mr/gr2β*, **e)** *gr1α/gr2α*, **f)** *gr1α/gr2β*, **g)** *gr1β/gr2α*, and **h)** *gr1β/gr2β* after 1-day post-thermal transfer, 58-days of acclimation and 1-h post-ACT confinement stressor. Values are presented as average ± SEM of each treatment (n = 8–10 per treatment/sampling). Uppercase letters indicate significant differences within a temperature treatment between samplings, whilst lowercase letters indicate a significant difference between temperature treatments within a sampling. A significant difference was held true at $P < 0.05$.

having significantly elevated expression compared to the 13 °C group. Contrastingly, post-smolts acclimated to 13 °C and 7 °C significantly downregulated their expression of *mr* (Fig. 2f) and both paralogs of *gr1* (α, β) (Fig. 2g, h) compared the respective pre-ACT state, however, this change in expression had no effect on corresponding *mr/gr1α* (Fig. 3a), *mr/gr2* (Fig. 3c, d) or *gr1/gr2* (Fig. 3e, f, g, h) ratio, which mirrored values seen during pre-ACT conditions. Only did stressed fish acclimated to 10 °C demonstrate a significant increase in *mr/gr1β* ratio (Fig. 3b) compared to the respective pre-ACT state, with stressed post-smolts acclimated to 4 °C, 7 °C and 10 °C having a significantly higher ratio compared to stressed fish at 13 °C.

3.2.3.2. Neural plasticity. In response to the ACTs, *neurod* expression (Fig. 4a) was maintained similarly to those observed during pre-ACT conditions, with post-smolts acclimated to 7 °C ($p < 0.05$) and 4 °C ($p < 0.05$) having elevated expression compared to the 13 °C group. In contrast, the expression of *pcna* (Fig. 4b) was unaffected in post-smolts when stressed, with all acclimation treatments displaying similar

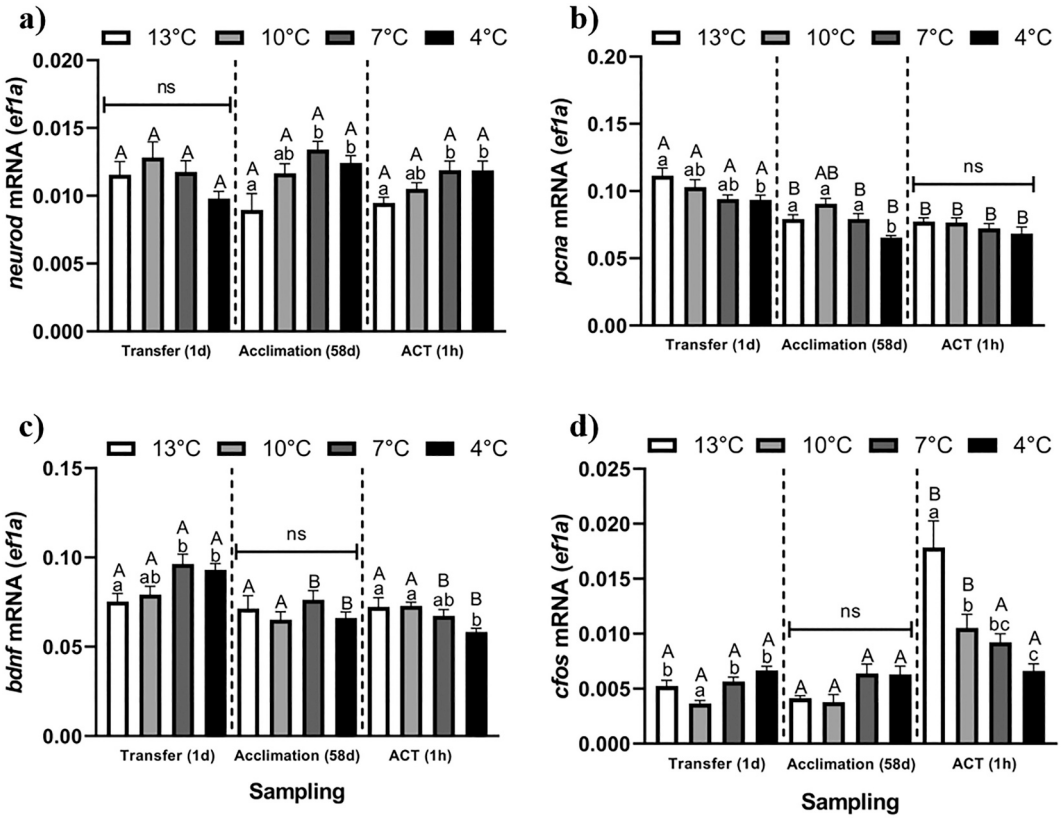

Fig. 4. Whole telencephalic changes in expression of a) neurogenic differentiation factor (*neurod*), b) proliferating nuclear antigen (*pcna*), c) brain-derived neurotrophic factor (*bdnf*), d) *c-fos* at 1-day post-thermal transfer, 58-days after acclimation and 1-h post-ACT confinement stressor. Values are presented as average ± SEM of each treatment (n = 8–10 per treatment/sampling). Uppercase letters indicate significant differences within a temperature treatment between samplings, whilst lowercase letters indicate a significant difference between temperature treatments within a sampling. A significant difference was held at $P < 0.05$.

levels, while stressed post-smolts acclimated to 4 °C displayed significantly lower *bdnf* expression compared to fish at 10 °C and 13 °C (Fig. 4c). Conversely, the expression of *cfos* (Fig. 4d) showed the largest response to the ACTs, demonstrating an upregulation in stressed post-smolts acclimated to 13 °C ($p < 0.0001$), 10 °C ($p < 0.0001$), and to a lesser extent in 7 °C ($p < 0.05$) compared to the respective pre-ACT state, but not for fish acclimated to 4 °C. Further, stressed post-smolts acclimated to 13 °C showed higher *cfos* expression (Fig. 4d) compared to fish at 10 °C ($p < 0.05$), 7 °C ($p < 0.01$), and 4 °C ($p < 0.0001$), with stressed post-smolts at 10 °C additionally having significantly elevated expression compared to the 4 °C group.

4. Discussion

In all animals, the interrelationship between the stress axis, neural activation and cognitive plasticity in coping with stressors is well documented and provides important underlying information as to the degree of stress load being experienced (Madaro et al., 2016, 2015; Manuel et al., 2016, 2014a; Samaras et al., 2018; Sørensen et al., 2013). It is known that early life experiences and stress history, due to differences in rearing strategies, may differentially influence stress responses and brain plasticity that extend later into life (Auperin and Geslin, 2008; Ebbesson and Braithwaite, 2012; Tsalafouta et al., 2015). Hence, when examining changes in stress responses and brain functions, one should keep in mind the potential influence of prior life history in experimental animals. Here we demonstrate how Atlantic salmon post-smolts exposed to the coldest temperatures, 7 °C and 4 °C, display the greatest alterations and perturbations in telencephalic regulation cued by a downregulation in *gr1* and a reduction in stress response capacities, signaling elevated stress loads that have reduced their capacity to mount a stress response. Conversely, neural plasticity was well maintained which became upregulated at these temperatures in a stressor dependent fashion, measured as elevated *bdnf* and *neurod* during the abrupt transfer (acute) and after acclimation (prolonged), respectively. These shifts in cognitive properties, in turn, signal key functions for maintaining stress resilience when stress loads accumulate at 7 °C and 4 °C. Taken together, this study recommends that subjecting post-smolts to temperatures reductions of 6 °C or greater should be avoided when going from 13 °C.

4.1. Acute temperature transfer

The severity of rapid stressors can either inhibit or maintain a capacity to mount a response to the challenge. If the stressor is overwhelming, this will inadvertently have detrimental impacts on physiology and stress functions, altering response capacities to adapt to the challenge. Aligning to prior reports, an acute temperature drop of 3 °C was enough to elicit a strong cortisol response in post-smolts (Fig. 1), while the magnitude of this response was the same going from 13 °C to 10 °C, 7 °C or 4 °C (Tanck et al., 2000; Van Den Burg et al., 2005). Hence, post-smolts rapidly exposed to the different cold temperatures still maintained a capacity to respond to all the transfer temperatures studied.

The level stress is known to impact neural regulation through CRs that manifest as disturbed expression and ratios in salmonids (Madaro et al., 2016, 2015). In line, despite the similarities seen in cortisol responses among the different abrupt transfer groups, a shift in CR expression was observed, with a downregulation of *gr1* (Fig. 2g, h) in the less extreme temperatures, 10 °C and 7 °C, and an upregulation of *mr* (Fig. 2f) and *gr2* (Fig. 2i, j) in post-smolts transferred to 4 °C, which elevated the *mr/gr1* ratio (Fig. 3a, b) with increasing temperature

change. This demonstrates that the increase in *mr/gr1* ratio is differentially attained between treatments, however, as *gr1* (Fig. 2g, h) did display an overall decreasing trend in all transfer treatments, the differential response in post-smolts transferred to 4 °C may simply reflect an alternative mechanism for further enhancing the *mr/gr1* ratio (Fig. 3a, b). This elevation in *mr/gr1* ratio may signal an attempt to lower Gr1 bioavailability suggesting suppression of receptor functions to prevent overstimulation that would otherwise harm neuronal integrity, a common symptom of elevated stress loads (Madaro et al., 2016, 2015; Samaras et al., 2018). Therefore, as post-smolts exposed to 7 °C and 4 °C displayed the largest increases in the *mr/gr1* ratio, this may reflect larger allostatic loads compared to fish at 10 °C. In contrast, as both *mr* and *gr2* regulate tissue functions during low stress conditions (Stolte et al., 2008), the increase in *mr/gr2* ratio (Fig. 3d) observed in post-smolts transferred to 10 °C could propose a capacity for baseline functions to alleviate stress loads, in complement with smaller to moderate shifts in the *mr/gr1* ratio (Fig. 3a, b). Taken together, these findings indicate transferring post-smolts from 13 °C to 10 °C may still be tolerable for maintaining normal stress functions, while rapid reductions of 6 °C or greater are associated with greater perturbations to CRs cueing larger stress loads, aligning to a prior report (Staurnes et al., 2001).

CRs play importance functions in regulating neural processes, therefore shifting CR patterns to mild or severe challenges can evoke rapid neuroplastic changes that modulate cognitive functions either beneficially or detrimentally (Ebbesson and Braithwaite, 2012; Sørensen et al., 2013). Aligning to this, post-smolts acutely exposed to 4 °C and to some extent 7 °C, exhibited lower telencephalic *pcna* (Fig. 4b) suggesting a reduction neurogenesis. Similar observations have been observed as stress levels converge to allostatic overloads aligning to the observations in CR regulation (*4.1.1.*) (Gould et al., 1992; Sørensen et al., 2011, 2012; Tea et al., 2019). Despite this, one should be mindful that a plausible alternative explanation for this *pcna* suppression may also be related to lower mitotic rates due to the direct influence of cold temperatures on cellular proliferation (Dunlap, 2016), although it has been argued that such effects can be abolished by additional challenges (Sørensen et al., 2012). In contrast, telencephalic *bdnf* (Fig. 4c) increased in fish exposed to 7 °C and 4 °C signaling changes in synaptic plasticity. As *bndf* is highly expressed in mature neurons, coupled with indices of decreased proliferation (*pcna*), this could suggest a larger network of maturing neurons when responding to rapid reductions in temperature. Further, as no transfer mortalities were observed, this elevation in *bdnf* indicates a surprisingly robust coping response, at least in protected environments (e.g. tank rearing), as prior studies demonstrate proactive salmon to exhibiting higher *bdnf* in association with elevated serotonergic activity, which improves stress resilience during large stress loads (Vindas et al., 2017). However, because Bdnf is also attributed to protecting neuronal circuits during adverse stimuli, such as cortisol overstimulation (Linz et al., 2019), its elevation in post-smolts transferred to 7 °C and 4 °C still suggests a larger degree of stress, consistent with the suppression in *pcna* and *gr1* observed. Therefore, despite this *bdnf* response being a robust indicator, these findings support an elevation in stress loads as an upregulation in these cognitive properties is required to battle larger stresses when rapidly transferred to 7 °C and 4 °C. Further, aligning with the emphasis that CRs and their bioavailability differentially regulate neural plasticity, the elevation in *mr/gr1* ratio (Fig. 3a, b) in post-smolts exposed to 7 °C and 4 °C coincided with the increase in *bdnf* (Fig. 4c) (Alfonso et al., 2019; Manuel et al., 2016; Sadoul et al., 2018; Sørensen et al., 2013). As Mr is noted to stimulating neural plasticity, one could argue that the diverging enhancement in *mr/gr1* ratio in post-smolts transferred to 7 °C and 4 °C could allow to enhance Mr bioavailability to necessitate these shifts in *bndf* regulation. However, due to the complexity of these regulatory pathways, further functional and neuroanatomical studies are still required.

4.2. Acclimation and response to ACTs

If the accumulation of challenges is still tolerable following prolonged exposure to the stressor, neural adjustments are pivotal for modulating cognitive and behavioral strategies to improve stress resilience and maintaining homeostasis (Ebbesson and Braithwaite, 2012; Sørensen et al., 2013). However, when experiencing stable and protected habitats, fish may be able to better cope and compensate under more severe challenges compared to being in exposed and fluctuating ones. Therefore, challenging the fish's ability to respond to an additional stressor (ACT), which in the present study was an ACT by confinement stress, may reveal detrimental cues to when stress loads overwhelm baseline capacities, measured as reductions in stress reactivity to the challenge (Samaras et al., 2018).

The prevailing acclimation temperature is noted to modifying the release of cortisol in Atlantic salmon (Madaro et al., 2018), green sturgeon (*Acipenser medirostris*) (Lankford et al., 2003) and sunshine seabass (*M. chrysops* × *M. saxatilis*) (Davis, 2004), yet limited studies have addressed the reasons behind these concentration differences and how important brain functions, such as the telencephalon crucial for stress resilience, are impacted. Aligning to these reports, the present study shows that the plasma cortisol (Fig. 1) and telencephalic *cfos* (Fig. 4d) response to ACTs decreased in post-smolts acclimated to declining temperatures, with the lowest responses observed for fish at 7 °C and especially 4 °C. Surprisingly, both the cortisol and *cfos* responses displayed striking resemblance, supporting the less commonly used marker, *cfos*, as an equally valid stress proxy in fish. As the severity of stress is known to dampen stress responses, this reduction in stress response capacity and reactivity at 7 °C and 4 °C could relate to an accumulation of challenges (Madaro et al., 2015). In support, counterintuitive to the traditional stepwise decrease in responsiveness one would expect related to changes in metabolism, the cortisol and *cfos* responses in the present study were substantially lower in post-smolts acclimated to the colder temperatures compared to the 13 °C control (Figs. 1, 4d), aligning to a recent report (Madaro et al., 2018). Taken together, these response patterns suggest that prolonged exposure to 7 °C and especially 4 °C begin to detrimentally influence stress reactivity in post-smolt Atlantic salmon.

Considering that the level of stress can disturb CR profiles (Madaro et al., 2016, 2015), post-smolts acclimated to 7 °C and 4 °C also maintained larger perturbations in telencephalic CR regulation, showing elevated *mr* (Fig. 2f) and a suppression of both *gr1* paralogs (Fig. 2g, h), respectively, elevating the respectful *mr/gr1* ratio (Fig. 3a, b). Coherent to the seen reductions in stress reactivity to ACTs (Figs. 1, 4d), this suggests stress induced disparities in CR stimulation are maintained even after acclimation to 7 °C and 4 °C. Moreover, this divergence in *mr* and *gr1* after acclimation to 7 °C and 4 °C inversely mirrored those observed during the initial abrupt transfer period (*4.1.1.*), potentially suggesting differential modes of regulation depending on stressor properties (e.g. acute vs prolonged), with similar effects also evidenced in European sea bass, an aspect requiring further study (Goikoetxea et al., 2021). Furthermore, *gr1α* (Fig. 2g) was more strongly regulated in stressed post-smolts compared to *gr1β* (Fig. 2h), which could signal differential modes of physiological regulation to acute or chronic stress, requiring further study into these paralog genes. Nevertheless, as the elevation in *mr/gr1* ratio (Fig. 3a, b) at 7 °C and 4 °C still indicates an attempt to maintain Gr1 suppression to preserve neuronal integrity, this supports a continuation of challenges and stress loads when exposure to 7 °C and 4 °C is prolonged (Fig. 2g, h), alternatively reasoning the reductions in stress reactivity (Figs. 1, 4d) (De Kloet et al., 2005; Koning et al., 2019; Madaro et al., 2016, 2015; Samaras et al., 2018). In line, the importance of full GR activation for IEG induction has been evidenced in mammals (Gutièrrez-Mecinas et al., 2011; Mifsud and Reul, 2018), possibly explaining the lower telencephalic *cfos* responses to ACTs towards the cold as Gr1 is suppressed. This could implicitly demonstrate the lacking *cfos* response (Fig. 4d) in stressed fish acclimated to 4 °C,

which coincidently displayed the strongest Gr1 suppression cued by a direct downregulation in *gr1* expression (Fig. 2g, h) and stronger elevation in the *mr/gr1* ratio (Fig. 3a, b) alongside a suppression in the CRF system (Fig. 2abc). Hence, the importance of GR in maintaining appropriate neuronal reactivity to stress should not be undermined in fish, as this study points to similar regulatory pathways as observed in mammals. Taken together, these data support the notion that temperature induced reductions in stress reactivity may not purely be a metabolic effect, but rather or in combination, due to an elevation in stress loads that suppress key stress functions at least in the telencephalon, requiring deeper mechanistic consideration (De Kloet et al., 2016; Koning et al., 2019; Madaro et al., 2015).

The Hsd11b2 is noted for important functions in preserving CR functions by regulating cortisol action (Alderman and Vijayan, 2012). Although still unclear, the differential regulation in *mr* and *gr1* could relate to differences in Hsd11b2 activity as post-smolts acclimated to 7 °C, but not 4 °C, displayed elevated *hsd11b2* expression (Fig. 2d, e) potentially suggesting a larger degree of control over cortisol action on CRs (Alderman and Vijayan, 2012; Madaro et al., 2016, 2015; Pizzolo et al., 2015). This could explain why *gr1* (Fig. 2d, e) was maintained and *mr* (Fig. 2f) even elevated in post-smolts acclimated to 7 °C, if enhanced Hsd11b2 functions generate a larger capacity to negate excess CR stimulation creating a lower demand to suppress functions, which starkly contrasts the downregulation in *gr1* (Fig. 2d, e) seen for post-smolts acclimated to 4 °C. However, a more simplistic explanation for the downregulation of *hsd11b2* (Fig. 2d, e) in post-smolts acclimated to 4 °C may be due to the stronger suppression in stress functions and dampened stress responses, which may inadvertently reduce the demand to maintain elevated Hsd11b2 activity if prevailing cortisol concentrations and respective receptor stimulation is readily retained low. In any case, this differential response in *hsd11b2* still suggests a greater accumulation of challenges at 4 °C, whereas at 7 °C proper CR functions may still conceivably be maintained.

Taken together, acclimating post-smolts to 7 °C and 4 °C clearly display a larger prevalence and continuation of challenges after transfer, impacting telencephalic functions in ways that drive a suppression in *gr1* and reductions in stress response capacities to additional challenges (ACTs), suggesting disparities in CR functions and elevated allostatic loads. However, post-smolts acclimated to 4 °C clearly showed the most adverse effects cued by the strongest suppression in functions as observed for the lower *hsd11b2* (Fig. 2d, e), stronger elevation in the *mr/gr1* ratio (Fig. 3a, b), and a direct downregulation in *gr1* (Fig. 2g, h) and the CRF system (Fig. 2a, b, c) consistent with indices of chronic stress (Madaro et al., 2016, 2015). This aligns to prior reports evidencing poor physiological functions at this temperature extreme (Handeland et al., 2014; Virtanen and Oikari, 1984).

Cognitive functions are crucial for stress resilience, providing beneficial physiological and behavioral adaptions via shifts in neuroplasticity to combat stressors (Ebbesson and Braithwaite, 2012; Manuel et al., 2016; Samaras et al., 2018; Sørensen et al., 2013). Following acclimation, this study shows that the suppression in *pcna* (Fig. 4b) was maintained in post-smolts at 4 °C, supporting that the continuation of stress loads are impeding the maintenance of neurogenesis, as similarly observed during the initial transfer period (*4.1.2.*) (Sørensen et al., 2011; Tea et al., 2019). Similarly, although no change in *bdnf* (Fig. 4c) was observed pre-ACT stress, following the ACTs post-smolts acclimated to 4 °C displayed a depression in levels. Given the importance of Bdnf in modulating synaptic plasticity, behavioral phenotypes and protecting neural circuits from cortisol, lower levels of these during stress conditions could propose an inferior cognitive capacity to handle additional challenges (Linz et al., 2019; Manuel et al., 2014a; Vindas et al., 2017). However, due to the clear suppression in stress functions alongside dampened stress responses at 4 °C, this may inherently reduce the physiological need to promote Bdnf to protect neuronal circuits from overstimulation, providing an alternative explanation for this depression in levels.

Conversely, neuromodulation shifted to enhancing *neurod* (Fig. 4a) at 7 °C and 4 °C, possibly suggesting a larger pool of differentiating neurons, which studies correlate to increased neuroplasticity and eustress conditions (Grassie et al., 2013; Johansen et al., 2012; Salvanes et al., 2013). However, as cold temperatures are clearly noted to having greater physiological challenges (Handeland et al., 2014), consistent with present findings observing the downregulation in *gr1*, the CRF system and stress response capacities (*4.2.1.*), this counterintuitive elevation in *neurod* at these presumably more "stressed states" may reason the progression of important functions in memory and learning processes to better manage the persisting stress loads, although behavioral designs are still needed to confirm this. Such neural adaptations to prolonged stressors may allow to better maintain a degree of stress resilience, showing that prevailing allostatic loads at 7 °C and 4 °C are still tolerable for allowing uninterrupted shifts in neural functions to play out the functional demand, *i.e.* in progressing important cognitive and behavioral adaptations (Grassie et al., 2013; Salvanes et al., 2013; Sørensen et al., 2013). Taken together, the upregulation in *neurod* at 7 °C and 4 °C (Fig. 4a) demonstrates a surprisingly robust response, despite still agreeing with a greater prevalence of challenges compared to post-smolts at 13 °C and 10 °C. This convincingly illustrates the importance of such cognitive manifestations for stress resilience when challenges accumulate at cold temperatures.

Our findings adhere to the notion that CRs, their ratio and bioavailability are linked to changes in neuroplasticity (Sørensen et al., 2013), showing that even after acclimation the maintained elevation in *mr/gr1* ratio (Fig. 2f, g) coincided with a modulation of *neurod* expression (Fig. 4a), reminiscent to the changes in *bdnf* (Fig. 4c) and *mr/gr1* ratio observed during the initial transfer period (*4.1.2.*). Aligning to reports implicating Neurod in the potentiation of Mr signaling in mammals (van Weert et al., 2019; Van Weert et al., 2017), one may argue that the enhancement in *mr/gr1* ratio could permit a larger bioavailability of Mr to facilitate these shifts in *neurod*. Similarly, impaired learning and memory have been detected in response to forebrain *mr* deletion suggesting decreases in Neurod functions (Berger et al., 2006), while *mr* overexpression shows to enhance memory and reduce anxiety (Lai et al., 2007; Rozeboom et al., 2007). Conversely, studies in zebrafish evidence that Hsd11b2 activity may indirectly provide important functions for maintaining the normal development of neurogenesis and neuroplasticity, by preventing untimely neural differentiation as discrepancies in GR stimulation arise (Alderman and Vijayan, 2012). Aligning to this, as post-smolts acclimated to 7 °C elevated telencephalic *hsd11b2* expression (Fig. 2d, e), which collectively showed to maintain both *gr1* (Fig. 2g, h) and *mr* expression (Fig. 2f) proposing retained CR functionality, this may explain the slightly larger response in *neurod* (Fig. 4a) or even the maintenance of *pcna* levels (Fig. 4b) in fish at 7 °C compared to 4 °C. Moreover, as Hsd11b2 has been reported for its tight regulation over Mr, one could argue that their co-elevation (Fig. 2d, e, f) at 7 °C supports this link, which may beneficially aid in maintaining functions of neural plasticity (Alderman and Vijayan, 2012; Fan et al., 2020). Taken together, these indicate that neural plasticity may be better maintained in post-smolts when exposure is prolonged at 7 °C compared to 4 °C, but also reasons that post-smolts overall retain a robust cognitive response to low thermal challenges.

5. Conclusion

In conclusion, post-smolts cope surprisingly well with rapid reductions in temperature as all treatments retained a capacity to mount a stress response (cortisol) to all the transfer temperatures. In observing no mortalities, the upregulation in *bdnf* to the acute transfer demonstrates a robust cognitive response, however, as post-smolts transferred to 7 °C and 4 °C evidently displayed larger perturbations in telencephalic CRs cueing a suppression in *gr1*, these still propose larger stress loads. Following acclimation, this suppression in *gr1* at 7 °C and 4 °C was

maintained, coinciding with reductions in stress response capacities to the ACT, that suggest a continuation of stress loads and challenges when exposure to these temperatures is prolonged. In retrospect, neuromodulation shifted from synaptic plasticity (*bdnf*) to memory and learning processes (*neurod*) after acclimation to 7 °C and 4 °C, showing that neural plasticity is robustly maintained within the studied thermal range. This supports for an importance of cognitive functions in maintaining stress resilience when challenges accumulate at these colder temperatures. Despite this, it should be noted that fish acclimated to 4 °C clearly began to display less adaptive signs in telencephalic regulation compared to fish at 7 °C. Taken together, although these modulations in neural plasticity support a robust response, together with the suppression in stress functions, these still also cue a larger degree of challenges in post-smolts transferred to 7 °C and 4 °C, therefore exposing post-smolts at 13 °C to colder magnitudes of 6 °C or greater should be avoided in aquaculture.

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CRediT authorship contribution statement

P.A. Tang: Conceptualization, Project administration, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **S.O. Stefansson:** Supervision, Writing – review & editing, Funding acquisition. **T.O. Nilsen:** Project administration, Supervision, Writing – review & editing. **N. Gharbi:** Project administration, Investigation. **F. Lai:** Methodology. **V. Tronci:** Investigation. **P. Balseiro:** Methodology, Writing – review & editing. **M. Gorissen:** Investigation, Writing – review & editing. **L.O.E. Ebbesson:** Conceptualization, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

Authors declare no conflict of interest.

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