Strong effect of season, but subtle responses to feed deprivation in the brain transcriptome of Arctic charr

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Abstract

Appetite controlling neuropeptides in mammals are found in fish, however, their role and function in appetite regulation in fish remains elusive. The Arctic charr (*Salvelinus alpinus*) has a highly seasonal feeding cycle which comprises long periods of voluntary fasting. Therefore, the charr represents an interesting species for studying appetite regulating mechanisms in fish. In this study we compared the brain transcriptomes of fed and feed deprived charr over a 4 weeks trial during their main summer feeding season. Despite prominent differences in body condition between the fed and feed deprived charr at the end of the trial, the results revealed only moderate effects of feed deprivation on the brain transcriptome. In contrast, the transcriptome differed markedly between the start and the end of the experiment in both the fed and feed deprived charr, indicating strong seasonal shifts in basic cell metabolic processes. The employment of a GO enrichment analysis revealed that many biological processes appeared to change in the same direction in both fed and feed deprived fish. In the feed deprived charr biological processes linked to oxygen transport and apoptosis were down- and up-regulated, respectively. A screen of the dataset for candidate genes did not indicate hunger- or satiety signalling by these in response to feed deprivation. Gene expression of *Deiodinase* 2 (*DIO2*), an enzyme implicated in the regulation of seasonal processes in mammals, was significantly lower expressed in response to season and feed deprivation. We further found a higher expression of *VGF* (non-acronymic) in the feed deprived than in the fed fish. The possible role of Dio2 and VGF in the regulation of energy homeostasis is being discussed and depicts a need for further studies of these in seasonal fish.

1. Introduction

Feeding is pivotal for animals in order to sustain their energy and substrate needs to live, grow and reproduce. In mammals, energy intake and expenditure are tightly regulated by a crosstalk of peripheral and central signalling actors and pathways (Wynne et al., 2005). Peripherally derived hunger (orexigenic) and satiety (anorexigenic) signals as well as long-term signals reporting about energy status are perceived and processed in a number of brain nuclei in order to control short-term (meal-to-meal) appetite and long-term energy homeostasis (Elmquist et al., 1999; Schwartz et al., 2000). Of these, the arcuate nucleus (ARC) in the hypothalamus represents the pivot for controlling food intake and energy balance (Cone et al., 2002). Here, two populations of “first order” neurons express anorexigenic proopiomelanocortin (POMC) and cocaine-and amphetamine regulated transcript (CART) and orexigenic agouti-related peptide (AgRP) and neuropeptide Y (NPY), respectively (Schwartz et al., 2000). These project to “second order” neurons which transduce orexigenic and anorexigenic signals via NPY and melanocortin receptors (Wynne et al., 2005). While NPY signalling through Y receptors causes an orexigenic response, signalling through MCRs results in either an anorexigenic response. POMC derived α-melanocyte-stimulating hormone (α-MSH) is a melanocortin 4 receptor (MC4R) agonists and a potent appetite suppressor in mammals (Cone, 1999). AgRP, on the other hand, is an inverse agonist to the constitutively active MCRs and increases food intake (Nijenhuis et al., 2001). These appetite and energy signalling neuropeptides have been shown to be evolutionary conserved (Cerda-Reverter et al., 2000; Cortés et al., 2014), and to be involved in the control of food intake in fish (Volkoff, 2016; Volkoff et al., 2005). However, there are major knowledge gaps on how appetite signals integrate in the control of food intake in fish, as the responses of central appetite regulators to energy perturbation vary across species and even within species depending on the experimental design (Hoskins and Volkoff, 2012). For example, *NPY* was higher expressed after 7 days feed deprivation in hypothalamus of zebrafish (*Danio rerio*) (Yokobori et al., 2012) and in the preoptic area of chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch)* (Silverstein et al., 1998), whereas in cunner (*Tautogolabrus adsperus*), hypothalamic *NPY* expression remained unaffected after 7 days feed deprivation (Babichuk and Volkoff, 2013). Such differences in the response to e.g. feed

deprivation are not unexpected as fish represent the most diverse group of vertebrates with a myriad of adaptations to spatially different and temporal changing environments. In the high-latitude inhabiting anadromous (sea-migrating) Arctic charr (*Salvelinus alpinus*) for example, food intake varies dramatically from little or no feeding while residing in fresh water during winter to voracious feeding during their short summer residence in the sea (Jørgensen and Johnsen, 2014; Swanson et al., 2011). This behaviour, which presumably developed as a response to seasonal and spatial differences in water temperature and food availability at high latitudes, now appears to be regulated independently of these factors; captive offspring of anadromous Arctic charr exhibit pronounced seasonal changes in appetite and growth when held at constant temperature and given food in excess (Tveiten et al., 1996). Despite such seasonal changes in food intake, a previous study did not show clear differences in the expression of orexigenic and anorexigenic appetite regulators in different brain compartments between anorexic winter charr and hyperphagic summer charr (Striberny et al., 2015). Furthermore, expressions of orexigenic and anorexigenic neuropeptides in the hypothalamus were unaffected by short- and long-term feed deprivation in this species (Striberny and Jørgensen, 2017). However, in these and in most other studies investigating appetite regulation in fish, expression levels of known appetite regulators have been measured by RT-qPCR, a method with the major drawback of restricting the focus to a limited number of genes. The fact that novel actors in the complex control of food intake are still being discovered, calls for a more global approach when investigating appetite regulation in fish. Today, high-throughput RNA sequencing is a powerful tool in experimental biology. Transcriptomic approaches have been applied in various contexts to improve knowledge of the biology of the seasonal Arctic charr (Magnanou et al., 2016; Norman et al., 2012), albeit not with focus on appetite regulation. Consequently, we sequenced the brain transcriptome of fed and feed deprived charr in an attempt to advance the knowledge on global responses to feed deprivation in the brain, assess alterations of central appetite signalling, and to identify new possible actors involved in the control of appetite and energy metabolism in fish.

2. Material and Methods

2.1 Ethics statement

Fish handling and euthanasia (see below) was performed by a competent person and in accordance with the European Union Regulations concerning the protection and welfare of experimental animals (European directive 91/492/CCE). The experiment was approved by the Norwegian Committee on Ethics in Animal Experimentation (ID 3630).

2.2 Experimental design and sampling of fish

The charr used in the present study were 2-years-old offspring of the anadromous Hammerfest strain, originating from wild charr caught in 1984 and since then bred at Tromsø Aquaculture Research Station, where the experiment was carried out. Until the start of the experiment they had been held on natural water temperature and light conditions (transparent roof) and fed a commercial Arctic charr feed (Skretting, Stavanger, Norway) *ad libitum* by automatic feeders. On June 25 (T_0), 2014, 42 fish were anesthetized in Benzocaine (60 ppm) and tattoo-tagged with Alcian Blue staining dye using a Pan Jet needleless injector (Wright Dental, Dundee, UK), measured for body mass and length and distributed among two 300 L tanks supplied with fresh water. Another 12 fish were sampled from the stock tank and euthanized by an overdose of Benzocaine (150 ppm). Body mass and length were then measured, after which the fish were decapitated. The belly was then cut open and the sex was determined. On a total of 5 males, brains were dissected out and separated into telencephalon, mesencephalon and hypothalamus. The tissues were subsequently stored in 1.5 ml Eppendorf tubes containing 1 ml of RNAlater. Samples were kept at 4 °C for 24 hours, and then frozen at -20 °C until RNA extraction.

On the same day, 220 fish from stock tank were distributed amongst the two tanks in which the tattooed fish had been placed, giving 130 fish per tank. From then on, the fish in one tank were fed (Fed) with the same commercial feed as before. They received two main meals at 08.00 AM and 3.00 PM by automatic feeders and were fed by hand in between the main meals to ensure excess feed availability. The fish in the other tank were feed deprived (FDP) until the end of the experiment. Fish were held at simulated natural photoperiod (69 °N), which was 24 h light at that time of the year, and ambient water

temperature (temperature range: 4.5 °C - 13.5 °C). On July 23 (T_1), 12 fish from each group were euthanized, from which 5 males were measured and sampled as described above. Finally the tattooed fish were anesthetized and measured for body mass and length.

The high number of fish in each treatment group compared to the number of fish sampled was justified by the need to avoid formation of social hierarchies in the fed group. The tattooed fish were included in order to monitor the body mass and condition factor development of the fish in the two treatment groups. Fulton's condition factor (K) was calculated according to Ricker (1975): $K = (W \times L^{-3}) \times 100$, where W is body mass in g, and L is fork length in cm.

2.3 Sample preparation

Tissues were disrupted using TissueLyser II (QIAGEN, Hilden, Germany), and RNA was extracted using the RNeasy Plus Universal Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer`s protocol. Concentration and purity of RNA were assessed using NanoDrop ND2000c (Thermo Scientific, MA USA) and when the 260/280 or 260/230 absorbance ratio was below the quality threshold (1.8), samples were further purified using ethanol precipitation. Genomic DNA was removed by treating the RNA with Ambion TURBO DNA-free™ Kit (Life Technologies, CA, USA). In order to obtain a representative view of the main brain areas that have been shown to be involved in central appetite control, 3 µg of RNA of each brain section (telencephalon, mesencephalon and hypothalamus) were pooled resulting in a total of 9 µg RNA per brain and individual. Finally, RNA quality was assessed with BioRad Experion Bioanalyzer. Samples were then shipped on dry-ice to the GenoToul sequencing platform, Toulouse, France for RNA-seq.

2.4 cDNA library construction and paired-end RNA-seq

RNA preparation and sequencing were performed at the GenoToul sequencing platform, Toulouse, France. Fifteen RNA banks were prepared using the TruSeq RNA sample preparation Kit from Illumina, involving the following steps. Poly-A containing mRNA were isolated from 3 µg of total RNA. The mRNA was then chemically fragmented. The cleaved RNA fragments were reverse transcribed into first stranded cDNA using random primers,

second strand cDNA was then synthesized. Adaptors were ligated to the end-repaired cDNA, which contributed to fragment selection after the PCR enrichment step. Each bank quality was validated measuring sample concentration and fragment size on an Agilent High Sensitivity DNA chip. Sequence hybridization to the flow cell and cluster generation was achieved using a cBot system and the cluster generation kit from Illumina. Hundred base pair fragments were sequenced in paired-end for the 15 samples. Sequencing By Synthesis (SBS) was achieved on an full flow cell of an Illumina HiSeq 2500 sequencer. Each sequencing lane of the flow cell was screened by a camera driven by the HiSeq Control Software. Image correction and base calling was performed using the Real Time Analysis (RTA) software.

2.5 General statistics, data assembly and annotation

2.5.1 Testing for differences in body weight and *K*

Data for weight and *K* were not normally distributed and therefore, statistical testing was conducted on LOG transformed data. Changes over time were tested using a repeated measures ANOVA. When differences were found, a pairwise comparison applying Bonferroni correction for multiple comparisons was used to determine main effects. Differences between treatment groups were tested using a 2-sample t-test. All statistical testing was done with SYSTAT 13 and figures were drawn using SigmaPlot 13 (both Systat Software, CA, USA). The significance level was set to $p < 0.05$.

2.5.2 Sequencing Data

a. RNA-Seq data assembly annotation and quality assessment

Read quality was checked within the ng6 environment (http://www.biomedcentral.com/1471-2164/13/462/abstract) using fastQC (fast Quality Control - http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and Burrows-Wheeler Aligner BWA (Li and Durbin, 2009) to search for contamination. The reads were assembled with the Drap pipeline (version 1.7) (Cabau et al., 2017). The individual sample assemblies were performed with runDrap using Oases with kmers 25, 31, 37, 43, 49, 55, 61, 65, 69. The individual contig files filtered by FPKM (Fragments Per Kilobase

per Million mapped reads) over one were then merged with runMeta and filtered again by FPKM over one to produce the reference contig set.

Contigs were annotated searching sequence homologies against the following Ensembl protein databases (blastx) *Danio rerio*, *Gadus morhua*, *Oreochromis niloticus*, *Oryzias latipes*, *Takifugu rubripes*, *Tetraodon nigroviridis*, *Xiphophorus maculatus*,, (release 79, May 2015) refseq_rna (blastn); swissprot (blastp); unigene_Takifugu_rubripes.9 (blastn); unigene_Oryzias_latipes.30 (blastn); unigene_Danio_rerio.126 (blastn); NCBI Arctic char ESTs (blastn); the contigs (blastn). Repeat were searched with repeatMasker (version open-4-0-3, with standard parameters) using Repbase database. The GO annotations where extracted from InterproScan (May 2015 version;(Jones et al., 2014)). The best SwissProt, RefSeq, or *S. Salar* NCBI ESTs hit result was used to classify species by best hits contribution.

Different approaches were used to verify the quality of the built contigs. First the contigs were processed with BUSCO V2 (Simão et al., 2015) to verify the number of actinopterygii_odb9 reference genes found and their reconstruction state (partial or complete). Then the *Salmo salar* protein sequences made available by the NCBI (GCF_000233375.1_ICSASG_v2_protein.faa) were aligned with BLAT (standard parameter, version 34) on the 6 frames translated contigs (Kent, 2002). The alignment was filtered to retain only hits with at least 80% identity and 80% coverage giving the size of the set of well reconstructed contigs. Finally, we validated our assembly t by (1) verifying the realignment rate of the reads of each individual sample on the contigs, and by (2) taking advantage of an Arctic charr public raw dataset (SRA accession: SRX314607). Reads from this gill transcriptome sequenced in 100bp paired-end, were also mapped on our contigs.

b. Polymorphism: SNP and microsatellite search

Reads were aligned back to the contigs with bwa mem (Li and Durbin, 2009). They were deduplicated with samtools rmdup, then GATK (Version 3.0-0-g6bad1c6) base quality score recalibration was applied (McKenna et al., 2010). Indel realignment, SNP and INDEL discovery were performed with HaplotypeCaller using standard hard filtering parameters according to GATK Best Practices recommendations (DePristo et al., 2011;

Van der Auwera et al., 2013). Indels and SNP were independently filtered; 3 per windows of 18b with a minimal quality of 30. The micro-satellites discovery was conducted using Tandem Repeat Finder Version 4.04 using the following parameters: 2 7 7 80 10 50 500 -f -d –m (http://www.ncbi.nlm.nih.gov/pubmed/9862982).

2.5.3 Assessment of seasonal and food deprivation effects on gene expression

a. Patterns of gene expression

Data exploration and gene expression analysis were performed using various packages implemented in R version 3.3.1 (2016-06-21). A sample correlation heatmap based on Pearson's coefficient of correlation was drawn with pheatmap.

We retrieved the number of reads counts per contig and normalized each sample, accounting for compositional differences between the libraries (calcNormFactors function EdgeR).

Paired comparisons of the treatments groups were performed in EdgeR package version 3.8.6 (Robinson et al., 2010) according to the user guide procedure. We identified differentially expressed contigs using a general linear model and a quasi-likelihood F-test, and correcting for false discovery rate (corrected Benjamini and Hochberg $p<0.05$). The seasonal effect on brain gene expression was assessed comparing fish at time zero (T_0) (June) with fish fed *ad libitum*, sampled in July (Fed). The effect of food deprivation over time was evaluated comparing time zero fish with the feed deprived group (FDP). Finally, the comparison of fed and feed deprived charr at T_1 would reveal the differences caused by feeding regime at the end of the experiment.

Log fold change (logFC) frequency distribution was plotted for transcripts showing a significant change in expression for any of the paired comparisons. The median was calculated respectively for up- and down regulation. Only transcripts presenting a logFC greater than 0.5 and smaller -0.5 were kept for comparison by JVenn and GO analyses. All differentially expressed transcripts were included in the search for candidate genes.

Lists of differentially expressed genes shared between the two time points of fed and unfed fish, for up- and down-regulations respectively were obtained in the JVenn interface developed by (Bardou et al., 2014). Resulting Venn diagrams where drawn in Venn Diagram Plotter. The Venn diagram generated three candidate gene lists: (1)

transcripts that were differentially expressed only in charr fed *ad libitum*, (2) transcripts that were only differentially expressed in feed deprived charr and (3) transcripts that differed over time irrespectively of diet, i.e. a seasonal effect independent of the feeding regime (the shared portion of the Venn diagram).

b. GO enrichment

Gene ontology (GO) term enrichment was searched using the TopGo package (Alexa and Rahnenfuhrer, 2010). It consisted of the identification of terms that host more differentially expressed genes than expected by chance between the two investigated experimental conditions. Enrichment of terms by differentially expressed genes was assessed using Fisher's exact test. This analysis focused on Biological Process.

Each of the three gene lists generated by JVenn were investigated for GO enrichment, lists of up- and down-regulated transcripts were analyzed separately. Finally, GO enrichment analysis was conducted with the two lists of up- and down-regulated transcripts of the endpoint comparison.

2.5.4 Data mining interface

The assembled contigs have been annotated using the RNA-seq *de novo* ngs-pipelines processing chain (https://mulcyber.toulouse.inra.fr/ngspipelines/) and the results have been uploaded to a web-based user interface build upon biomart (http://www.biomart.org/).

3. Results and Discussion

3.1 Feed deprived and fed charr showed a diverging development of weight and condition

Over the four weeks period there was a strong increase in *K* and body mass in the fed charr (Fig. 1). This was consistent with the seasonal feeding behaviour of anadromous Arctic charr which is characterized by high food intake and growth during summer in order to replenish body mass and fat reserves that had been lost during winter (Jørgensen et al., 1997; Tveiten et al., 1996). The feed deprived charr underwent a strong mobilization of energy reserves during the experiment resulting in a markedly lower *K* and body mass

in feed deprived charr than in fed charr at T_1 (Fig. 1). This effect of treatment was also evident in the 5 fish from each treatment sampled for transcriptomics; mean body masses (g)/*K* were 108.3±10.2/1.05±0.03 at T_0, and 157.2±8.3/1.25±0.03 and 80.6±6.3/0.92±0.04 in fed and feed deprived at T_1, respectively.

3.2 A reliable *de novo* brain transcriptome

The present *de novo* assembly produced 49,829 contigs with a FPKM greater than 1 for at least one of the 15 sample. Their total length equalled 84,028,148 base pairs. The N50 reached 2,663bp (i.e., the contig length such that, using equal or longer contigs, produces half the bases of the assembly) (Table 1).

Table 1 General statistics of contigs generated by RNAseq technology for brain gene expression characterization. Only contigs possessing a FPKM greater than 1 for at least one bank were considered for annotation and further expression analysis

Number of base pairs in the reads	42,491,174,498
Number of reads	420,704,698
Number of base pairs in the contigs (FPKM>1)	84,028,148
Number of contigs (FPKM>1)	49,829
N50	2,663
N90	816
Number of putative micro-satellites	34,440
Number of putative SNPs	420,406
Number of contigs including SNP	39,484

The annotation rate reached 85.07 % of the 49,829 contigs. Atlantic salmon (*Salmo salar*) contributed most to the annotation of the Arctic charr brain transcriptome (Fig. 2). This was expected as Atlantic salmon is phylogenetically the closest related species with a sequenced genome (Davidson et al., 2010). Note that all other species but *Homo sapiens* contributing to the annotation were exclusively teleosts including another salmonid, the rainbow trout (*Oncorhynchus mykiss*). The human genome is extremely well

characterized and might have brought annotations of genes that have only been described for this species.

Different approaches were used to verify the quality of the built contigs. First, the BUSCO analysis revealed the good assembly quality and annotation completeness of the transcriptome. Out of 4,584 single-copy ortholog genes common to Actinopterygii our assembly is 67.2% complete (2,560 complete single-copy BUSCOs and 524 complete duplicated BUSCOs), while 2.8% of contigs are fragmented (130 BUSCOs) and 30.0% are missing (1,370 BUSCOs). These results confirm that our assembly produced correctly built contigs. Even if it is known for its complex transcriptomic signature, the brain alone cannot represent the entire diversity of the Arctic charr transcriptome. Moreover, other fish transcriptome *de novo* assemblies brought results in the same order of magnitude with 70.2% completeness for the gut tench (*Tinca tinca*) (Panicz et al., 2017) and 64% for 4 combined tissues of the noble crayfish (*Astacus astacus*) (Theissinger et al., 2016). Second, the comparison to a phylogenetically close species reference proteome was achieved using the Atlantic salmon database from the NCBI. Out of 97,555 *S. salar* proteins, 47,419 got aligned with at least 80% identity over 80% of their length on our assembly, which corresponded to 12,238 Arctic charr contigs. Third, the contigs being a sum-up of the initial reads the higher the reads and pairs alignment rate are, the better the contigs reflected the initial information. We built compact sets of contigs with high realignment rates that ranged between 88 and 89% depending on the sample (15 banks). We finally validated the construction of our RNAseq data set taking the advantage an Arctic charr public raw data that was mapped to our set of contigs. An average of 79.35% of reads from this gill transcriptome (SRA accession: SRX314607) got aligned on the 15 Arctic charr banks.

3.3 Season strongly shapes global brain gene expression patterns while diet has a moderate impact

All samples from T_0 clustered together and were markedly different from samples taken at T_1. Remarkably, samples of the fed and feed deprived charr at T_1 did not cluster in accordance with the treatment group (Fig.3). These results stand in contrast with the strong divergence in *K* and weight between the fed and feed deprived charr. However,

353 feed deprivation may only have affected very specific processes in the brain
354 transcriptome leaving the overall expression pattern less strongly affected.
355 Seasonal changes, including the increased water temperature, caused 5.7 % of
356 the sequenced transcripts to differ over time in fed charr, and 9.2 % to differ over time in
357 feed deprived charr (Tab 2). However, only 175 transcripts (0.4 %) were found to be
358 differentially expressed comparing feed deprived with fed charr at T_1 (Table 2). In other
359 words, there is a higher number of differing transcripts from start to end of the experiment
360 and only minor differences between the treatment groups at the endpoint. This pattern
361 depicts strong shifts in the charr brain gene expression during a one month period during
362 summer which occurred irrespective of feeding regime. These results underline that
363 seasonal processes and changes in water temperature output much stronger effects on
364 the charr transcriptome than one month of feed deprivation during the feeding season.
365 However, the two times higher number of differentially expressed transcripts over time in
366 the feed deprived charr than in the fed conspecifics suggests an enhancement of
367 seasonal differences by feed deprivation (Table 2).
368 The alteration of seasonal changes by feed deprivation was further unveiled by
369 matching of the lists of differentially expressed transcripts using Venn diagrams. Hence,
370 we were able to discriminate differences that resulted uniquely from feed deprivation or
371 feeding over time from those that were found irrespective of the feeding trial (Fig.4).
372 Indeed, 55.4% of all the transcripts that were higher expressed in the brain of deprived
373 charr after one month of feed deprivation, were uniquely found in this comparison. The
374 remaining 44.6% were also found to be up-regulated in the T_1_Fed *versus* T_0 comparison.
375 On the other hand, only the minor part (23.9%) of all up-regulated transcripts over time in
376 the brain of fed charr was exclusively found in this comparison and three-fourths (76.1%)
377 were also found in the T_1_ FDP *versus* T_0 comparison (Fig.4).
378 Similarly, more than half (57.2%) of the down-regulated transcripts over time in
379 feed deprived charr constituted of transcripts that were uniquely lower expressed in the
380 feed deprived charr with the other 42.8% also found to be lower expressed in the fed
381 charr. In contrast, only 34.9 % of the lower expressed transcripts over time were uniquely
382 found to differ in the fed charr and more than half (65.1%) were as also found when
383 comparing feed deprived charr with T_0 charr (Fig. 4).

In summary, there were not only more transcripts differentially expressed in the feed deprived charr at T_1 *versus* T_0 compared with the fed charr at T_1 *versus* T_0, the larger fraction of both up- and down-regulated transcripts was also unique to this treatment and hence represents the charr's specific response to feed deprivation. Most of the differences found in the fed fish were at the same time found in the feed deprived charr. These shared differences over time depict robust seasonal processes that remain unaffected by feed availability.

Table 2 Number of up- and down-regulated transcripts in the different comparisons returned by EdgeR analysis. Cut-off at FDR<0.05 and at LogFC 0.5/-0.5.

T_1_Fed versus T_0		T_1_FDP versus T_0		T_1_ FDP *versus* T_1_Fed	
Up	Down	Up	Down	Up	Down
1,534	1,285	2,616	1,954	68	107

3.4 Food deprivation partially offsets seasonal increase in brain metabolism

GO enrichment analysis for biological processes was employed to identify global effects of a 4 weeks feed deprivation during summer on the charr brain transcriptome. One or more GO identifier could be assigned to 13,231 out of the 49,829 contigs.

All GO enrichment analyses (Table 3-10) were conducted with the lists of up- and down-regulated contigs of the comparisons over time that were either shared by both treatment groups, and thus representing differences over time irrespective of feeding, or uniquely found in one of the treatment groups, hence denoting feeding regime specific differences (Fig. 4). Furthermore, GO enrichment analyses were conducted with the lists of up-and down-regulated transcripts of the end point comparison (T_1). The GO enrichment analysis was focused on biological processes and involved 7039 contigs.

3.4.1 Specific differences over time in fed charr

Up-regulated transcripts over time confined to fed charr denoted foremost oxygen transport, constituted of genes encoding for several haemoglobin (Hb) subunits, and protein related biological processes (Table 3). Brain *Hb* mRNA has been found in rodents and humans (Richter et al., 2009) and it has been suggested that neural haemoglobin

may facilitate oxygen transport in neurons (Schelshorn et al., 2009), but the exact mechanism remains unknown. The metabolic rate of ectotherms is directly linked to ambient temperature. Specifically, the temperature of optimal growth performance of Arctic charr from North-Norway has been shown to be 14 ºC (Jobling, 1983). The increase in water temperature by almost 10 ºC during our study likely involved an increase in metabolic rate of Arctic charr. Hence, the observed increase in expression of genes encoding oxygen transporters, likely occurred in order to meet increased oxygen demands at higher temperatures.

Furthermore, the feeding related terms such as “feeding behaviour” and “negative regulation of appetite” appeared in the list of GO terms of down-regulated transcripts. The transcript that contributed most to these GO terms was the anorexigenic neuropeptide *CART*, thus suggesting an increase in appetite in the fed charr over time (Table 4). The decrease in *CART* expression is discussed in detail in the paragraph on candidate appetite regulators.

3.4.2 Differences over time irrespective of feeding regime

Over time, there was an up-regulation of transcripts involved in biological processes that included, among others, DNA replication, RNA metabolism, response to steroid hormones and immune response (Table 5). The increase in these basic cell metabolism processes may indicate that cell proliferation and neuronal development were positively affected by season. At the same time, there was a down-regulation of transcripts affecting biological processes such as ion transport, protein related processes and wnt signalling from start to end of the experiment (Table 6).

Interestingly, in adult zebrafish, activation and deactivation of wnt signalling in a sequential manner has been shown to accommodate proliferation and differentiation of progenitor cells in the hypothalamus (Wang et al., 2012). Furthermore, the finding that wnt signalling in the ARC was stimulated by leptin in mouse (Benzler et al., 2013) and by both leptin and long photoperiod in the seasonal Djungarian hamster (*Phodopus sungorus*) (Boucsein et al., 2016) have triggered a discussion for a role of hypothalamic wnt signalling in the seasonal control of energy balance (Helfer and Tups, 2016). Yet, we

found only differences in expression for *WNT4*, and testing for a possible seasonal related function of wnt signalling in charr requires further experiments.

Taken together, seasonal changes including the rise in water temperature, affected many physiological processes in the charr brain and many of these were also seen in feed deprived charr.

3.4.3 Feed deprivation specific differences over time

In the feed deprived charr, up-regulated genes at the end of the experiment were associated with processes such as catabolism, apoptosis, and immune function (Table 7). In contrast, no such trends were found in brain transcriptome analyses of 21-day feed-deprived zebrafish (Drew et al., 2008). The finding that apoptotic related processes were increased is puzzling, given the consensus that the brain is well protected from starvation in both mammals (McCue, 2010) and fish (Tidwell et al., 1992). However, in mammals, there is a debate to what extend feed deprivation may initiate a degeneration of the central nervous system, as different studies have given indication for both absence (Mizushima et al., 2004) and presence (Alirezaei et al., 2014) of autophagy in the brain of feed deprived mice. Further experiments are needed to test whether the observed increase in transcripts involved in apoptosis were a sign of neuronal degradation in charr.

Furthermore, we found the GO term “ketone body catabolic process”, comprising the gene encoding 3-oxoacid CoA transferase, to be up-regulated in the feed deprived charr, pointing towards an increase in ketone catabolic activity from start to the end of experiment (Table 7). This is in line with previous studies on Atlantic salmon and rainbow trout, where ketone bodies were found to serve as an important energy source for the brain when food is absent (Soengas et al., 1996; Soengas et al., 1998a).

In contrast to the fed charr, biological processes referring to oxygen transport were down-regulated in feed deprived charr from start to the end of the experiment (Table 8). This finding is in agreement with the down-regulation of transcripts related to oxygen transport in response to feed deprivation previously seen in Atlantic salmon liver transcriptome (Martin et al., 2010), rainbow trout liver transcriptome (Salem et al., 2007) and in zebrafish brain transcriptome (Drew et al., 2008). Given a potential role for neural haemoglobin in oxygen transport in the fish brain, the lower expression of *Hb* in feed

deprived charr may be a sign of metabolic suppression, despite the increase in water temperature. Brain metabolic suppression, indicated by a reduction of glucose oxidation has previously been observed in feed deprived rainbow trout (Soengas et al., 1998b). However, in the previously cited study, the feed deprived rainbow trout showed a decrease in hexokinase and 6-phosphofruktokinase activity in the brain. In contrast, expression of these glycolytic enzymes did not differ between fed and feed deprived charr in the present study.

3.4.4 Differences between fed and feed deprived charr at the end of the experiment

Similar to the T_1_FDP versus T_0 comparison we found an up-regulation of biological processes related to apoptosis of the T_1_FDP versus T_1_Fed comparison as well as a down-regulation of biological processes referring to oxygen transport (Table 9, Table 10). These findings underline the possibility that brain metabolic process may have been impaired in feed deprived charr.

Taken together, the results from the GO analyses give rise to the assumption that the artificially inflicted energy perturbation by feed deprivation affected several metabolic processes in the brain at the mRNA level.

Yet, many elementary biological processes, including cell division processes and immune response, differed similarly over time in both fed and feed deprived charr. Furthermore, the finding that most of the brain metabolic processes were regulated on the same level in the feed deprived as in the fed charr at the endpoint (Table 5, Table 6) may indicate that most biological processes were adjusted to the absence of feed over time. Anadromous charr feed little to nothing for several months during winter (Jørgensen and Johnsen, 2014) but this study was carried out in summer, and encompassed a rise in water temperature. Given the marked increase in body mass and *K* in the control group in contrast to the reduction of both in feed deprived charr, our results illustrate that even during summer the anadromous charr have a vast ability and flexibility to deal with food limitation.

506 **Table 3** Biological processes enriched by up-regulated contigs only found in the fed group
507 over time. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0006810	Transport	1483	25	14.54	0.0026
GO:0051234	establishment of localization	1484	25	14.55	0.0026
GO:0051179	Localization	1501	25	14.71	0.0031
GO:0044765	single-organism transport	883	19	8.66	0.0006
GO:1902578	single-organism localization	893	19	8.75	0.0007
GO:0015669	gas transport	16	10	0.16	3.20E-17
GO:0015671	oxygen transport	16	10	0.16	3.20E-17
GO:0006457	protein folding	104	5	1.02	0.0034
GO:0003333	amino acid transmembrane transport	15	2	0.15	0.0092
GO:0006865	amino acid transport	15	2	0.15	0.0092
GO:1903825	organic acid transmembrane transport	15	2	0.15	0.0092
GO:0098656	anion transmembrane transport	16	2	0.16	0.0104
GO:0015849	organic acid transport	27	2	0.26	0.0284
GO:0046942	carboxylic acid transport	27	2	0.26	0.0284
GO:0006414	translational elongation	30	2	0.29	0.0345
GO:0051258	protein polymerization	36	2	0.35	0.0482
GO:0071705	nitrogen compound transport	36	2	0.35	0.0482
GO:0009249	protein lipoylation	1	1	0.01	0.0098
GO:0018065	protein-cofactor linkage	3	1	0.03	0.0291
GO:0006284	base-excision repair	4	1	0.04	0.0386
GO:0046836	glycolipid transport	4	1	0.04	0.0386
GO:0006555	methionine metabolic process	5	1	0.05	0.0481
GO:0009086	methionine biosynthetic process	5	1	0.05	0.0481
GO:0019509	L-methionine biosynthetic process from methylthioadenosine	5	1	0.05	0.0481
GO:0043102	amino acid salvage	5	1	0.05	0.0481
GO:0046168	glycerol-3-phosphate catabolic process	5	1	0.05	0.0481
GO:0071265	L-methionine biosynthetic process	5	1	0.05	0.0481
GO:0071267	L-methionine salvage	5	1	0.05	0.0481

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509

510 **Table 4** Biological processes enriched by down-regulated contigs only found in the fed
511 group over time. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0044765	single-organism transport	883	11	5.77	0.02405
GO:1902578	single-organism localization	893	11	5.84	0.02593
GO:0006811	ion transport	532	10	3.48	0.0019
GO:0006461	protein complex assembly	173	4	1.13	0.0258

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0070271	protein complex biogenesis	173	4	1.13	0.0258
GO:0065003	macromolecular complex assembly	189	4	1.24	0.03419
GO:0071822	protein complex subunit organization	189	4	1.24	0.03419
GO:0009966	regulation of signal transduction	212	4	1.39	0.04877
GO:0010646	regulation of cell communication	213	4	1.39	0.04947
GO:0023051	regulation of signalling	213	4	1.39	0.04947
GO:0008272	sulfate transport	3	2	0.02	0.00012
GO:0072348	sulfur compound transport	3	2	0.02	0.00012
GO:0015698	inorganic anion transport	31	2	0.2	0.01723
GO:0048522	positive regulation of cellular process	47	2	0.31	0.03749
GO:0048585	negative regulation of response to stimulus	51	2	0.33	0.04351
GO:0051260	protein homooligomerization	55	2	0.36	0.04985
GO:0008614	pyridoxine metabolic process	2	1	0.01	0.01303
GO:0008615	pyridoxine biosynthetic process	2	1	0.01	0.01303
GO:0042816	vitamin B6 metabolic process	2	1	0.01	0.01303
GO:0042819	vitamin B6 biosynthetic process	2	1	0.01	0.01303
GO:0007172	signal complex assembly	3	1	0.02	0.01948
GO:0009110	vitamin biosynthetic process	3	1	0.02	0.01948
GO:0042364	water-soluble vitamin biosynthetic process	3	1	0.02	0.01948
GO:0001678	cellular glucose homeostasis	5	1	0.03	0.03226
GO:0007631	feeding behaviour	5	1	0.03	0.03226
GO:0008343	adult feeding behaviour	5	1	0.03	0.03226
GO:0009267	cellular response to starvation	5	1	0.03	0.03226
GO:0009991	response to extracellular stimulus	5	1	0.03	0.03226
GO:0030534	adult behaviour	5	1	0.03	0.03226
GO:0031667	response to nutrient levels	5	1	0.03	0.03226
GO:0031668	cellular response to extracellular stimulus	5	1	0.03	0.03226
GO:0031669	cellular response to nutrient levels	5	1	0.03	0.03226
GO:0032094	response to food	5	1	0.03	0.03226
GO:0032095	regulation of response to food	5	1	0.03	0.03226
GO:0032096	negative regulation of response to food	5	1	0.03	0.03226
GO:0032098	regulation of appetite	5	1	0.03	0.03226
GO:0032099	negative regulation of appetite	5	1	0.03	0.03226
GO:0032101	regulation of response to external stimulus	5	1	0.03	0.03226
GO:0032102	negative regulation of response to external stimulus	5	1	0.03	0.03226
GO:0032104	regulation of response to extracellular stimulus	5	1	0.03	0.03226
GO:0032105	negative regulation of response to extracellular stimulus	5	1	0.03	0.03226
GO:0032107	regulation of response to nutrient levels	5	1	0.03	0.03226
GO:0032108	negative regulation of response to nutrients	5	1	0.03	0.03226
GO:0033500	carbohydrate homeostasis	5	1	0.03	0.03226

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0042593	glucose homeostasis	5	1	0.03	0.03226
GO:0042594	response to starvation	5	1	0.03	0.03226
GO:0044708	single-organism behaviour	5	1	0.03	0.03226
GO:0071496	cellular response to external stimulus	5	1	0.03	0.03226
GO:0000186	activation of MAPKK activity	6	1	0.04	0.03859
GO:0006766	vitamin metabolic process	6	1	0.04	0.03859
GO:0006767	water-soluble vitamin metabolic process	6	1	0.04	0.03859
GO:0007610	Behaviour	6	1	0.04	0.03859
GO:0010506	regulation of autophagy	6	1	0.04	0.03859
GO:0032147	activation of protein kinase activity	6	1	0.04	0.03859
GO:0033674	positive regulation of kinase activity	6	1	0.04	0.03859
GO:0043405	regulation of MAP kinase activity	6	1	0.04	0.03859
GO:0045860	positive regulation of protein kinase activity	6	1	0.04	0.03859
GO:0051347	positive regulation of transferase activity	6	1	0.04	0.03859
GO:0000165	MAPK cascade	7	1	0.05	0.04488
GO:0001934	positive regulation of protein phosphorylation	7	1	0.05	0.04488
GO:0010562	positive regulation of phosphorus metabolic process	7	1	0.05	0.04488
GO:0023014	signal transduction by protein phosphorylation	7	1	0.05	0.04488
GO:0031329	regulation of cellular catabolic process	7	1	0.05	0.04488
GO:0031401	positive regulation of protein modification process	7	1	0.05	0.04488
GO:0042327	positive regulation of phosphorylation	7	1	0.05	0.04488
GO:0043408	regulation of MAPK cascade	7	1	0.05	0.04488
GO:0043410	positive regulation of MAPK cascade	7	1	0.05	0.04488
GO:0045937	positive regulation of phosphate metabolic process	7	1	0.05	0.04488

Table 5 Biological processes enriched by up-regulated contigs found in both treatment groups over time. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0019438	aromatic compound biosynthetic process	782	28	16	0.0021
GO:0018130	heterocycle biosynthetic process	796	28	16.28	0.0027
GO:1901362	organic cyclic compound biosynthetic process	803	28	16.43	0.0031
GO:0034654	nucleobase-containing compound biosynthetic process	750	25	15.34	0.0092
GO:0080090	regulation of primary metabolic process	622	24	12.72	0.0017
GO:0031323	regulation of cellular metabolic process	632	24	12.93	0.0021
GO:0019222	regulation of metabolic process	641	24	13.11	0.0025
GO:0019219	regulation of nucleobase-containing compound metabolic process	577	23	11.8	0.0014

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0051171	regulation of nitrogen compound metabolic process	587	23	12.01	0.0017
GO:0060255	regulation of macromolecule metabolic process	619	23	12.66	0.0034
GO:0006355	regulation of transcription, DNA-templated	568	22	11.62	0.0025
GO:1903506	regulation of nucleic acid-templated transcription	568	22	11.62	0.0025
GO:2001141	regulation of RNA biosynthetic process	569	22	11.64	0.0026
GO:0051252	regulation of RNA metabolic process	572	22	11.7	0.0028
GO:0010556	regulation of macromolecule biosynthetic process	580	22	11.87	0.0033
GO:2000112	regulation of cellular macromolecule biosynthetic process	580	22	11.87	0.0033
GO:0031326	regulation of cellular biosynthetic process	581	22	11.89	0.0034
GO:0009889	regulation of biosynthetic process	582	22	11.91	0.0034
GO:0010468	regulation of gene expression	583	22	11.93	0.0035
GO:0006351	transcription, DNA-templated	630	22	12.89	0.0087
GO:0097659	nucleic acid-templated transcription	630	22	12.89	0.0087
GO:0032774	RNA biosynthetic process	633	22	12.95	0.0092
GO:0051276	chromosome organization	94	7	1.92	0.003
GO:0006325	chromatin organization	72	6	1.47	0.0034
GO:0006955	immune response	50	5	1.02	0.0034
GO:0002376	immune system process	52	5	1.06	0.004
GO:0016571	histone methylation	11	3	0.23	0.0012
GO:0018022	peptidyl-lysine methylation	11	3	0.23	0.0012
GO:0034968	histone lysine methylation	11	3	0.23	0.0012
GO:0018205	peptidyl-lysine modification	22	3	0.45	0.0097
GO:0016569	covalent chromatin modification	23	3	0.47	0.011
GO:0016570	histone modification	23	3	0.47	0.011
GO:0019882	antigen processing and presentation	26	3	0.53	0.0154
GO:0006479	protein methylation	27	3	0.55	0.0171
GO:0008213	protein alkylation	27	3	0.55	0.0171
GO:0033993	response to lipid	28	3	0.57	0.0189
GO:0043401	steroid hormone mediated signaling pathway	28	3	0.57	0.0189
GO:0048545	response to steroid hormone	28	3	0.57	0.0189
GO:0071383	cellular response to steroid hormone stimulus	28	3	0.57	0.0189
GO:0071396	cellular response to lipid	28	3	0.57	0.0189
GO:0014070	response to organic cyclic compound	29	3	0.59	0.0208
GO:0071407	cellular response to organic cyclic compound	29	3	0.59	0.0208
GO:0009725	response to hormone	31	3	0.63	0.0248
GO:0009755	hormone-mediated signaling pathway	31	3	0.63	0.0248
GO:0032870	cellular response to hormone stimulus	31	3	0.63	0.0248
GO:0018193	peptidyl-amino acid modification	34	3	0.7	0.0316
GO:0006334	nucleosome assembly	35	3	0.72	0.0341

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0016568	chromatin modification	35	3	0.72	0.0341
GO:0031497	chromatin assembly	35	3	0.72	0.0341
GO:0034728	nucleosome organization	35	3	0.72	0.0341
GO:0006323	DNA packaging	36	3	0.74	0.0366
GO:0006333	chromatin assembly or disassembly	37	3	0.76	0.0393
GO:0033014	tetrapyrrole biosynthetic process	14	2	0.29	0.0322
GO:0007093	mitotic cell cycle checkpoint	1	1	0.02	0.0205
GO:0007094	mitotic spindle assembly checkpoint	1	1	0.02	0.0205
GO:0009888	tissue development	1	1	0.02	0.0205
GO:0009895	negative regulation of catabolic process	1	1	0.02	0.0205
GO:0015696	ammonium transport	1	1	0.02	0.0205
GO:0031099	regeneration	1	1	0.02	0.0205
GO:0031330	negative regulation of cellular catabolic process	1	1	0.02	0.0205
GO:0031396	regulation of protein ubiquitination	1	1	0.02	0.0205
GO:0031397	negative regulation of protein ubiquitination	1	1	0.02	0.0205
GO:0032434	regulation of proteasomal ubiquitin-dependent catabolic process	1	1	0.02	0.0205
GO:0032435	negative regulation of proteasomal ubiquitin dependent protein catabolic process	1	1	0.02	0.0205
GO:0033046	negative regulation of sister chromatid segregation	1	1	0.02	0.0205
GO:0033048	negative regulation of mitotic sister chromatid segregation	1	1	0.02	0.0205
GO:0042177	negative regulation of protein catabolic process	1	1	0.02	0.0205
GO:0042246	tissue regeneration	1	1	0.02	0.0205
GO:0045839	negative regulation of mitotic nuclear division	1	1	0.02	0.0205
GO:0045841	negative regulation of mitotic metaphase/anaphase transition	1	1	0.02	0.0205
GO:0045861	negative regulation of proteolysis	1	1	0.02	0.0205
GO:0045930	negative regulation of mitotic cell cycle	1	1	0.02	0.0205
GO:0048589	developmental growth	1	1	0.02	0.0205
GO:0051340	regulation of ligase activity	1	1	0.02	0.0205
GO:0051352	negative regulation of ligase activity	1	1	0.02	0.0205
GO:0051436	negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	1	1	0.02	0.0205
GO:0051438	regulation of ubiquitin-protein transferase activity	1	1	0.02	0.0205
GO:0051439	regulation of ubiquitin-protein ligase activity	1	1	0.02	0.0205
GO:0051444	negative regulation of ubiquitin-protein transferase activity	1	1	0.02	0.0205
GO:0051725	protein de-ADP-ribosylation	1	1	0.02	0.0205
GO:0051782	negative regulation of cell division	1	1	0.02	0.0205
GO:0051784	negative regulation of nuclear division	1	1	0.02	0.0205
GO:0051985	negative regulation of chromosome segregation	1	1	0.02	0.0205
GO:0061136	regulation of proteasomal protein catabolic process	1	1	0.02	0.0205
GO:0071173	spindle assembly checkpoint	1	1	0.02	0.0205

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0071174	mitotic spindle checkpoint	1	1	0.02	0.0205
GO:1901799	negative regulation of proteasomal protein catabolic process	1	1	0.02	0.0205
GO:1901988	negative regulation of cell cycle phase transition	1	1	0.02	0.0205
GO:1901991	negative regulation of mitotic cell cycle phase transition	1	1	0.02	0.0205
GO:1902100	negative regulation of metaphase/anaphase transition of cell cycle	1	1	0.02	0.0205
GO:1903050	regulation of proteolysis involved in cellular protein catabolic process	1	1	0.02	0.0205
GO:1903051	negative regulation of proteolysis involed in cellular protein catabolic process	1	1	0.02	0.0205
GO:1903320	regulation of protein modification by small protein conjugation or removal	1	1	0.02	0.0205
GO:1903321	negative regulation of protein modification by small protein conjugation or removal	1	1	0.02	0.0205
GO:1903362	regulation of cellular protein catabolic process	1	1	0.02	0.0205
GO:1903363	negative regulation of cellular protein catabolic process	1	1	0.02	0.0205
GO:1904666	regulation of ubiquitin protein ligase activity	1	1	0.02	0.0205
GO:1904667	negative regulation of ubiquitin protein ligase activity	1	1	0.02	0.0205
GO:2000816	negative regulation of mitotic sister chromatid separation	1	1	0.02	0.0205
GO:2001251	negative regulation of chromosome organization	1	1	0.02	0.0205

Table 6 Biological processes enriched by down-regulated contigs found in both treatment groups over time. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0044699	single-organism process	3629	54	45.37	0.04
GO:0044765	single-organism transport	883	17	11.04	0.0443
GO:1902578	single-organism localization	893	17	11.16	0.0485
GO:0006811	ion transport	532	12	6.65	0.0322
GO:0006470	protein dephosphorylation	126	6	1.58	0.0048
GO:0016311	dephosphorylation	162	6	2.03	0.0156
GO:0015672	monovalent inorganic cation transport	175	6	2.19	0.0219
GO:0006813	potassium ion transport	88	4	1.1	0.024
GO:0006457	protein folding	104	4	1.3	0.0408
GO:0051258	protein polymerization	36	3	0.45	0.01
GO:0006304	DNA modification	6	2	0.08	0.0022
GO:0006305	DNA alkylation	6	2	0.08	0.0022
GO:0006306	DNA methylation	6	2	0.08	0.0022
GO:0044728	DNA methylation or demethylation	6	2	0.08	0.0022
GO:0006835	dicarboxylic acid transport	9	2	0.11	0.0053
GO:0016055	Wnt signaling pathway	11	2	0.14	0.0079
GO:0045892	negative regulation of transcription. DNA templated	12	2	0.15	0.0094

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0051253	negative regulation of RNA metabolic process	12	2	0.15	0.0094
GO:1902679	negative regulation of RNA biosynthetic synthetic process	12	2	0.15	0.0094
GO:1903507	negative regulation of nucleic acid-templated transcription	12	2	0.15	0.0094
GO:0006071	glycerol metabolic process	13	2	0.16	0.011
GO:0019400	alditol metabolic process	13	2	0.16	0.011
GO:0045934	negative regulation of nucleobase-containing compound metabolic process	16	2	0.2	0.0165
GO:0019751	polyol metabolic process	17	2	0.21	0.0186
GO:0010558	negative regulation of macromolecule biosynthetic process	19	2	0.24	0.023
GO:2000113	negative regulation of cellular macromolecule biosynthetic process	19	2	0.24	0.023
GO:0007205	protein kinase C-activating G-protein coupled receptor	20	2	0.25	0.0254
GO:0010629	negative regulation of gene expression	20	2	0.25	0.0254
GO:0031327	negative regulation of cellular biosynthetic process	20	2	0.25	0.0254
GO:0009890	negative regulation of biosynthetic process	21	2	0.26	0.0278
GO:0010605	negative regulation of macromolecule metabolic process	22	2	0.28	0.0304
GO:0051172	negative regulation of nitrogen compound metabolic process	23	2	0.29	0.033
GO:0006066	alcohol metabolic process	25	2	0.31	0.0385
GO:0031324	negative regulation of cellular metabolic process	25	2	0.31	0.0385
GO:0009892	negative regulation of metabolic process	27	2	0.34	0.0443
GO:0015849	organic acid transport	27	2	0.34	0.0443
GO:0046942	carboxylic acid transport	27	2	0.34	0.0443
GO:0000087	mitotic M phase	1	1	0.01	0.0125
GO:0000279	M phase	1	1	0.01	0.0125
GO:0022403	cell cycle phase	1	1	0.01	0.0125
GO:0044848	biological phase	1	1	0.01	0.0125
GO:0098763	mitotic cell cycle phase	1	1	0.01	0.0125
GO:0017182	peptidyl-diphthamide metabolic process	2	1	0.03	0.0248
GO:0017183	peptidyl-diphthamide biosynthetic process	2	1	0.03	0.0248
GO:0018202	peptidyl-histidine modification	2	1	0.03	0.0248
GO:0006189	*'de novo*' IMP biosynthetic process	3	1	0.04	0.037
GO:0009186	deoxyribonucleoside diphosphate metabolic process	3	1	0.04	0.037
GO:0006359	regulation of transcription from RNA polymerase III	4	1	0.05	0.0491
GO:0016480	negative regulation of transcription from RNA polymerase III	4	1	0.05	0.0491

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520 **Table 7** Biological processes enriched by up-regulated contigs only found in the feed
521 deprived group over time. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0065007	biological regulation	2021	62	47.66	0.00925
GO:0050789	regulation of biological process	1973	61	46.53	0.00837
GO:0050794	regulation of cellular process	1934	59	45.61	0.01308
GO:0006725	cellular aromatic compound metabolic process	1375	42	32.43	0.03914
GO:0090304	nucleic acid metabolic process	1097	35	25.87	0.03448
GO:0016070	RNA metabolic process	884	30	20.85	0.02407
GO:0060255	regulation of macromolecule metabolic process	619	22	14.6	0.03315
GO:0080090	regulation of primary metabolic process	622	22	14.67	0.03473
GO:0031323	regulation of cellular metabolic process	632	22	14.9	0.04041
GO:0019222	regulation of metabolic process	641	22	15.12	0.04609
GO:0051252	regulation of RNA metabolic process	572	20	13.49	0.04778
GO:0006396	RNA processing	189	10	4.46	0.01367
GO:0006955	immune response	50	7	1.18	0.00015
GO:0002376	immune system process	52	7	1.23	0.0002
GO:0008219	cell death	58	6	1.37	0.00231
GO:0016265	death	58	6	1.37	0.00231
GO:0010941	regulation of cell death	45	5	1.06	0.00391
GO:0042981	regulation of apoptotic process	45	5	1.06	0.00391
GO:0043067	regulation of programmed cell death	45	5	1.06	0.00391
GO:0006915	apoptotic process	57	5	1.34	0.0107
GO:0012501	programmed cell death	57	5	1.34	0.0107
GO:0048518	positive regulation of biological process	67	5	1.58	0.02048
GO:0006397	mRNA processing	68	5	1.6	0.0217
GO:0016071	mRNA metabolic process	79	5	1.86	0.03824
GO:0019882	antigen processing and presentation	26	4	0.61	0.00297
GO:0051726	regulation of cell cycle	40	4	0.94	0.01409
GO:0015074	DNA integration	55	4	1.3	0.04013
GO:0010942	positive regulation of cell death	13	3	0.31	0.0031
GO:0043065	positive regulation of apoptotic process	13	3	0.31	0.0031
GO:0043068	positive regulation of programmed cell death	13	3	0.31	0.0031
GO:0007050	cell cycle arrest	18	3	0.42	0.0081
GO:0045786	negative regulation of cell cycle	21	3	0.5	0.01254
GO:0008380	RNA splicing	26	3	0.61	0.0225
GO:0006729	tetrahydrobiopterin biosynthetic process	5	2	0.12	0.00528
GO:0046146	tetrahydrobiopterin metabolic process	5	2	0.12	0.00528
GO:0006405	RNA export from nucleus	7	2	0.17	0.01074
GO:0006406	mRNA export from nucleus	7	2	0.17	0.01074
GO:0051028	mRNA transport	7	2	0.17	0.01074
GO:0051168	nuclear export	7	2	0.17	0.01074
GO:0071166	ribonucleoprotein complex localization	7	2	0.17	0.01074

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0071426	ribonucleoprotein complex export from nucleus	7	2	0.17	0.01074
GO:0071427	mRNA-containing ribonucleoprotein complex export from nucleus	7	2	0.17	0.01074
GO:0006403	RNA localization	9	2	0.21	0.01785
GO:0050657	nucleic acid transport	9	2	0.21	0.01785
GO:0050658	RNA transport	9	2	0.21	0.01785
GO:0051236	establishment of RNA localization	9	2	0.21	0.01785
GO:0042559	pteridine-containing compound biosynthetic process	11	2	0.26	0.02644
GO:0042558	pteridine-containing compound metabolic process	13	2	0.31	0.03636
GO:0015931	nucleobase-containing compound transport	14	2	0.33	0.04178
GO:0000270	peptidoglycan metabolic process	1	1	0.02	0.02358
GO:0006027	glycosaminoglycan catabolic process	1	1	0.02	0.02358
GO:0009253	peptidoglycan catabolic process	1	1	0.02	0.02358
GO:0042133	neurotransmitter metabolic process	1	1	0.02	0.02358
GO:0042135	neurotransmitter catabolic process	1	1	0.02	0.02358
GO:0046950	cellular ketone body metabolic process	1	1	0.02	0.02358
GO:0046952	ketone body catabolic process	1	1	0.02	0.02358
GO:1902224	ketone body metabolic process	1	1	0.02	0.02358
GO:0006026	aminoglycan catabolic process	2	1	0.05	0.04661
GO:0006535	cysteine biosynthetic process from serine	2	1	0.05	0.04661
GO:0006584	catecholamine metabolic process	2	1	0.05	0.04661
GO:0009712	catechol-containing compound metabolic process	2	1	0.05	0.04661
GO:0019344	cysteine biosynthetic process	2	1	0.05	0.04661
GO:0051090	regulation of sequence-specific DNA binding transcription factor activity	2	1	0.05	0.04661

Table 8 Biological processes enriched by down-regulated contigs only found in the feed deprived group over time. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0006807	nitrogen compound metabolic process	1736	66	53.76	0.03251
GO:1901360	organic cyclic compound metabolic process	1400	55	43.36	0.0299
GO:0009058	biosynthetic process	1439	55	44.57	0.04759
GO:1901576	organic substance biosynthetic process	1374	54	42.55	0.03127
GO:0044249	cellular biosynthetic process	1358	53	42.06	0.03699
GO:0006725	cellular aromatic compound metabolic process	1375	53	42.58	0.0453
GO:1901564	organonitrogen compound metabolic process	620	28	19.2	0.02644
GO:0016043	cellular component organization	466	23	14.43	0.01725
GO:0071840	cellular component organization or biogenesis	499	23	15.45	0.03484
GO:1901566	organonitrogen compound biosynthetic process	451	21	13.97	0.039
GO:0006508	proteolysis	298	18	9.23	0.00495

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0022607	cellular component assembly	222	13	6.88	0.02012
GO:0034622	cellular macromolecular complex assembly	127	12	3.93	0.00054
GO:0007017	microtubule-based process	132	12	4.09	0.00076
GO:0006461	protein complex assembly	173	12	5.36	0.00728
GO:0070271	protein complex biogenesis	173	12	5.36	0.00728
GO:0065003	macromolecular complex assembly	189	12	5.85	0.01414
GO:0071822	protein complex subunit organization	189	12	5.85	0.01414
GO:0006082	organic acid metabolic process	226	12	7	0.04764
GO:0019752	carboxylic acid metabolic process	226	12	7	0.04764
GO:0043436	oxoacid metabolic process	226	12	7	0.04764
GO:0006520	cellular amino acid metabolic process	126	10	3.9	0.00554
GO:0051258	protein polymerization	36	9	1.11	1.00E-06
GO:0043623	cellular protein complex assembly	76	9	2.35	0.00052
GO:0006457	protein folding	104	9	3.22	0.00477
GO:0030163	protein catabolic process	118	8	3.65	0.02963
GO:1902582	single-organism intracellular transport	88	7	2.73	0.01889
GO:0044257	cellular protein catabolic process	100	7	3.1	0.03502
GO:0051603	proteolysis involved in cellular protein catabolic process	100	7	3.1	0.03502
GO:1901605	alpha-amino acid metabolic process	58	5	1.8	0.03302
GO:0006270	DNA replication initiation	4	4	0.12	9.00E-07
GO:0006261	DNA-dependent DNA replication	5	4	0.15	4.40E-06
GO:0006839	mitochondrial transport	16	4	0.5	0.00121
GO:0009069	serine family amino acid metabolic process	20	4	0.62	0.00293
GO:0008652	cellular amino acid biosynthetic process	31	4	0.96	0.01461
GO:1901607	alpha-amino acid biosynthetic process	31	4	0.96	0.01461
GO:0071103	DNA conformation change	44	4	1.36	0.04628
GO:0009070	serine family amino acid biosynthetic process	7	3	0.22	0.00094
GO:0031032	actomyosin structure organization	7	3	0.22	0.00094
GO:0000278	mitotic cell cycle	14	3	0.43	0.00828
GO:0006720	isoprenoid metabolic process	14	3	0.43	0.00828
GO:0008299	isoprenoid biosynthetic process	14	3	0.43	0.00828
GO:1903047	mitotic cell cycle process	14	3	0.43	0.00828
GO:0015669	gas transport	16	3	0.5	0.01218
GO:0015671	oxygen transport	16	3	0.5	0.01218
GO:0006564	L-serine biosynthetic process	4	2	0.12	0.0055
GO:0006563	L-serine metabolic process	7	2	0.22	0.01809
GO:0006544	glycine metabolic process	11	2	0.34	0.04369
GO:0008202	steroid metabolic process	11	2	0.34	0.04369
GO:0000281	mitotic cytokinesis	1	1	0.03	0.03097
GO:0000917	barrier septum assembly	1	1	0.03	0.03097

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0006231	dTMP biosynthetic process	1	1	0.03	0.03097
GO:0006426	glycyl-tRNA aminoacylation	1	1	0.03	0.03097
GO:0006427	histidyl-tRNA aminoacylation	1	1	0.03	0.03097
GO:0006545	glycine biosynthetic process	1	1	0.03	0.03097
GO:0009157	deoxyribonucleoside monophosphate biosynthetic process	1	1	0.03	0.03097
GO:0009162	deoxyribonucleoside monophosphate metabolic process	1	1	0.03	0.03097
GO:0009176	pyrimidine deoxyribonucleoside monophosphate metabolic process	1	1	0.03	0.03097
GO:0009177	pyrimidine deoxyribonucleoside monophosphate biosynthetic process	1	1	0.03	0.03097
GO:0016973	poly(A)+ mRNA export from nucleus	1	1	0.03	0.03097
GO:0030186	melatonin metabolic process	1	1	0.03	0.03097
GO:0030187	melatonin biosynthetic process	1	1	0.03	0.03097
GO:0032506	cytokinetic process	1	1	0.03	0.03097
GO:0034754	cellular hormone metabolic process	1	1	0.03	0.03097
GO:0042445	hormone metabolic process	1	1	0.03	0.03097
GO:0042446	hormone biosynthetic process	1	1	0.03	0.03097
GO:0046073	dTMP metabolic process	1	1	0.03	0.03097
GO:0048033	heme o metabolic process	1	1	0.03	0.03097
GO:0048034	heme O biosynthetic process	1	1	0.03	0.03097
GO:0061640	cytoskeleton-dependent cytokinesis	1	1	0.03	0.03097
GO:0090529	cell septum assembly	1	1	0.03	0.03097
GO:1902410	mitotic cytokinetic process	1	1	0.03	0.03097

Table 9 Biological processes enriched by up-regulated contigs comparing feed deprived *versus* fed charr at end of experiment. Terms sorted by the number of contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0044763	single-organism cellular process	2834	10	6.04	0.03531
GO:0055114	oxidation-reduction process	404	4	0.86	0.0088
GO:0016310	phosphorylation	631	4	1.34	0.03927
GO:0006119	oxidative phosphorylation	11	2	0.02	0.00023
GO:0042773	ATP synthesis coupled electron transport	11	2	0.02	0.00023
GO:0022904	respiratory electron transport chain	12	2	0.03	0.00028
GO:0022900	electron transport chain	18	2	0,04	0,00064
GO:0045333	cellular respiration	32	2	0,07	0,00203
GO:0015980	energy derivation by oxidation of organic compounds	41	2	0,09	0,00331
GO:0010941	regulation of cell death	45	2	0,1	0,00398
GO:0042981	regulation of apoptotic process	45	2	0,1	0,00398
GO:0043067	regulation of programmed cell death	45	2	0,1	0,00398

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0006915	apoptotic process	57	2	0,12	0,00632
GO:0012501	programmed cell death	57	2	0,12	0,00632
GO:0008219	cell death	58	2	0.12	0.00654
GO:0016265	death	58	2	0.12	0.00654
GO:0046034	ATP metabolic process	77	2	0.16	0.01131
GO:0006091	generation of precursor metabolites and energy	81	2	0.17	0.01246
GO:0009144	purine nucleoside triphosphate metabolic process	82	2	0.17	0.01276
GO:0009199	ribonucleoside triphosphate metabolic process	82	2	0.17	0.01276
GO:0009205	purine ribonucleoside triphosphate metabolic process	82	2	0.17	0.01276
GO:0009141	nucleoside triphosphate metabolic process	85	2	0.18	0.01366
GO:0009126	purine nucleoside monophosphate metabolic process	88	2	0.19	0.0146
GO:0009167	purine ribonucleoside monophosphate metabolic process	88	2	0.19	0.0146
GO:0009161	ribonucleoside monophosphate metabolic process	93	2	0.2	0.01621
GO:0009123	nucleoside monophosphate metabolic process	94	2	0.2	0.01655
GO:0042278	purine nucleoside metabolic process	99	2	0.21	0.01825
GO:0046128	purine ribonucleoside metabolic process	99	2	0.21	0.01825
GO:0009119	ribonucleoside metabolic process	105	2	0.22	0.02039
GO:0009150	purine ribonucleotide metabolic process	107	2	0.23	0.02113
GO:0009259	ribonucleotide metabolic process	110	2	0.23	0.02225
GO:0009116	nucleoside metabolic process	112	2	0.24	0.02302
GO:1901657	glycosyl compound metabolic process	112	2	0.24	0.02302
GO:0006163	purine nucleotide metabolic process	116	2	0.25	0.02458
GO:0019693	ribose phosphate metabolic process	122	2	0.26	0.027
GO:0072521	purine-containing compound metabolic process	125	2	0.27	0.02824
GO:0006536	glutamate metabolic process	1	1	0	0.00213
GO:0006537	glutamate biosynthetic process	1	1	0	0.00213
GO:0006562	proline catabolic process	1	1	0	0.00213
GO:0009065	glutamine family amino acid catabolic process	1	1	0	0.00213
GO:0043650	dicarboxylic acid biosynthetic process	1	1	0	0.00213
GO:0006560	proline metabolic process	5	1	0.01	0.01061
GO:0009084	glutamine family amino acid biosynthetic process	10	1	0.02	0.02112
GO:0043648	dicarboxylic acid metabolic process	10	1	0.02	0.02112
GO:0010942	positive regulation of cell death	13	1	0.03	0.02737
GO:0043065	positive regulation of apoptotic process	13	1	0.03	0.02737
GO:0043068	positive regulation of programmed cell death	13	1	0.03	0.02737
GO:0009064	glutamine family amino acid metabolic process	15	1	0.03	0.03152
GO:1901606	alpha-amino acid catabolic process	15	1	0.03	0.03152
GO:0007050	cell cycle arrest	18	1	0.04	0.03772
GO:0009063	cellular amino acid catabolic process	18	1	0.04	0.03772

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0045786	negative regulation of cell cycle	21	1	0.04	0.04387

529

530 **Table 10** Biological processes enriched by down-regulated contigs comparing feed
531 deprived *versus* fed charr at end of experiment. Terms sorted by the number of
532 contributing transcripts.

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0006810	transport	1483	15	8.85	0.02027
GO:0051234	establishment of localization	1484	15	8.85	0.02039
GO:0051179	localization	1501	15	8.96	0.0225
GO:0044765	single-organism transport	883	14	5.27	0.00038
GO:1902578	single-organism localization	893	14	5.33	0.00043
GO:0015669	gas transport	16	11	0.1	3.50E-22
GO:0015671	oxygen transport	16	11	0.1	3.50E-22
GO:0006259	DNA metabolic process	228	6	1.36	0.00214
GO:0006260	DNA replication	113	5	0.67	0.00052
GO:0006270	DNA replication initiation	4	4	0.02	1.10E-09
GO:0006261	DNA-dependent DNA replication	5	4	0.03	5.50E-09
GO:0051258	protein polymerization	36	4	0.21	5.60E-05
GO:0043623	cellular protein complex assembly	76	4	0.45	0.00103
GO:0034622	cellular macromolecular complex assembly	127	4	0.76	0.00666
GO:0006461	protein complex assembly	173	4	1.03	0.01908
GO:0070271	protein complex biogenesis	173	4	1.03	0.01908
GO:0065003	macromolecular complex assembly	189	4	1.13	0.02546
GO:0071822	protein complex subunit organization	189	4	1.13	0.02546
GO:0022607	cellular component assembly	222	4	1.32	0.04232
GO:0007017	microtubule-based process	132	3	0.79	0.04352
GO:0006231	dTMP biosynthetic process	1	1	0.01	0.00597
GO:0006275	regulation of DNA replication	1	1	0.01	0.00597
GO:0009157	deoxyribonucleoside monophosphate biosynthetic process	1	1	0.01	0.00597
GO:0009162	deoxyribonucleoside monophosphate metabolic process	1	1	0.01	0.00597
GO:0009176	pyrimidine deoxyribonucleoside monophosphate metabolic process	1	1	0.01	0.00597
GO:0009177	pyrimidine deoxyribonucleoside monophosphate biosynthetic process	1	1	0.01	0.00597
GO:0046073	dTMP metabolic process	1	1	0.01	0.00597
GO:0051052	regulation of DNA metabolic process	1	1	0.01	0.00597
GO:0009221	pyrimidine deoxyribonucleotide biosynthetic process	2	1	0.01	0.0119
GO:0009263	deoxyribonucleotide biosynthetic process	2	1	0.01	0.0119
GO:0009265	2'-deoxyribonucleotide biosynthetic process	2	1	0.01	0.0119

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0046385	deoxyribose phosphate biosynthetic process	2	1	0.01	0.0119
GO:0009186	deoxyribonucleoside diphosphate metabolic process	3	1	0.02	0.0178
GO:0009129	pyrimidine nucleoside monophosphate metabolic process	4	1	0.02	0.02366
GO:0009130	pyrimidine nucleoside monophosphate biosynthetic process	4	1	0.02	0.02366
GO:0009219	pyrimidine deoxyribonucleotide metabolic process	4	1	0.02	0.02366
GO:0009394	2'-deoxyribonucleotide metabolic process	4	1	0.02	0.02366
GO:0019692	deoxyribose phosphate metabolic process	4	1	0.02	0.02366
GO:0006595	polyamine metabolic process	5	1	0.03	0.02949
GO:0006596	polyamine biosynthetic process	5	1	0.03	0.02949
GO:0009262	deoxyribonucleotide metabolic process	5	1	0.03	0.02949
GO:0009309	amine biosynthetic process	5	1	0.03	0.02949
GO:0042401	cellular biogenic amine biosynthetic process	5	1	0.03	0.02949
GO:0006694	steroid biosynthetic process	6	1	0.04	0.03528
GO:0009396	folic acid-containing compound biosynthetic process	6	1	0.04	0.03528
GO:0006265	DNA topological change	8	1	0.05	0.04677
GO:0006576	cellular biogenic amine metabolic process	8	1	0.05	0.04677
GO:0006760	folic acid-containing compound metabolic process	8	1	0.05	0.04677
GO:0044106	cellular amine metabolic process	8	1	0.05	0.04677

3.5 Effect of feed deprivation on candidate genes involved in the regulation of appetite and energy metabolism in fish

In an attempt to unravel whether central appetite signalling pathways in the charr brain transcriptome were modulated by feed-deprivation, we screened the lists of differentially expressed transcripts systematically for candidate genes that have previously been demonstrated to be involved in the regulation of appetite and energy homeostasis in fish (Volkoff, 2016; Volkoff et al., 2005). Further, due to a strong effect of season and temperature seen on the brain transcriptome (Fig. 3), genes involved in seasonal rhythms were included in the search (Table 11). Lastly, we searched the dataset for possible new actors involved in the regulation of energy homeostasis and food intake, not previously described in fish.

Table 11 Differentially expressed candidate genes involved in food intake control and seasonality

Gene Name	ContigID	Accession	T_1_Fed *vs* T_0		T_1_FDP *vs* T_0		T_1_FDP *vs* T_1_Fed	
			LOG FC	P value	LOG FC	P value	LOG FC	P value
Apelin receptor A	Fishapp_brain_apja.2.3	NM_001140368.1	0.99	0.003	-	-	-	-
Cocaine and amphetamine regulated transcript	Fishapp_brain_contig_33002	NM_001146680.1	-0.66	9.87E-06	-0.33	0.006	-	-
Cholecystokinin	Fishapp_brain_contig_18503	NM_001139522.1	-	-	-	-	-	-
	Fishapp_brain_contig_21023		-	-	-	-	-	-
	Fishapp_brain_contig_17948		-	-	-0.37	0.003	-	-
Corticotropin-releasing factor	Fishapp_brain_contig_16188	NM_001124627.1	-	-	-0.55	0.008	-	-
Deiodinase 2b	Fishapp_brain_contig_18436	NM_001124268.1	-0.60	0.003	-1.79	1.79E-8	-1.19	6.9E-06
	Fishapp_brain_contig_15175		-1.21	2.90E-07	-2.02	1.79E-10	-0.81	6.01E-05
Insulin like growth factor 1	Fishapp_brain_IGF1	GU933431.1	-	-	-0.81	0.003	-	-
Leptin	Fishapp_brain_lepb1	JX131305.1	1,701	5,22E-07	1,372	8,49E-06	-	-
Neuropeptide Y	Fishapp_brain_npy	NM_001146681.1	-0.55	0.0004	-	-	-	-
Proopiomelanocortin	Fishapp_brain_contig_04399	XM_024143555.1	-	-	5,99	0,001	-	-
Tachykinin 1	Fishapp_brain_contig_09262	XM_023974799.1	-	-	-0.51	0.002	-	-

Genes found in the transcriptome, but no difference in expression:
Apelin, Agouti related peptide, Arginine vasotocin, Galanin, Melanin concentrating hormone, Peptide YY, Thyroid releasing hormone

Genes searched, but not present in the transcriptome:
Ghrelin, Kisspeptin, Leptin receptor, Melanocortin receptor 4, Obestatin, Octadecaneuropeptide, Orexin, Pituitary adenylate cyclase-activating polypeptide, Prolactin releasing peptide, Secretoneurin

3.5.1 Expression patterns of anorexigenic neuropeptides do not indicate a stronger satiety signalling in fed compared with feed deprived charr

There was a lower expression of *CART* at T_1 in both fed and feed deprived charr than in fed charr at T_0. However, the difference was larger in fed fish (LogFC = -0.66) than in feed deprived (LogFC = -0.33). No significant difference was found when comparing fed and feed deprived fish at T_1 (Table 11). If CART exhibits an anorexigenic function in charr, the lower expression of *CART* in both fed and feed deprived fish over time points towards a seasonal increase in hunger signalling. This contradicts the expectation to find an increased hunger signal by feed deprivation, but correspond to the lack of responses in hypothalamic CART expression seen in previous long-term feed deprivation studies with rainbow trout (4 months) and Arctic charr (4 weeks) (Jørgensen et al., 2016; Striberny and Jørgensen, 2017). In contrast, *CART* expression decreased in zebrafish brain after three days feed deprivation (Nishio et al., 2012), in salmon brain after six days of feed deprivation (Murashita et al., 2009) and in Atlantic cod (*Gadus morhua*) after seven days feed deprivation (Kehoe and Volkoff, 2007). Partly in line with our results, there was no effect on hypothalamic *CART* expression after 4 months feed deprivation in rainbow trout (Jørgensen et al., 2016).

Being described as a potent satiety signal in mammals and several fish species, hypothalamic *POMCA* was, unexpectedly, markedly (LogFC = 5.99) higher expressed in feed deprived charr at T_1 than in fed charr at T_0. Unlike for *CART*, the result would give support for a decrease in hunger signalling in feed deprived charr. Being aware of the fact that *POMCA* paralogues could not be distinguished in the present study, this result corresponds with an increased expression of *POMCA*1 and *POMCB* in rainbow trout after 4 months of feed deprivation (Jørgensen et al., 2016). Conversely, in another experiment with rainbow trout, hypothalamic *POMCA1* was downregulated after 28 days of feed deprivation (Leder and Silverstein, 2006). However, no difference in *POMCA* expression was seen between fed and feed deprived charr at T_1. This paradox was due to an insignificant increase in POMCA expression also in fed charr, reflecting a seasonal change enhanced by feed deprivation.

Similar to *CART*, hypothalamic *CRF* was lower expressed in feed deprived charr at T_1 than in fed charr at T_0 (LogFC -0.55) in the present study, while no difference was seen

between feed deprived and fed charr at T_1. Previously, a reduced *CRF* brain expression was observed in goldfish (*Carassius auratus*) after seven days of feed deprivation (Maruyama et al., 2006) whereas no changes were observed after short- and long-term feed deprivation in charr (Striberny and Jørgensen, 2017) or after long-term feed deprivation in rainbow trout (Jørgensen et al., 2016).

In rat, the preprotachykinin 1 (PPT) protein, encoded by the *TAC1* gene, has been shown to be negatively regulated by ghrelin and high fat diets, and hence is assumed to be involved in regulating adiposity in rodents (Trivedi et al., 2015). In goldfish, the post-prandial increase of hypothalamic expression of *μ-PPT* has led to the suggestion that μ-PPT may signal satiety (Peyon et al., 2000). To the best of our knowledge, no other long-term feed deprivation study has focused on the effect on central *TAC1* expression. In the present study, brain *TAC1* expression was down-regulated in feed deprived charr at T_1 compared to fed fish at T_0 (LogFC -0.51), without being different from *TAC1* expression in fed fish at T_1.

The function of locally produced LEP in the brain is still a matter of debate, both in mammals and in fish (Morash et al., 1999; Rønnestad et al., 2010; Tinoco et al., 2014), and results from other studies have so far not provided evidence for a role of central LEP in appetite regulation in fish (Striberny et al., 2015; Tinoco et al., 2014). This study revealed a higher *LEP* expression in both fed (LogFC 1.7) and feed deprived fish (LogFC 1.3) at T_1 compared with fed fish at T_0. However, there was no significant difference in the expression of *LEP* between fed and feed deprived charr at T_1, despite a profound difference in condition factor (Fig. 1), and hence, adiposity, between these. In a previous study, *LEPA1* was found to be higher expressed in the hypothalamus of hyperphagic charr in July than of anorexic charr in May and January (Striberny et al., 2015). Due to the lack of *LEPR* in the dataset we were unable to investigate potential feed deprivation induced modulations of LEP sensitivity in the brain. Taken together, these results provide no evidence for an anorexigenic role of central LEP in appetite regulation, nor in the regulation of lipid and energy metabolism.

3.5.2 Expression patterns of orexigenic neuropeptides do not indicate hunger signalling in response to feed deprivation

Previous studies have given evidence for a conserved orexigenic function of NPY in fish (Aldegunde and Mancebo, 2006; Narnaware et al., 2000; Yokobori et al., 2012). *NPY* was lower expressed in fed fish at T_1 compared to T_0 (LogFC -0.55) while no difference was seen between feed deprived and fed fish at T_0 or T_1. As such, based on the brain transcriptome, no hunger signalling by up-regulation of *NPY* could be found in the feed deprived fish. This finding is in accordance with the lack of responses seen in other feed deprivation studies with in Atlantic cod (Kehoe and Volkoff, 2007), Atlantic salmon (Murashita et al., 2009), charr (Striberny and Jørgensen, 2017) and rainbow trout (Jørgensen et al., 2016). The same was the case for *AGRP* expression which was not found to be differentially expressed in any of the comparisons.

Apelin is considered as another potent orexigenic actor in fish (Lin et al., 2014; Volkoff and Wyatt, 2009). Our data did not reveal an effect of long-term feed deprivation on apelin expression. Brain expression of apelin receptor (*APJA*), was, however, higher in fed fish at T_1 than in fed fish at T_0 (LogFC = 0.99) but not different between feed deprived and fed fish at T_0 or T_1.

In summary, the results in the present study did not show expected responses to feed deprivation in the expression of candidate genes involved in appetite regulation in fish. This result does not necessarily contradict an appetite regulatory role of these actors in fish. In a previous study with charr, no differences in the hypothalamic expression of *AgRP*, *MC4R*, *CRF*, *NPY*, *CART*, *POMC*s and *LEPR* were seen between fed and 4 weeks feed deprived fish, whereas the expression of, *CART*, *MC4R* and *AgRP* responded when the feed deprived fish subsequently was re-fed for 1 or 5 hours or exposed to feed flavour during the same time interval (Striberny and Jørgensen, 2017). This indicates that changes in gene expression are more likely to be seen during transition stages than during steady-state situations, when regulation may be output through post-transcriptional mechanism. In support of this, only few and weak differences in brain expression of appetite regulators were noted in winter and summer adapted charr, despite their dramatic difference in feeding status from anorexia in winter to hyperphagia in summer (Striberny et al., 2015).

Taken together, the results in the present study adds to the strongly varying and contradictory results previously obtained in long-term feed deprivation studies with fish, most likely reflecting methodological insufficiencies, differences in experimental designs and/or species-specific adaptations. The latter may be exemplified by the finding of a strong increase in brain expression of the anorexic *POMCA* by feed deprivation, and only an insignificant change in the same direction in fed fish from T_0 to T_1, possibly reflecting a satiety output by *POMCA* in the feed deprived charr. An up-regulation of satiety signalling in the feed deprived charr in the present study seems unlikely, but corresponds with findings from a previous long-term (4 months) feed deprivation study on rainbow trout, in which there was seen a marked up-regulation of hypothalamic *POMCA1* and *POMCB* expression (Jørgensen et al., 2016). Such paradoxical results may be interpreted as an adaptation in high-latitude fish to save energy by reducing feed searching behaviour when feed is absent.

3.5.3 Genes related to energy metabolism and seasonality

Insulin-like growth factor 1 is key growth regulating hormone in vertebrates, and plasma levels of IGF1 usually correlate positively with growth in fish (Beckman, 2011). Accordingly, it has been shown that plasma IGF1 levels vary proportionally with increases and decreases in feeding rate in Arctic charr (Cameron et al., 2007). Further, hypothalamic *IGF1* expression was reduced by one month feed deprivation resulting in a positive correlation also between hypothalamic *IGF1* expression and *K* of fed and feed deprived fish (Striberny and Jørgensen, 2017). In the present study, *IGF1* expression was, as expected, downregulated in feed deprived charr at T_1 compared to fed charr at T_0. However, no difference was found in central *IGF1* expression between fed and feed deprived charr at T_1, despite the huge difference in *K* between feed deprived and fed charr at T_1 (Fig. 1). This discrepancy in results between studies with Arctic charr may relate to the fact that hypothalamic *IGF1* expression was measured in the former study by Striberny and Jørgensen (2017), while brain *IGF1* expression was measured in the present study.

Deiodinase 2 (Dio2) converses thyroxin (T4) to the biologically active triiodothyronine (T3) which, in turn, is known as an enhancer of several biological

processes and exerts pleiotropic functions in the mammalian brain (Bernal, 2002). In mammals and birds, the increase in day length in spring stimulates hypothalamic *Dio2* expression, thereby stimulating a range of processes related to seasonal phenotype transitions, including appetite (Nakane and Yoshimura, 2014). Similarly, it was recently shown that brain expression of the paralogue *Dio2b* was elevated in response to an increased day length in Atlantic salmon (Lorgen et al., 2015). We found a significantly lower hypothalamic *Dio2b* expression at T_1 than at T_0 in both fed and feed deprived charr, and a lower expression in feed deprived charr than in fed charr at T_1 (LogFC = -1.19). Our findings may be interpreted as a general decline in *Dio2b* expression during summer after the spring peak, a decline that was enhanced by feed deprivation. Indeed, in the seasonal Djungarian hamster (*Phodopus sungorus*), hypothalamic *Dio2* expression was reduced in response to fasting induced torpor during summer (Bank et al., 2017). Further experiments are needed to characterize the function of Dio2 in seasonal processes, including feeding behaviour, in the highly seasonal Arctic charr.

3.6 Nerve growth factor inducible (VGF) - a novel candidate involved in the control of appetite and energy homeostasis in fish?

The present study could not reveal any clear patterns of up- or down-regulation of central hunger and satiety signals by feed deprivation in charr. Therefore, we searched the lists of top differentially expressed annotated transcripts of the different comparisons for neuropeptides that may be involved in the central control of appetite regulation and energy metabolism. Most of the top annotated differentially expressed transcripts represented genes involved in the biological processes that were also overrepresented by GO enrichment analysis, e.g. genes encoding for haemoglobin subunits, and genes involved in basic cell metabolic processes (see supplementary tables S1-S6).

Interestingly, we found brain *VGF* (non-acronymic, nerve growth factor inducible) to be higher expressed (LogFC = 0.54) in feed deprived than in fed charr at T_1. In mammals, the *VGF* gene encodes for a 68 kDa protein precursor that is abundantly expressed in the brain, particularly in the hypothalamus. VGF cleaves into several smaller peptides that have been shown to be involved in a multitude of processes including nerve growth upon injury, seasonality, and food intake/energy metabolism (Lewis et al., 2015). Several

studies in rodents have given evidence for a role of VGF in the control of energy metabolism (Foglesong et al., 2016; Hahm et al., 2002; Hahm et al., 1999) and food intake (Jethwa et al., 2007). Targeted deletion of *VGF* produces a lean, small, and hyperactive mouse (Hahm et al., 1999). In mammals, the function of *VGF* is complex and not entirely understood. For example, 48h feed deprivation in mice caused in one study an up-regulation of hypothalamic *VGF* expression (Hahm et al. 1999), and down-regulation in another study (Foglesong et al. 2016). In mice, *VGF* derived neuropeptide TLQP-21 increases energy expenditure without affecting expression of *POMC*/*CART* and *AgRP*/*NPY*, suggesting that TLQP-21 exerts its effects downstream of MC4R signalling (Bartolomucci et al., 2006). Furthermore, in Siberian hamster, ARC *VGF* expression was induced by a decrease in photoperiod (Barrett et al., 2005) and reduced by T_3 (Lewis et al., 2016), raising evidence that *VGF* is involved in the control of seasonal feeding in this species. We found *Dio2b* to be lower expressed in feed deprived charr than in fed charr at T_1. This indicates a reduced thyroid hormone action, which based on the results from Siberian hamster, could be underlying the increased *VGF* expression seen in feed deprived charr.

To the best of our knowledge, a role of *VGF* in fish has so far not been reported. Given its role in the control of appetite and energy homeostasis in mammals, its putative role in appetite regulation in fish needs to be investigated. Its response to photoperiod in Siberian hamster is of particular interest in the strongly seasonal Arctic charr.

4. Conclusion

In conclusion, the general gene expression patterns in brain transcriptome of fed and feed deprived charr displayed strong shifts in expression of transcripts involved in basic cell metabolic processes over time, and only minor differences were seen in response to feed deprivation. However, these seasonal changes appeared to be enhanced by feed deprivation, indicated by a higher number of differentially expressed transcripts over time in feed deprived than in fed charr. A decrease in the expression of haemoglobin subunits together with an increase in expression of genes involved in apoptosis, revealed from GO analysis, may indicate a negative effect of feed deprivation on brain metabolism. This is

also supported by a substantially stronger reduction in *DIO2* expression in feed deprived than in fed charr from start to end of the experiment.

Despite the marked divergence of body mass and *K*, no clear hunger signalling was found between fed and feed deprived charr, when searching the lists of differentially expressed transcripts for anorexigenic and orexigenic candidate genes known to be involved in appetite regulation in fish. This result indicates that appetite regulators were expressed in a similar manner in main brain compartments of both feed deprived and fed charr. On the other hand, pooling of different brain structures may have masked possible differences in gene expression of appetite regulators at more confined brain areas. Also, it must be taken into account that differences were only measured on the RNA level and thus it is not possible to conclude about any phenotypic consequences by long-term feed deprivation on the Arctic charr brain.

5. References

Aldegunde, M., and M. Mancebo. 2006. Effects of neuropeptide Y on food intake and brain biogenic amines in the rainbow trout (Oncorhynchus mykiss). *Peptides*. 27:719-727.

Alexa, A., and J. Rahnenfuhrer. 2010. topGO: enrichment analysis for gene ontology. *R package version*. 2.

Alirezaei, M., C.C. Kemball, C.T. Flynn, M.R. Wood, J.L. Whitton, and W.B. Kiosses. 2014. Short-term fasting induces profound neuronal autophagy. *Autophagy*. 6:702-710.

Babichuk, N.A., and H. Volkoff. 2013. Changes in expression of appetite-regulating hormones in the cunner (Tautogolabrus adspersus) during short-term fasting and winter torpor. *Physiol Behav*. 120:54-63.

Bank, J.H., C. Cubuk, D. Wilson, E. Rijntjes, J. Kemmling, H. Markovsky, P. Barrett, and A. Herwig. 2017. Gene expression analysis and microdialysis suggest hypothalamic triiodothyronine (T3) gates daily torpor in Djungarian hamsters (Phodopus sungorus). *Journal of Comparative Physiology B*. 187:857-868.

Bardou, P., J. Mariette, F. Escudié, C. Djemiel, and C. Klopp. 2014. jvenn: an interactive Venn diagram viewer. *BMC bioinformatics*. 15:293.

Barrett, P., A.W. Ross, A. Balik, P.A. Littlewood, J.G. Mercer, K.M. Moar, T. Sallmen, J. Kaslin, P. Panula, and S. Schuhler. 2005. Photoperiodic regulation of histamine H3 receptor and VGF messenger ribonucleic acid in the arcuate nucleus of the Siberian hamster. *Endocrinology*. 146:1930-1939.

Bartolomucci, A., G. La Corte, R. Possenti, V. Locatelli, A. Rigamonti, A. Torsello, E. Bresciani, I. Bulgarelli, R. Rizzi, and F. Pavone. 2006. TLQP-21, a VGF-derived peptide, increases energy expenditure and prevents the early phase of diet-induced obesity. *Proceedings of the National Academy of Sciences*. 103:14584-14589.

Beckman, B.R. 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. *Gen Comp Endocrinol*. 170:233-252.

774 Benzler, J., Z.B. Andrews, C. Pracht, S. Stohr, P.R. Shepherd, D.R. Grattan, and A. Tups. 2013.
775 Hypothalamic WNT signalling is impaired during obesity and reinstated by leptin treatment in
776 male mice. *Endocrinology*. 154:4737-4745.
777 Bernal, J. 2002. Action of thyroid hormone in brain. *Journal of endocrinological investigation*. 25:268-
778 288.
779 Boucsein, A., J. Benzler, C. Hempp, S. Stohr, G. Helfer, and A. Tups. 2016. Photoperiodic and Diurnal
780 Regulation of WNT Signaling in the Arcuate Nucleus of the Female Djungarian Hamster,
781 Phodopus sungorus. *Endocrinology*. 157:799-809.
782 Cabau, C., F. Escudié, A. Djari, Y. Guiguen, J. Bobe, and C. Klopp. 2017. Compacting and correcting Trinity
783 and Oases RNA-Seq *de novo* assemblies. *PeerJ*. 5:e2988.
784 Cameron, C., R. Moccia, P.A. Azevedo, and J.F. Leatherland. 2007. Effect of diet and ration on the
785 relationship between plasma GH and IGF-1 concentrations in Arctic charr, Salvelinus alpinus (L.).
786 *Aquaculture Research*. 38:877-886.
787 Cerda-Reverter, J., G. Martınez-Rodrıguez, S. Zanuy, M. Carrillo, and D. Larhammar. 2000. Molecular
788 evolution of the neuropeptide Y (NPY) family of peptides: cloning of three NPY-related peptides
789 from the sea bass (Dicentrarchus labrax). *Regulatory peptides*. 95:25-34.
790 Cone, R., M. Cowley, A. Butler, W. Fan, D. Marks, and M. Low. 2002. The arcuate nucleus as a conduit for
791 diverse signals relevant to energy homeostasis. *International Journal of Obesity*. 25:S63.
792 Cone, R.D. 1999. The Central Melanocortin System and Energy Homeostasis. *Trends Endocrinol Metab*.
793 10:211-216.
794 Cortés, R., S. Navarro, M.J. Agulleiro, R. Guillot, V. García-Herranz, E. Sánchez, and J.M. Cerdá-Reverter.
795 2014. Evolution of the melanocortin system. *General and comparative endocrinology*. 209:3-10.
796 Davidson, W.S., B.F. Koop, S.J. Jones, P. Iturra, R. Vidal, A. Maass, I. Jonassen, S. Lien, and S.W. Omholt.
797 2010. Sequencing the genome of the Atlantic salmon (Salmo salar). *Genome Biol*. 11.
798 DePristo, M.A., E. Banks, R. Poplin, K.V. Garimella, J.R. Maguire, C. Hartl, A.A. Philippakis, G. Del Angel,
799 M.A. Rivas, and M. Hanna. 2011. A framework for variation discovery and genotyping using next-
800 generation DNA sequencing data. *Nature genetics*. 43:491.
801 Drew, R.E., K.J. Rodnick, M. Settles, J. Wacyk, E. Churchill, M.S. Powell, R.W. Hardy, G.K. Murdoch, R.A.
802 Hill, and B.D. Robison. 2008. Effect of starvation on transcriptomes of brain and liver in adult
803 female zebrafish (Danio rerio). *Physiol Genomics*. 35:283-295.
804 Elmquist, J.K., C.F. Elias, and C.B. Saper. 1999. Hypothalamic control of body weight. *Neuron*. 22:221-
805 232.
806 Foglesong, G.D., W. Huang, X. Liu, A.M. Slater, J. Siu, V. Yildiz, S.R. Salton, and L. Cao. 2016. Role of
807 hypothalamic VGF in energy balance and metabolic adaption to environmental enrichment in
808 mice. *Endocrinology*. 2016:34-46.
809 Hahm, S., C. Fekete, T.M. Mizuno, J. Windsor, H. Yan, C.N. Boozer, C. Lee, J.K. Elmquist, R.M. Lechan, and
810 C.V. Mobbs. 2002. VGF is required for obesity induced by diet, gold thioglucose treatment, and
811 agouti and is differentially regulated in pro-opiomelanocortin-and neuropeptide Y-containing
812 arcuate neurons in response to fasting. *Journal of Neuroscience*. 22:6929-6938.
813 Hahm, S., T.M. Mizuno, T.J. Wu, J.P. Wisor, C.A. Priest, C.A. Kozak, C.N. Boozer, B. Peng, R.C. McEvoy,
814 and P. Good. 1999. Targeted deletion of the Vgf gene indicates that the encoded secretory
815 peptide precursor plays a novel role in the regulation of energy balance. *Neuron*. 23:537-548.
816 Helfer, G., and A. Tups. 2016. Hypothalamic Wnt Signalling and its Role in Energy Balance Regulation. *J*
817 *Neuroendocrinol*. 28:12368.
818 Hoskins, L.J., and H. Volkoff. 2012. The comparative endocrinology of feeding in fish: insights and
819 challenges. *Gen Comp Endocrinol*. 176:327-335.

Jethwa, P.H., A. Warner, K.N. Nilaweera, J.M. Brameld, J.W. Keyte, W.G. Carter, N. Bolton, M. Bruggraber, P.J. Morgan, and P. Barrett. 2007. VGF-derived peptide, TLQP-21, regulates food intake and body weight in Siberian hamsters. *Endocrinology*. 148:4044-4055.

Jobling, M. 1983. Influence of body weight and temperature on growth rates of Arctic charr, Salvelinus alpinus (L.). *Journal of Fish Biology*. 22:471-475.

Jones, P., D. Binns, H.-Y. Chang, M. Fraser, W. Li, C. McAnulla, H. McWilliam, J. Maslen, A. Mitchell, and G. Nuka. 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics*. 30:1236-1240.

Jørgensen, E.H., N.J. Bernier, A.G. Maule, and M.M. Vijayan. 2016. Effect of long-term fasting and a subsequent meal on mRNA abundances of hypothalamic appetite regulators, central and peripheral leptin expression and plasma leptin levels in rainbow trout. *Peptides*. 86:162-170.

Jørgensen, E.H., S.J.S. Johansen, and M. Jobling. 1997. Seasonal patterns of growth, lipid deposition and lipid depletion in anadromous Arctic charr. *Journal of Fish Biology*. 51:312-326.

Jørgensen, E.H., and H.K. Johnsen. 2014. Rhythmic life of the Arctic charr: adaptations to life at the edge. *Mar Genomics*. 14:71-81.

Kehoe, A.S., and H. Volkoff. 2007. Cloning and characterization of neuropeptide Y (NPY) and cocaine and amphetamine regulated transcript (CART) in Atlantic cod (Gadus morhua). *Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology*. 146:451-461.

Kent, W.J. 2002. BLAT—the BLAST-like alignment tool. *Genome research*. 12:656-664.

Leder, E.H., and J.T. Silverstein. 2006. The pro-opiomelanocortin genes in rainbow trout (Oncorhynchus mykiss): duplications, splice variants, and differential expression. *J Endocrinol*. 188:355-363.

Lewis, J.E., J.M. Brameld, P. Hill, D. Wilson, P. Barrett, F.J. Ebling, and P.H. Jethwa. 2016. Thyroid hormone and vitamin D regulate VGF expression and promoter activity. *J Mol Endocrinol*. 56:123-134.

Lewis, J.E., J.M. Brameld, and P.H. Jethwa. 2015. Neuroendocrine role for VGF. *Frontiers in endocrinology*. 6:3.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*. 25:1754-1760.

Lin, F., H. Wu, H. Chen, Z. Xin, D. Yuan, T. Wang, J. Liu, Y. Gao, X. Zhang, and C. Zhou. 2014. Molecular and physiological evidences for the role in appetite regulation of apelin and its receptor APJ in Ya-fish (Schizothorax prenanti). *Molecular and cellular endocrinology*. 396:46-57.

Lorgen, M., E. Casadei, E. Król, A. Douglas, M.J. Birnie, L.O. Ebbesson, T.O. Nilsen, W.C. Jordan, E.H. Jørgensen, and H. Dardente. 2015. Functional divergence of type 2 deiodinase paralogs in the Atlantic salmon. *Current Biology*. 25:936-941.

Magnanou, E., C. Noirot, J. Falcón, and E.H. Jørgensen. 2016. Sequencing and characterization of a multi-organ Arctic charr transcriptome: a toolbox for investigating polymorphism and seasonal life in a high Arctic fish. *Marine genomics*. 29:45-53.

Martin, S.A., A. Douglas, D.F. Houlihan, and C.J. Secombes. 2010. Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (Salmo salar). *BMC genomics*. 11:418.

Maruyama, K., T. Miura, M. Uchiyama, S. Shioda, and K. Matsuda. 2006. Relationship between anorexigenic action of pituitary adenylate cyclase-activating polypeptide (PACAP) and that of corticotropin-releasing hormone (CRH) in the goldfish, Carassius auratus. *Peptides*. 27:1820-1826.

McCue, M.D. 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp Biochem Physiol A Mol Integr Physiol*. 156:1-18.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, and M. Daly. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome research*. 20:1297-1303.

Mizushima, N., A. Yamamoto, M. Matsui, T. Yoshimori, and Y. Ohsumi. 2004. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol Biol Cell*. 15:1101-1111.

Morash, B., A. Li, P.R. Murphy, M. Wilkinson, and E. Ur. 1999. Leptin Gene Expression in the Brain and Pituitary Gland. *Endocrinology*. 140:5995.

Murashita, K., T. Kurokawa, L.O. Ebbesson, S.O. Stefansson, and I. Rønnestad. 2009. Characterization, tissue distribution, and regulation of agouti-related protein (AgRP), cocaine- and amphetamine-regulated transcript (CART) and neuropeptide Y (NPY) in Atlantic salmon (Salmo salar). *Gen Comp Endocrinol*. 162:160-171.

Nakane, Y., and T. Yoshimura. 2014. Universality and diversity in the signal transduction pathway that regulates seasonal reproduction in vertebrates. *Frontiers in neuroscience*. 8:115.

Narnaware, Y.K., P. Peyon, X. Lin, and R.E. Peter. 2000. Regulation of food intake by neuropeptide Y in goldfish *Am J Physiol Regul Integr Comp Physiol*. 279:1025-1034.

Nijenhuis, W.A., J. Oosterom, and R.A. Adan. 2001. AgRP (83–132) acts as an inverse agonist on the human-melanocortin-4 receptor. *Molecular Endocrinology*. 15:164-171.

Nishio, S.-I., Y. Gibert, L. Berekelya, L. Bernard, F. Brunet, E. Guillot, J.-C. Le Bail, J.A. Sánchez, A.M. Galzin, and G. Triqueneaux. 2012. Fasting induces CART down-regulation in the zebrafish nervous system in a cannabinoid receptor 1-dependent manner. *Molecular endocrinology*. 26:1316-1326.

Norman, J.D., M. Robinson, B. Glebe, M.M. Ferguson, and R.G. Danzmann. 2012. Genomic arrangement of salinity tolerance QTLs in salmonids: a comparative analysis of Atlantic salmon (Salmo salar) with Arctic charr (Salvelinus alpinus) and rainbow trout (Oncorhynchus mykiss). *BMC genomics*. 13:420.

Panicz, R., C. Klopp, R. Igielski, P. Hofsoe, J. Sadowski, and J.A. Coller Jr. 2017. Tench (Tinca tinca) high-throughput transcriptomics reveal feed dependent gut profiles. *Aquaculture*. 479:200-207.

Peyon, P., H. Saied, X. Lin, and R. Peter. 2000. Preprotachykinin gene expression in goldfish brain:: Sexual, seasonal, and postprandial variations☆. *Peptides*. 21:225-231.

Richter, F., B.H. Meurers, C. Zhu, V.P. Medvedeva, and M.F. Chesselet. 2009. Neurons express hemoglobin alpha- and beta-chains in rat and human brains. *J Comp Neurol*. 515:538-547.

Robinson, M.D., D.J. McCarthy, and G.K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 26:139-140.

Rønnestad, I., T.O. Nilsen, K. Murashita, A.R. Angotzi, A.G. Gamst Moen, S.O. Stefansson, P. Kling, B. Thrandur Björnsson, and T. Kurokawa. 2010. Leptin and leptin receptor genes in Atlantic salmon: Cloning, phylogeny, tissue distribution and expression correlated to long-term feeding status. *Gen Comp Endocrinol*. 168:55-70.

Salem, M., J. Silverstein, C.E. Rexroad, 3rd, and J. Yao. 2007. Effect of starvation on global gene expression and proteolysis in rainbow trout (Oncorhynchus mykiss). *BMC Genomics*. 8:328.

Schelshorn, D.W., A. Schneider, W. Kuschinsky, D. Weber, C. Kruger, T. Dittgen, H.F. Burgers, F. Sabouri, N. Gassler, A. Bach, and M.H. Maurer. 2009. Expression of hemoglobin in rodent neurons. *J Cereb Blood Flow Metab*. 29:585-595.

Schwartz, M.W., S.C. Woods, D. Porte Jr., R.J. Seeley, and D.G. Baskin. 2000. Central nervous system control of food intake. *Nature*. 404:661-671.

Silverstein, J.T., J. Breininger, D.G. Baskin, and E.M. Plisetskaya. 1998. Neurpepeptide-Y like Gene Expression in the Salmon Brain Increases with Fasting. *Gen Comp Endocrinol*. 110:157-165.

913 Simão, F.A., R.M. Waterhouse, P. Ioannidis, E.V. Kriventseva, and E.M. Zdobnov. 2015. BUSCO: assessing
914 genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*.
915 31:3210-3212.
916 Soengas, J., E. Strong, J. Fuentes, J. Veira, and M. Andrés. 1996. Food deprivation and refeeding in
917 Atlantic salmon, Salmo salar: effects on brain and liver carbohydrate and ketone bodies
918 metabolism. *Fish Physiology and Biochemistry*. 15:491-511.
919 Soengas, J.L., E.F. Strong, and M.D. Andres. 1998a. Glucose, lactate, and b-hydroxybutyrate utilization by
920 rainbow trout brain: changes during food deprivation. *Physiological zoology*. 71:285-293.
921 Soengas, J.L., E.F. Strong, and M.D. Andres. 1998b. Glucose, Lactate, and b-Hydroxybutyrate Utilization
922 by Rainbow Trout Brain: Changes during Food Deprivation. *Physioligal Zoology*. 71:285-293.
923 Striberny, A., and E.H. Jørgensen. 2017. Feedback from Arctic charr: Feed flavour stimulation and re-
924 feeding after feed deprivation stimulate genes encoding both orexigenic and anorexigenic
925 neuropeptides. *Gen Comp Endocrinol*. 246:71-80.
926 Striberny, A., C.S. Ravuri, M. Jobling, and E.H. Jorgensen. 2015. Seasonal Differences in Relative Gene
927 Expression of Putative Central Appetite Regulators in Arctic Charr (Salvelinus alpinus) Do Not
928 Reflect Its Annual Feeding Cycle. *PLoS One*. 10:e0138857.
929 Swanson, H.K., K.A. Kidd, J.D. Reist, and M. Trudel. 2011. Quantifying importance of marine prey in the
930 diets of two partially anadromous fishes. *Canadian Journal of Fisheries and Aquatic Sciences*.
931 68:2020-2028.
932 Theissinger, K., C. Falckenhayn, D. Blande, A. Toljamo, J. Gutekunst, J. Makkonen, J. Jussila, F. Lyko, A.
933 Schrimpf, and R. Schulz. 2016. *De Novo* assembly and annotation of the freshwater crayfish
934 Astacus astacus transcriptome. *Marine genomics*. 28:7-10.
935 Tidwell, J.H., C.D. Webster, and J.A. Clark. 1992. Effects of feeding, starvation, and refeeding on the fatty
936 acid composition of channel catfish, Ictalurus punctatus, tissues. *Comp Biochem Physiol*.
937 103A:365-368.
938 Tinoco, A.B., L.G. Nisembaum, N. de Pedro, M.J. Delgado, and E. Isorna. 2014. Leptin expression is
939 rhythmic in brain and liver of goldfish (Carassius auratus). Role of feeding time. *Gen Comp*
940 *Endocrinol*. 204:239-247.
941 Trivedi, C., X. Shan, Y.-C.L. Tung, D. Kabra, J. Holland, S. Amburgy, K. Heppner, H. Kirchner, G.S. Yeo, and
942 D. Perez-Tilve. 2015. Tachykinin-1 in the central nervous system regulates adiposity in rodents.
943 *Endocrinology*. 156:1714-1723.
944 Tveiten, H., H.K. Johnsen, and M. Jobling. 1996. Influence of the maturity status on the annual cycles of
945 feeding and growth in Arctic charr reared at constant temperature. *Journal of Fish Biology*.
946 48:910-924.
947 Van der Auwera, G.A., M.O. Carneiro, C. Hartl, R. Poplin, G. Del Angel, A. Levy-Moonshine, T. Jordan, K.
948 Shakir, D. Roazen, and J. Thibault. 2013. From FastQ data to high-confidence variant calls: the
949 genome analysis toolkit best practices pipeline. *Current protocols in bioinformatics*:11.10. 11-
950 11.10. 33.
951 Volkoff, H. 2016. The Neuroendocrine Regulation of Food Intake in Fish: A Review of Current Knowledge.
952 *Frontiers in Neuroscience*. 10.
953 Volkoff, H., L.F. Canosa, S. Unniappan, J.M. Cerda-Reverter, N.J. Bernier, S.P. Kelly, and R.E. Peter. 2005.
954 Neuropeptides and the control of food intake in fish. *Gen Comp Endocrinol*. 142:3-19.
955 Volkoff, H., and J.L. Wyatt. 2009. Apelin in goldfish (Carassius auratus): cloning, distribution and role in
956 appetite regulation. *Peptides*. 30:1434-1440.
957 Wang, X., D. Kopinke, J. Lin, A.D. McPherson, R.N. Duncan, H. Otsuna, E. Moro, K. Hoshijima, D.J.
958 Grunwald, F. Argenton, C.B. Chien, L.C. Murtaugh, and R.I. Dorsky. 2012. Wnt signaling regulates
959 postembryonic hypothalamic progenitor differentiation. *Dev Cell*. 23:624-636.
960 Wynne, K., S. Stanley, B. McGowan, and S. Bloom. 2005. Appetite control. *J Endocrinol*. 184:291-318.

Yokobori, E., M. Azuma, R. Nishiguchi, K.S. Kang, M. Kamijo, M. Uchiyama, and K. Matsuda. 2012. Neuropeptide Y stimulates food intake in the Zebrafish, Danio rerio. *J Neuroendocrinol*. 24:766-773.

6. Figures and figure captions

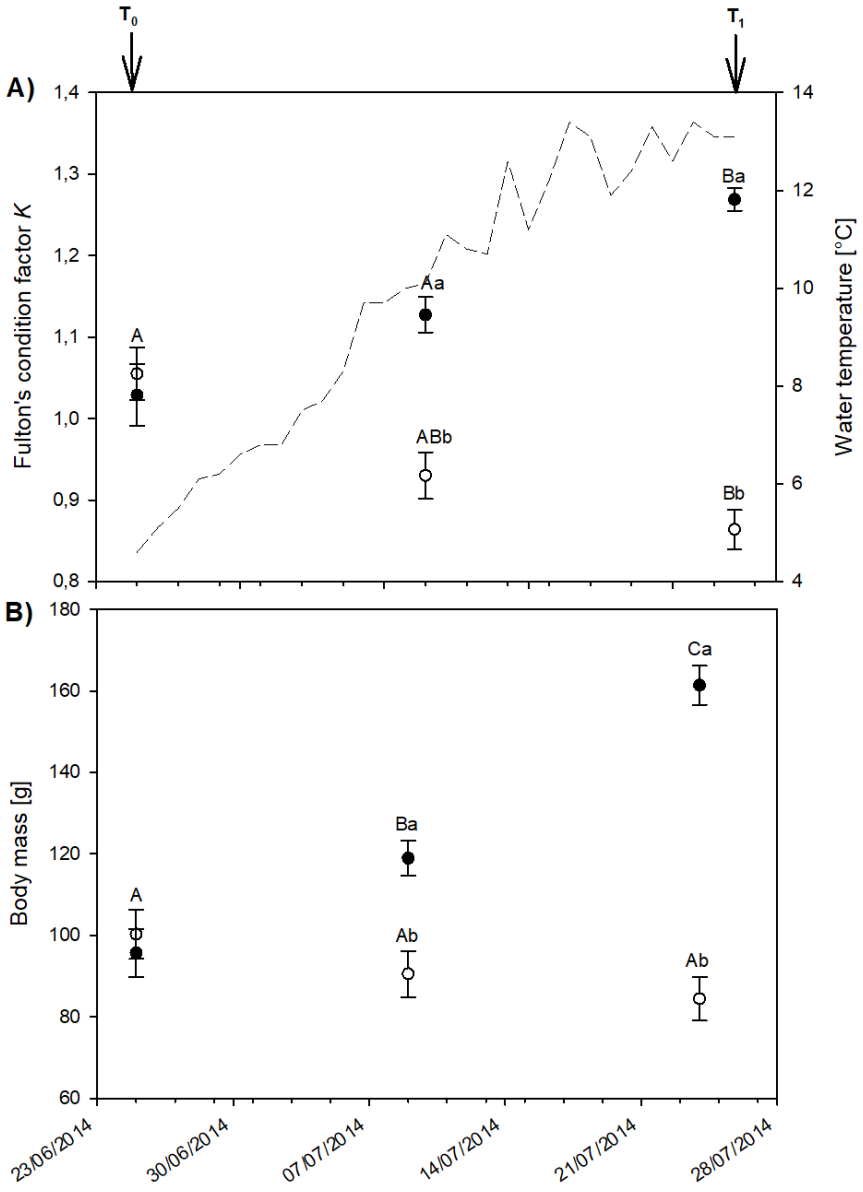

Fig. 1 Fulton's condition factor (A) and body mass (B) of fed (black dots) and feed deprived Arctic charr (white dots) during the experiment. Charr sampled at T_0 were measured before distribution to Tank 1 and Tank 2. Dashed line: water temperature. n = 21 per treatment group. Values are shown as mean ± SEM. Different capital and lowercase letters denote differences within treatment group at different time points and differences between treatment groups at the given time point, respectively.

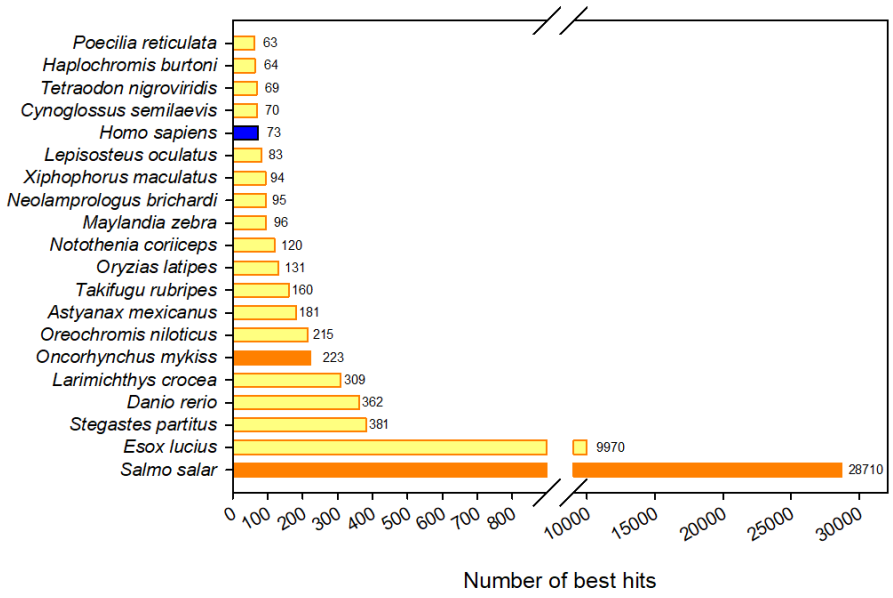

Fig. 2 Top 20 of the species contributing the most to best hit annotations. Best hits were based on all databases involved in the annotation process. Yellow bars: teleosts, orange bars: salmonids, blue bar: mammals.

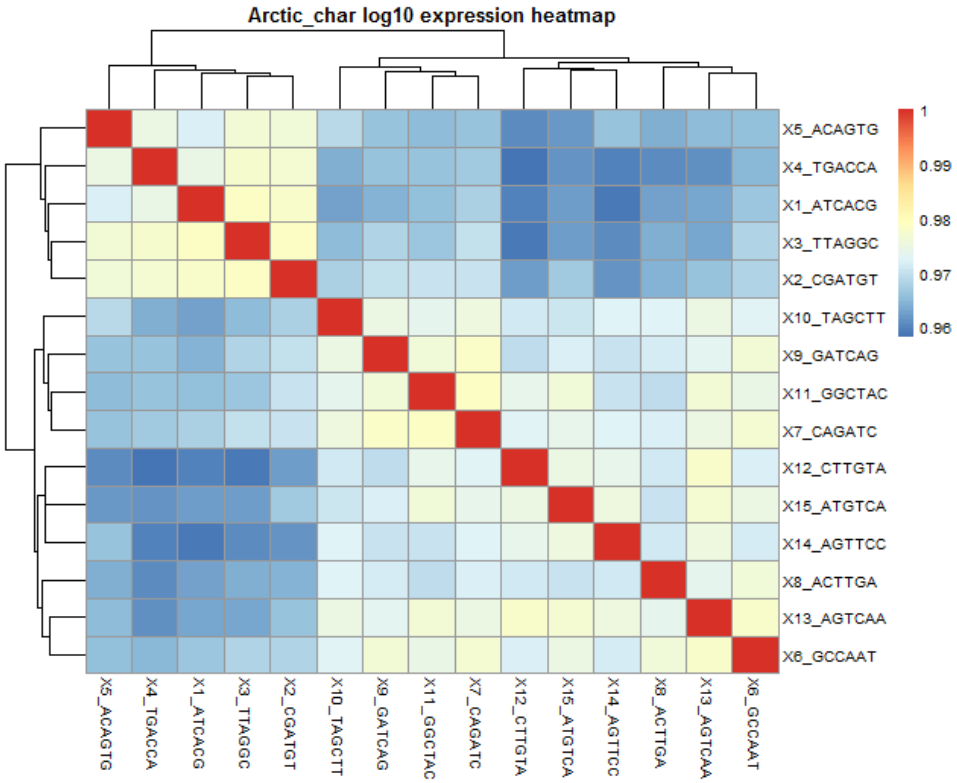

Fig. 3 Correlation heatmap based on raw counts of the 49,829 contigs possessing a FPKM greater than 1 for at least one sample.

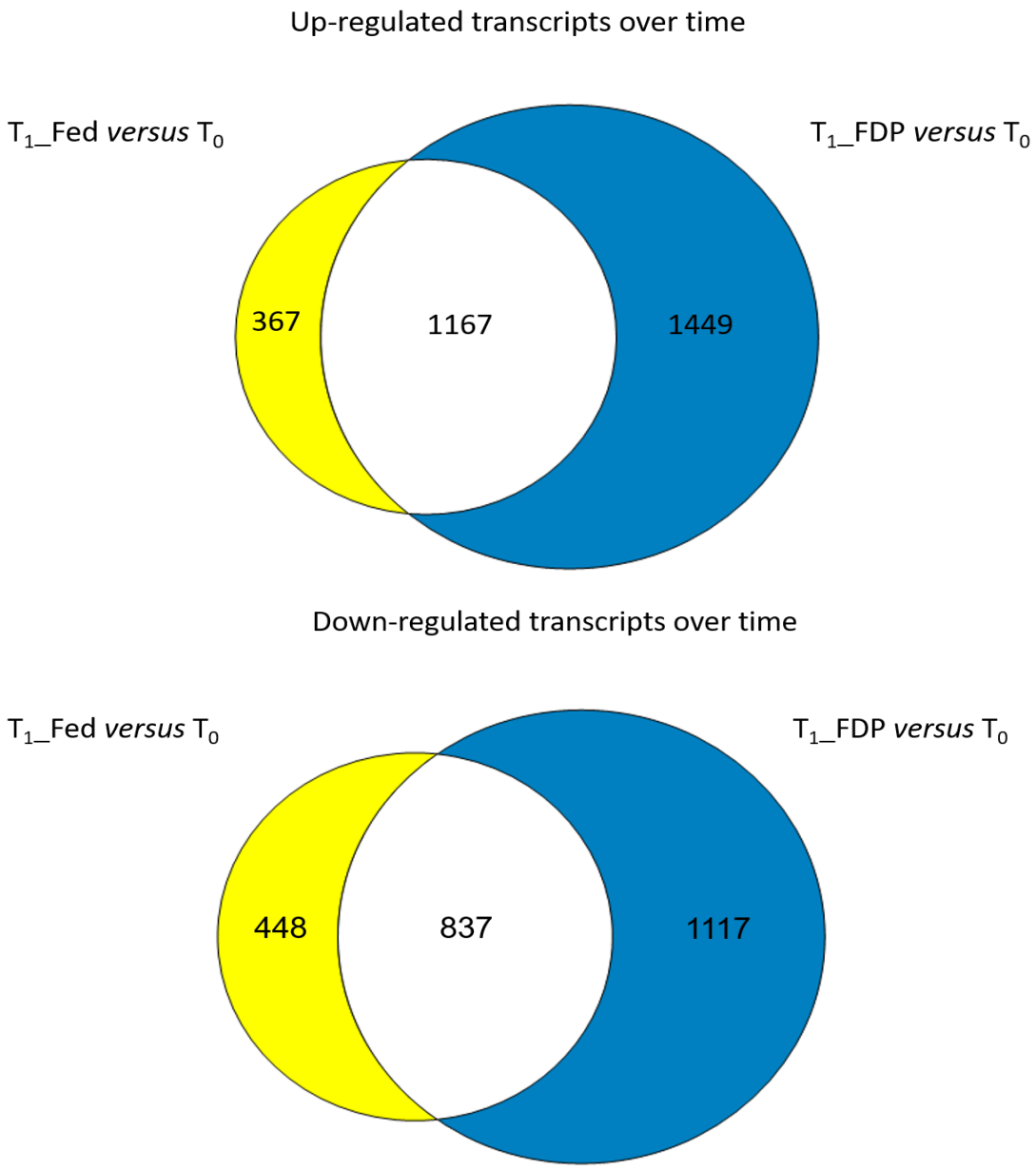

Fig. 4 Venn diagrams comparing up- and down-regulations over time between the two treatments: T_1_Fed *versus* T_0 and T_1_FDP *versus* T_0 (FDR<0.05. LogFC cut-off 0.5/-0.5). Yellow: transcripts uniquely differentially expressed in T_1_Fed *versus* T_0 comparison, blue: transcripts uniquely differentially expressed in T_1_FDP *versus* T_0 comparison. White: transcripts that were found to be differentially expressed over time irrespective of feeding regime.

6. Supplementary Tables

Supplementary table 1 FDP versus Fed Top annotated up-regulated genes. logFC = Log fold change, logCPM = log counts per million, F = F statistic, FDR = false discovery rate. Differential gene expression was analysed in R (EdgeR package version 3.8.6) using a general linear model and a quasi-likelihood F-test.

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_WIPI4.3.3	1.12	3.76	57.78	0.00	0.00
Fishapp_brain_LOC101475809	0.96	4.10	23.64	0.00	0.03
Fishapp_brain_LOC105005941	0.88	2.68	24.86	0.00	0.03
Fishapp_brain_ddx17.2.2	0.88	4.16	55.66	0.00	0.00
Fishapp_brain_LOC105013210.1.2	0.86	5.88	51.09	0.00	0.00
Fishapp_brain_bcr.1.2	0.81	5.18	25.79	0.00	0.02
Fishapp_brain_LOC105016071	0.68	4.43	28.42	0.00	0.02
Fishapp_brain_LOC105017087	0.68	3.84	29.00	0.00	0.02
Fishapp_brain_txnip	0.64	4.41	35.80	0.00	0.01
Fishapp_brain_LOC105027064	0.62	3.01	22.27	0.00	0.04
Fishapp_brain_adck3.2.2	0.59	3.93	41.55	0.00	0.00
Fishapp_brain_ezh1.2.2	0.58	3.36	32.99	0.00	0.01
Fishapp_brain_LOC104942424.1.2	0.58	4.23	20.98	0.00	0.05
Fishapp_brain_ezh1.1.2	0.56	6.29	67.55	0.00	0.00
Fishapp_brain_carns1.1.3	0.55	4.06	45.66	0.00	0.00
Fishapp_brain_TKTL2	0.55	3.35	30.77	0.00	0.02
Fishapp_brain_vgf.3.3	0.54	5.30	23.82	0.00	0.03
Fishapp_brain_nat14	0.54	5.99	20.97	0.00	0.05
Fishapp_brain_LOC105011516	0.52	4.50	24.08	0.00	0.03

Supplementary table 2 FDP versus Fed Top annotated down-regulated genes. logFC = Log fold change, logCPM = log counts per million, F = F statistic, FDR = false discovery rate. Differential gene expression was analysed in R (EdgeR package version 3.8.6) using a general linear model and a quasi-likelihood F-test.

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_HBA	-2.10	1.96	60.64	0.00	0.00
Fishapp_brain_B3AT	-1.76	4.24	44.58	0.00	0.00
Fishapp_brain_LOC105028541.4.4	-1.68	0.65	33.75	0.00	0.01
Fishapp_brain_HBB	-1.57	5.90	57.76	0.00	0.00
Fishapp_brain_tieg3.1.2	-1.23	5.73	67.01	0.00	0.00
Fishapp_brain_tieg3.2.2	-1.16	4.88	58.95	0.00	0.00
Fishapp_brain_cdc2	-1.11	3.22	37.31	0.00	0.01
Fishapp_brain_klf11	-1.06	3.35	43.52	0.00	0.00
Fishapp_brain_tysy	-1.06	2.63	30.46	0.00	0.01
Fishapp_brain_mki67	-1.05	1.09	25.72	0.00	0.02
Fishapp_brain_LOC103037264	-0.90	6.83	24.08	0.00	0.03
Fishapp_brain_CP1A1	-0.84	4.16	25.49	0.00	0.02
Fishapp_brain_ccna2	-0.84	2.97	47.17	0.00	0.00
Fishapp_brain_TUBB4B	-0.84	1.72	30.20	0.00	0.01
Fishapp_brain_top2a	-0.82	4.40	63.15	0.00	0.00
Fishapp_brain_nusap1	-0.73	2.85	32.80	0.00	0.01
Fishapp_brain_LOC105021720	-0.63	7.68	25.65	0.00	0.02
Fishapp_brain_TBB5	-0.63	4.07	37.43	0.00	0.01
Fishapp_brain_cdk2	-0.62	2.81	26.81	0.00	0.03
Fishapp_brain_rir2	-0.61	4.35	28.37	0.00	0.02
Fishapp_brain_prep.1.2	-0.59	2.99	36.44	0.00	0.03
Fishapp_brain_lss	-0.58	4.14	23.30	0.00	0.03
Fishapp_brain_mcm5	-0.58	3.44	32.69	0.00	0.01
Fishapp_brain_mcm6	-0.58	3.83	36.35	0.00	0.01
Fishapp_brain_lmnb1	-0.57	4.85	43.51	0.00	0.00
Fishapp_brain_mthfd2	-0.56	4.10	36.75	0.00	0.01
Fishapp_brain_TBB1	-0.56	3.52	33.59	0.00	0.01
Fishapp_brain_g3bp2.2.3	-0.53	3.71	25.04	0.00	0.03
Fishapp_brain_mcm4	-0.53	3.30	26.11	0.00	0.03
Fishapp_brain_nsdhl	-0.52	3.79	23.53	0.00	0.03
Fishapp_brain_mcm2	-0.51	3.97	32.76	0.00	0.01

Supplementary table 3 T1_Fed versus T0 Top annotated up-regulated genes. logFC = Log fold change, logCPM = log counts per million, F = F statistic, FDR = false discovery rate. Differential gene expression was analysed in R (EdgeR package version 3.8.6) using a general linear model and a quasi-likelihood F-test.

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_LOC102194111	2.35	4.36	77.45	0.00	0.00
Fishapp_brain_hsp47	1.98	3.97	58.37	0.00	0.00
Fishapp_brain_nattl	1.93	2.34	20.02	0.00	0.01
Fishapp_brain_LOC105026715.1.2	1.87	0.94	11.64	0.00	0.04
Fishapp_brain_IscW_ISCW010708	1.86	1.89	68.86	0.00	0.00
Fishapp_brain_p2rx4	1.81	1.22	81.71	0.00	0.00
Fishapp_brain_LOC105027984.1.3	1.78	0.20	23.78	0.00	0.00
Fishapp_brain_lepb1	1.70	3.07	58.20	0.00	0.00
Fishapp_brain_LOC105012776.1.2	1.67	4.50	708.40	0.00	0.00
Fishapp_brain_JARD2	1.67	3.74	217.72	0.00	0.00
Fishapp_brain_LOC105028541.4.4	1.60	0.65	31.26	0.00	0.00
Fishapp_brain_ddhd1.2.5	1.50	2.00	89.09	0.00	0.00
Fishapp_brain_tax1bp1.1.4	1.48	1.50	42.60	0.00	0.00
Fishapp_brain_DJC27	1.48	-0.17	31.58	0.00	0.00
Fishapp_brain_LOC105007533	1.45	4.41	19.01	0.00	0.01
Fishapp_brain_ef2.2.3	1.44	7.34	462.66	0.00	0.00
Fishapp_brain_LOC102310373	1.39	-0.25	37.12	0.00	0.00
Fishapp_brain_LOC105015765	1.38	-1.05	14.52	0.00	0.02
Fishapp_brain_LOC105010020	1.34	5.22	59.97	0.00	0.00
Fishapp_brain_5ntc	1.33	3.48	97.85	0.00	0.00
Fishapp_brain_LOC101475809	1.33	4.10	39.75	0.00	0.00
Fishapp_brain_hps3.1.2	1.31	4.35	49.73	0.00	0.00
Fishapp_brain_ninj2	1.29	5.09	17.00	0.00	0.01
Fishapp_brain_WDR37	1.28	0.21	26.11	0.00	0.00
Fishapp_brain_LOC105005941	1.26	2.68	41.97	0.00	0.00
Fishapp_brain_LOC102222545	1.25	1.13	17.24	0.00	0.01
Fishapp_brain_LOC101067200	1.23	-0.20	13.75	0.00	0.02
Fishapp_brain_upp.2.2	1.22	1.78	26.59	0.00	0.00
Fishapp_brain_LOC105028591.1.2	1.21	0.22	17.17	0.00	0.01
Fishapp_brain_bpi	1.21	2.76	56.10	0.00	0.00
Fishapp_brain_fam135b	1.21	1.19	50.97	0.00	0.00
Fishapp_brain_LOC103035992	1.20	2.93	63.04	0.00	0.00
Fishapp_brain_HBB	1.19	5.90	34.02	0.00	0.00
Fishapp_brain_TBA1D.1.2	1.19	5.43	457.20	0.00	0.00
Fishapp_brain_anxa3	1.17	3.76	140.34	0.00	0.00
Fishapp_brain_LOC105006249	1.15	2.96	28.62	0.00	0.00
Fishapp_brain_dnaja4	1.15	3.37	57.20	0.00	0.00

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_LOC105028005	1.13	1.42	40.39	0.00	0.00
Fishapp_brain_agpat4	1.13	4.02	33.99	0.00	0.00
Fishapp_brain_nup155.4.5	1.12	0.27	31.74	0.00	0.00
Fishapp_brain_sag	1.12	2.23	28.59	0.00	0.00
Fishapp_brain_UBP13	1.10	2.37	47.40	0.00	0.00
Fishapp_brain_LOC105022989.2.2	1.10	1.59	37.15	0.00	0.00
Fishapp_brain_man2a2.3.3	1.09	2.84	14.03	0.00	0.02
Fishapp_brain_ST7.1.3	1.09	0.05	11.75	0.00	0.04
Fishapp_brain_CNEPA	1.09	0.48	14.17	0.00	0.02
Fishapp_brain_LOC105011288.2.2	1.09	3.73	51.87	0.00	0.00
Fishapp_brain_LOC105029373	1.08	2.92	70.03	0.00	0.00
Fishapp_brain_LOC101493977	1.07	1.88	41.28	0.00	0.00

Supplementary table 4 T1_Fed versus T0 Top annotated down-regulated genes. logFC = Log fold change, logCPM = log counts per million, F = F statistic, FDR = false discovery rate. Differential gene expression was analysed in R (EdgeR package version 3.8.6) using a general linear model and a quasi-likelihood F-test.

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_jarid2.1.2	-3.57	4.22	720.52	0.00	0.00
Fishapp_brain_jarid2.2.2	-3.04	6.19	1369.02	0.00	0.00
Fishapp_brain_JARID2	-2.85	1.79	223.80	0.00	0.00
Fishapp_brain_TBAT	-1.93	3.28	365.32	0.00	0.00
Fishapp_brain_LOC102112523	-1.80	0.65	26.13	0.00	0.00
Fishapp_brain_ppp1r17.1.2	-1.66	0.86	29.49	0.00	0.00
Fishapp_brain_pggt1b.2.2	-1.47	3.35	12.27	0.00	0.03
Fishapp_brain_suz12.2.2	-1.38	5.63	500.10	0.00	0.00
Fishapp_brain_LOC105025099.2.3	-1.36	0.68	22.74	0.00	0.00
Fishapp_brain_LOC105015657	-1.35	3.67	38.86	0.00	0.00
Fishapp_brain_LOC105024451.2.2	-1.33	0.90	14.44	0.00	0.02
Fishapp_brain_contig_16122	-1.33	-0.16	10.45	0.00	0.05
Fishapp_brain_p4r2a.2.3	-1.27	3.26	201.07	0.00	0.00
Fishapp_brain_sec23ip.2.2	-1.25	0.72	47.78	0.00	0.00
Fishapp_brain_LOC103044328	-1.21	5.57	445.66	0.00	0.00
Fishapp_brain_LOC105006814.2.2	-1.20	3.43	80.64	0.00	0.00
Fishapp_brain_LOC105028880.2.3	-1.16	2.53	74.07	0.00	0.00
Fishapp_brain_LOC105006814.1.2	-1.16	3.60	80.94	0.00	0.00
Fishapp_brain_suz12.1.2	-1.15	4.84	270.75	0.00	0.00
Fishapp_brain_csmd1.1.3	-1.15	0.13	19.26	0.00	0.01
Fishapp_brain_env.2.8	-1.14	4.25	33.39	0.00	0.00
Fishapp_brain_slc13a5	-1.13	4.20	143.78	0.00	0.00
Fishapp_brain_LOC105015451.2.2	-1.11	5.74	351.54	0.00	0.00
Fishapp_brain_LOC105028964.4.4	-1.09	0.19	18.61	0.00	0.01
Fishapp_brain_iqcb1	-1.08	1.56	29.81	0.00	0.00
Fishapp_brain_pon2.2.3	-1.08	1.87	30.23	0.00	0.00
Fishapp_brain_kiaa0100.6.12	-1.08	2.08	35.53	0.00	0.00
Fishapp_brain_LOC105028880.1.3	-1.07	2.58	90.49	0.00	0.00
Fishapp_brain_SLC47A1	-1.07	1.45	31.25	0.00	0.00
Fishapp_brain_EML6	-1.07	5.66	135.40	0.00	0.00
Fishapp_brain_shank3.1.2	-1.06	0.60	16.61	0.00	0.01
Fishapp_brain_kiaa0100.7.12	-1.04	2.23	35.41	0.00	0.00
Fishapp_brain_stambp	-1.03	2.54	48.48	0.00	0.00
Fishapp_brain_CREB1	-1.01	2.38	46.37	0.00	0.00
Fishapp_brain_dennd5a.2.3	-1.00	-0.46	11.50	0.00	0.04
Fishapp_brain_p4r2a.3.3	-1.00	2.29	38.07	0.00	0.00
Fishapp_brain_phf20l1.1.2	-0.99	5.01	217.88	0.00	0.00

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_tbcel.4.4	-0.99	0.13	10.97	0.00	0.04
Fishapp_brain_mybl1	-0.98	3.84	52.56	0.00	0.00
Fishapp_brain_LOC104927480.1.2	-0.98	2.37	49.16	0.00	0.00
Fishapp_brain_LOC104531997	-0.98	1.16	19.50	0.00	0.01
Fishapp_brain_prkd1.2.7	-0.92	0.68	18.07	0.00	0.01
Fishapp_brain_LOC104934062	-0.91	5.28	151.53	0.00	0.00
Fishapp_brain_ppp1r17.2.2	-0.91	1.24	20.73	0.00	0.01
Fishapp_brain_crfa.2.2	-0.91	1.42	17.55	0.00	0.01
Fishapp_brain_kcnq2.2.3	-0.90	1.52	28.00	0.00	0.00
Fishapp_brain_sgk1.1.2	-0.90	1.58	28.53	0.00	0.00
Fishapp_brain_LOC105013965.2.2	-0.90	2.60	21.15	0.00	0.01
Fishapp_brain_ARNT.1.2	-0.90	2.65	30.15	0.00	0.00

Supplementary table 5 T1_FDP versus T0 Top annotated up-regulated genes. logFC = Log fold change, logCPM = log counts per million, F = F statistic, FDR = false discovery rate. Differential gene expression was analysed in R (EdgeR package version 3.8.6) using a general linear model and a quasi-likelihood F-test.

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_LOC105007533	2.41	4.41	52.54	0.00	0.00
Fishapp_brain_ninj2	2.33	5.09	55.00	0.00	0.00
Fishapp_brain_LOC102194111	2.30	4.36	74.35	0.00	0.00
Fishapp_brain_LOC101475809	2.28	4.10	119.26	0.00	0.00
Fishapp_brain_LOC105005941	2.14	2.68	127.98	0.00	0.00
Fishapp_brain_LOC105027984.1.3	2.13	0.20	36.32	0.00	0.00
Fishapp_brain_LOC105010626.1.2	2.04	2.87	18.63	0.00	0.01
Fishapp_brain_LOC105021410	2.02	2.43	20.85	0.00	0.00
Fishapp_brain_JARD2	1.99	3.74	315.14	0.00	0.00
Fishapp_brain_LOC105006249	1.92	2.96	83.40	0.00	0.00
Fishapp_brain_LOC102310373	1.91	-0.25	78.29	0.00	0.00
Fishapp_brain_tax1bp1.1.4	1.89	1.50	72.94	0.00	0.00
Fishapp_brain_LOC105012776.1.2	1.87	4.50	893.83	0.00	0.00
Fishapp_brain_p2rx4	1.76	1.22	78.62	0.00	0.00
Fishapp_brain_env.8.8	1.75	3.95	16.32	0.00	0.01
Fishapp_brain_ef2.1.3	1.75	1.83	9.49	0.01	0.04
Fishapp_brain_LOC102222545	1.73	1.13	35.14	0.00	0.00
Fishapp_brain_ddhd1.2.5	1.70	2.00	117.94	0.00	0.00
Fishapp_brain_nattl	1.65	2.34	14.72	0.00	0.01
Fishapp_brain_ddx17.2.2	1.64	4.16	182.71	0.00	0.00
Fishapp_brain_LOC105011288.2.2	1.62	3.73	118.49	0.00	0.00
Fishapp_brain_LOC105010020	1.62	5.22	87.82	0.00	0.00
Fishapp_brain_LOC105016071	1.56	4.43	139.66	0.00	0.00
Fishapp_brain_WDR37	1.55	0.21	40.78	0.00	0.00
Fishapp_brain_LOC105021444	1.54	3.64	26.31	0.00	0.00
Fishapp_brain_hps3.1.2	1.52	4.35	66.78	0.00	0.00
Fishapp_brain_GMAN2	1.48	1.33	23.34	0.00	0.00
Fishapp_brain_bpi	1.47	2.76	84.52	0.00	0.00
Fishapp_brain_WIPI4.3.3	1.46	3.76	96.20	0.00	0.00
Fishapp_brain_UBP13	1.46	2.37	85.75	0.00	0.00
Fishapp_brain_LOC105022989.2.2	1.45	1.59	68.25	0.00	0.00
Fishapp_brain_LOC105011288.1.2	1.43	3.80	92.34	0.00	0.00
Fishapp_brain_fam135b	1.40	1.19	71.81	0.00	0.00
Fishapp_brain_spata5l1.2.2	1.40	-0.29	19.79	0.00	0.00
Fishapp_brain_WIPI4.2.3	1.37	1.24	42.50	0.00	0.00
Fishapp_brain_lepb1	1.37	3.07	38.04	0.00	0.00
Fishapp_brain_LOC105028591.1.2	1.35	0.22	22.55	0.00	0.00

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_ef2.2.3	1.34	7.34	404.42	0.00	0.00
Fishapp_brain_upp.2.2	1.33	1.78	32.83	0.00	0.00
Fishapp_brain_LOC105019889.3.6	1.33	5.69	199.42	0.00	0.00
Fishapp_brain_LOC105013210.1.2	1.31	5.88	116.09	0.00	0.00
Fishapp_brain_LOC105025510.4.5	1.31	1.67	50.68	0.00	0.00
Fishapp_brain_SLC25A42	1.30	-0.28	14.08	0.00	0.01
Fishapp_brain_kdm5b.5.5	1.30	3.43	30.29	0.00	0.00
Fishapp_brain_LOC105030671.2.2	1.30	4.08	304.09	0.00	0.00
Fishapp_brain_LOC104922467	1.28	5.16	43.22	0.00	0.00
Fishapp_brain_CRAM	1.28	-1.01	9.63	0.01	0.04
Fishapp_brain_LOC105029087.1.2	1.28	4.06	125.60	0.00	0.00
Fishapp_brain_LOC105010626.2.2	1.28	3.76	40.97	0.00	0.00

Supplementary table 6 T1_FDP versus T0 Top annotated down-regulated genes. logFC = Log fold change, logCPM = log counts per million, F = F statistic, FDR = false discovery rate. Differential gene expression was analysed in R (EdgeR package version 3.8.6) using a general linear model and a quasi-likelihood F-test.

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_jarid2.1.2	-3.36	4.22	676.78	0.00	0.00
Fishapp_brain_jarid2.2.2	-2.79	6.19	1197.53	0.00	0.00
Fishapp_brain_JARID2	-2.50	1.79	191.13	0.00	0.00
Fishapp_brain_TBAT	-2.34	3.28	520.71	0.00	0.00
Fishapp_brain_LOC105024451.2.2	-1.72	0.90	23.83	0.00	0.00
Fishapp_brain_ppp1r17.1.2	-1.65	0.86	30.85	0.00	0.00
Fishapp_brain_LOC102112523	-1.62	0.65	22.70	0.00	0.00
Fishapp_brain_iqcb1	-1.60	1.56	63.03	0.00	0.00
Fishapp_brain_LOC102199920	-1.51	-0.66	13.38	0.00	0.02
Fishapp_brain_klf11	-1.48	3.35	87.06	0.00	0.00
Fishapp_brain_MSI2H	-1.43	-0.13	27.65	0.00	0.00
Fishapp_brain_mki67	-1.41	1.09	48.28	0.00	0.00
Fishapp_brain_LOC105017390	-1.34	1.47	33.00	0.00	0.00
Fishapp_brain_cdc2	-1.33	3.22	53.83	0.00	0.00
Fishapp_brain_LOC105028880.2.3	-1.32	2.53	95.79	0.00	0.00
Fishapp_brain_suz12.2.2	-1.31	5.63	461.76	0.00	0.00
Fishapp_brain_nup93.1.2	-1.30	-0.80	11.97	0.00	0.02
Fishapp_brain_LOC104965831	-1.30	-1.15	8.87	0.01	0.05
Fishapp_brain_dnmt3a	-1.29	4.23	275.86	0.00	0.00
Fishapp_brain_LOC103044328	-1.29	5.57	509.35	0.00	0.00
Fishapp_brain_HBA	-1.26	1.96	21.26	0.00	0.00
Fishapp_brain_ppp1r17.2.2	-1.25	1.24	39.24	0.00	0.00
Fishapp_brain_suz12.1.2	-1.24	4.84	315.24	0.00	0.00
Fishapp_brain_pon2.2.3	-1.22	1.87	38.97	0.00	0.00
Fishapp_brain_slc13a5	-1.20	4.20	165.01	0.00	0.00
Fishapp_brain_chd8.2.3	-1.18	-0.79	10.53	0.00	0.03
Fishapp_brain_LOC105015657	-1.18	3.67	31.03	0.00	0.00
Fishapp_brain_prkd1.2.7	-1.16	0.68	29.10	0.00	0.00
Fishapp_brain_LOC105028964.4.4	-1.16	0.19	21.94	0.00	0.00
Fishapp_brain_s22a6	-1.16	3.16	21.18	0.00	0.00
Fishapp_brain_LOC105015451.2.2	-1.15	5.74	378.41	0.00	0.00
Fishapp_brain_T265_09854.2.2	-1.14	1.72	41.10	0.00	0.00
Fishapp_brain_LOC105007920.1.3	-1.13	0.08	17.00	0.00	0.01
Fishapp_brain_contig_30115	-1.13	-0.33	10.71	0.00	0.03
Fishapp_brain_mybl1	-1.13	3.84	70.28	0.00	0.00
Fishapp_brain_LOC105025099.2.3	-1.13	0.68	17.04	0.00	0.01
Fishapp_brain_FEN1.1.2	-1.13	3.42	87.70	0.00	0.00

	logFC	logCPM	F	PValue	FDR
Fishapp_brain_env.2.8	-1.13	4.25	33.19	0.00	0.00
Fishapp_brain_p4r2a.2.3	-1.12	3.26	164.64	0.00	0.00
Fishapp_brain_tysy	-1.11	2.63	33.88	0.00	0.00
Fishapp_brain_LOC105006814.1.2	-1.09	3.60	73.21	0.00	0.00
Fishapp_brain_wfdc1	-1.08	1.16	16.65	0.00	0.01
Fishapp_brain_ccna2	-1.08	2.97	79.99	0.00	0.00
Fishapp_brain_ncoa4.4.4	-1.07	1.06	13.62	0.00	0.02
Fishapp_brain_shank3.2.2	-1.06	0.08	16.67	0.00	0.01
Fishapp_brain_LOC104934062	-1.06	5.28	206.48	0.00	0.00
Fishapp_brain_LOC104531997	-1.06	1.16	23.64	0.00	0.00
Fishapp_brain_lss	-1.05	4.14	76.77	0.00	0.00
Fishapp_brain_tieg3.2.2	-1.04	4.88	47.60	0.00	0.00

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