Journal of Comparative Physiology A
https://doi.org/10.1007/s00359-024-01716-3

RESEARCH

Tanycytes from a bird's eye view: gene expression profiling of the tanycytic region under different seasonal states in the Svalbard ptarmigan

Daniel Appenroth[1] · Alexander C. West[1] · Shona H. Wood[1] · David G. Hazlerigg[1]

Received: 27 June 2024 / Revised: 3 September 2024 / Accepted: 6 September 2024
© The Author(s) 2024

Abstract

In mammals and birds, tanycytes are known to regulate thyroid hormone conversion, and this process is central to the control of seasonal reproduction. In mammals, this cell type is also implicated in retinoic acid signalling, neurogenesis, and nutritional gatekeeping, all of which have been linked to hypothalamic regulation of energy metabolism. Less is known about these potential wider roles of tanycytes in birds. To address this gap, we combined LASER capture microdissection and transcriptomics to profile the tanycytic region in male Svalbard ptarmigan, a High Arctic species with photoperiod-dependent seasonal rhythms in reproductive activation and body mass. Short photoperiod (SP) adapted birds were transferred to constant light (LL) to trigger breeding and body mass loss. After five months under LL, the development of photorefractoriness led to spontaneous re-emergence of the winter phenotype, marked by the termination of breeding and gain in body mass. The transfer from SP to LL initiated gene expression changes in both thyroid hormone and retinoic acid pathways, as described in seasonal mammals. Furthermore, transcriptomic signatures of cell differentiation and migration were observed. Comparison to data from Siberian hamsters demonstrated that a photoperiod-dependent re-organisation of the hypothalamic tanycytic region is likely a conserved feature. Conversely, the spontaneous development of photorefractoriness showed a surprisingly small number of genes that reverted in expression level, despite reversal of the reproductive and metabolic phenotype. Our data suggest general conservation of tanycyte biology between photoperiodic birds and mammals and raise questions about the mechanistic origins of the photorefractory state.

Keywords Tanycytes · Seasonal · Photoperiod · Metabolism · Birds

Introduction

Temperate and polar latitudes are characterised by yearly cycles in daylength, temperature and nutrient availability, known as the seasons. Animals inhabiting those seasonal habitats synchronise their behaviour, physiology and metabolism to the annual cycle. For example, birds and mammals breed seasonally to ensure that the raising of young coincides with the favourable nutritional conditions of spring and summer, allowing the energy demands of the juvenile period to be met. The change in photoperiod (daily duration of the light phase) is the primary environmental signal by which seasonal animals anticipate upcoming seasons and synchronise their life cycles accordingly. To do so, the environmental photoperiod must be perceived, integrated, and then ultimately trigger transitions between seasonal phenotypes. The photoreceptive pathways involved in photoperiodic synchronisation differ between birds and mammals, with the former employing deep encephalic photoreception (Halford et al. 2009; Nakane et al. 2010; Pérez et al. 2019) while the latter employs a retina-suprachiasmatic nucleus-pineal melatonin relay pathway (Dardente et al. 2010; Masumoto et al. 2010; Wood et al. 2020). For comparative review, see (Hazlerigg and Loudon 2008; West and Wood 2018; Nakane and Yoshimura 2019).

Handling Editor: Charlotte Helfrich-Förster.

✉ Daniel Appenroth
daniel.appenroth@uit.no

[1] Arctic Seasonal Timekeeping Initiative (ASTI), Arctic Chronobiology & Physiology, Arctic & Marine Biology, BFE, UiT - Arctic University of Norway, Tromsø, Norway

Published online: 20 September 2024

Despite this, there is remarkable conservation in the downstream pathways of photoperiodic integration (Yoshimura et al. 2003; Hanon et al. 2008; Hazlerigg and Loudon 2008; Nakane et al. 2013). Transitions to long photoperiod (LP) trigger the expression of thyroid-stimulating hormone subunit beta (*Tshb*) and its release from the *pars tuberalis* (PT) of the anterior pituitary gland (Hanon et al. 2008; Nakao et al. 2008a). PT-derived TSH binds to its receptor expressed in specialised cells lining the third ventricle, known as tanycytes (Horstmann 1954), triggering a cAMP-dependent pathway ultimately leading to increased expression of type II iodothyronine deiodinase (*Dio2*) (Hanon et al. 2008; Nakao et al. 2008a; Ono et al. 2008). DIO2 locally converts thyroxine (T4) into triiodothyronine (T3) by outer ring deiodination, leading to increased hypothalamic concentration of bioactive thyroid hormone under LP. Conversely, short photoperiod (SP) coincides with increased expression of type III iodothyronine deiodinase (*Dio3*) within the tanycytes causing thyroid hormone deactivation (Yasuo et al. 2005; Nakao et al. 2008b; Sáenz de Miera et al. 2013; Milesi et al. 2017).

In birds and long-day breeding mammals, increased T3 levels in the hypothalamus lead to increased release of gonadotropin hormone-releasing hormone (GnRH), ultimately causing increased release of gonadotropins from the anterior pituitary and gonadal activation under LP (Yoshimura et al. 2003; Yamamura et al. 2004, 2006; Henson et al. 2013; Klosen et al. 2013; Dardente et al. 2014; Quignon et al. 2020). Contrarily, in short day-breeding mammals (e.g. sheep) increased T3 availability under long days leads to decreased GnRH release and gonadal quiescence (Hanon et al. 2008; Sáenz de Miera et al. 2013; Hut et al. 2014; Dardente and Simonneaux 2022). Despite this opposing coupling between T3 availability and GnRH release between long-day and short-day breeders, it is clear that artificial manipulation of thyroid hormones in seasonal birds and mammals affects seasonal breeding cycles (Woitkewitsch 1940; Follett and Nicholls 1988; Anderson and Barrell 1998; Billings et al. 2002; Anderson et al. 2003; Yoshimura et al. 2003; Yamamura et al. 2006; Barrett et al. 2007). The mechanism by which thyroid hormones control GnRH release is not completely understood, but effects on hypothalamic neuronal activity, and on the encasement of the axonal terminals of GnRH neurons by tanycytic end-feet has been proposed (Yamamura et al. 2004, 2006; Wood et al. 2015; Dardente and Simonneaux 2022).

Several lines of evidence suggest a central role of tanycytes in seasonal rhythms of energy metabolism in addition to their role in seasonal breeding (Appenroth and Cázarez-Márquez 2024). In the Siberian hamster (*Phodopus sungorus*), a mammalian model organism with seasonal rhythm in energy balance, hypothalamic T3 administration triggers responses in body mass and appetite (Barrett et al. 2007; Murphy et al. 2012). In mice, tanycytes respond to metabolites within the cerebrospinal fluid, such as glucose, suggesting sensitivity to the animal's energetic state (Frayling et al. 2011; Orellana et al. 2012; Benford et al. 2017; Elizondo-Vega et al. 2019). Tanycytes also seem to control access of bloodborne substances to the hypothalamus parenchyma via the cerebrospinal fluid and vessel fenestration (Langlet et al. 2013a, b; Balland et al. 2014; Langlet 2014). Tanycytic processes extend into hypothalamic nuclei related to energy homeostasis, i.e. the arcuate nucleus, ventromedial nucleus and dorsomedial nucleus (Dale 2011; Bolborea and Dale 2013; Pasquettaz et al. 2021; Dali et al. 2023). Tanycytes are also a hypothalamic stem cell niche and at least a subset of tanycytes-derived neurons migrate to populate the aforementioned hypothalamic nuclei and acquire appetite-regulatory neuropeptidergic phenotypes (Lee et al. 2012a; Haan et al. 2013; Yoo et al. 2021). The retinoic acid pathway is thought to regulate cell proliferation and many elements of this pathway are known to be under photoperiodic control within tanycytes in seasonal rodents, e.g. Siberian hamster (Melum et al. 2024) and photoperiodic F344 rats (Ross et al. 2004; Shearer et al. 2012).

Although studies in Japanese quail (*Coturnix japonica*) were the first to describe seasonal hypothalamic thyroid hormone conversion as a key regulatory mechanism of seasonal breeding (Yoshimura et al. 2003; Yasuo et al. 2005; Nakao et al. 2008a), subsequent research on the role of tanycytes in seasonal metabolism, body mass control and appetite, derives exclusively from studies in mammals (Ebling 2014, 2015; Ebling and Lewis 2018; Helfer et al. 2019; Langlet 2019). In this experiment, we aimed to characterise changes in the tanycytic region with photoperiod in a highly seasonal bird species. For this purpose, we used captive Svalbard ptarmigan (*Lagopus muta hyperborea*, Sundevall 1845). The Svalbard ptarmigan (Fig. 1A) is a subspecies of the rock ptarmigan and a permanent resident of the archipelago of Svalbard (78°N) (Fuglei et al. 2017) (Fig. 1A). Svalbard ptarmigan display strong seasonal rhythms in breeding, body mass, appetite and coat colour both in the wild and in captivity (Mortensen et al. 1983; Steen and Unander 1985; Stokkan et al. 1986a, b, 1988, 1995; Lindgård and Stokkan 1989; Lindgård et al. 1995). The winter phenotype is characterised by reproductive quiescence, accumulation of body fat (high body mass) and white plumage. Exposure to LP triggers the summer phenotype characterised by breeding, depletion of fat stores (low body mass) and transition to a brown summer plumage. In common with many seasonally breeding bird species (Dawson et al. 2001; Watanabe et al. 2007), Svalbard ptarmigan subsequently develop photorefractoriness with extended exposure to LP and this is marked by termination of breeding, moulting from brown

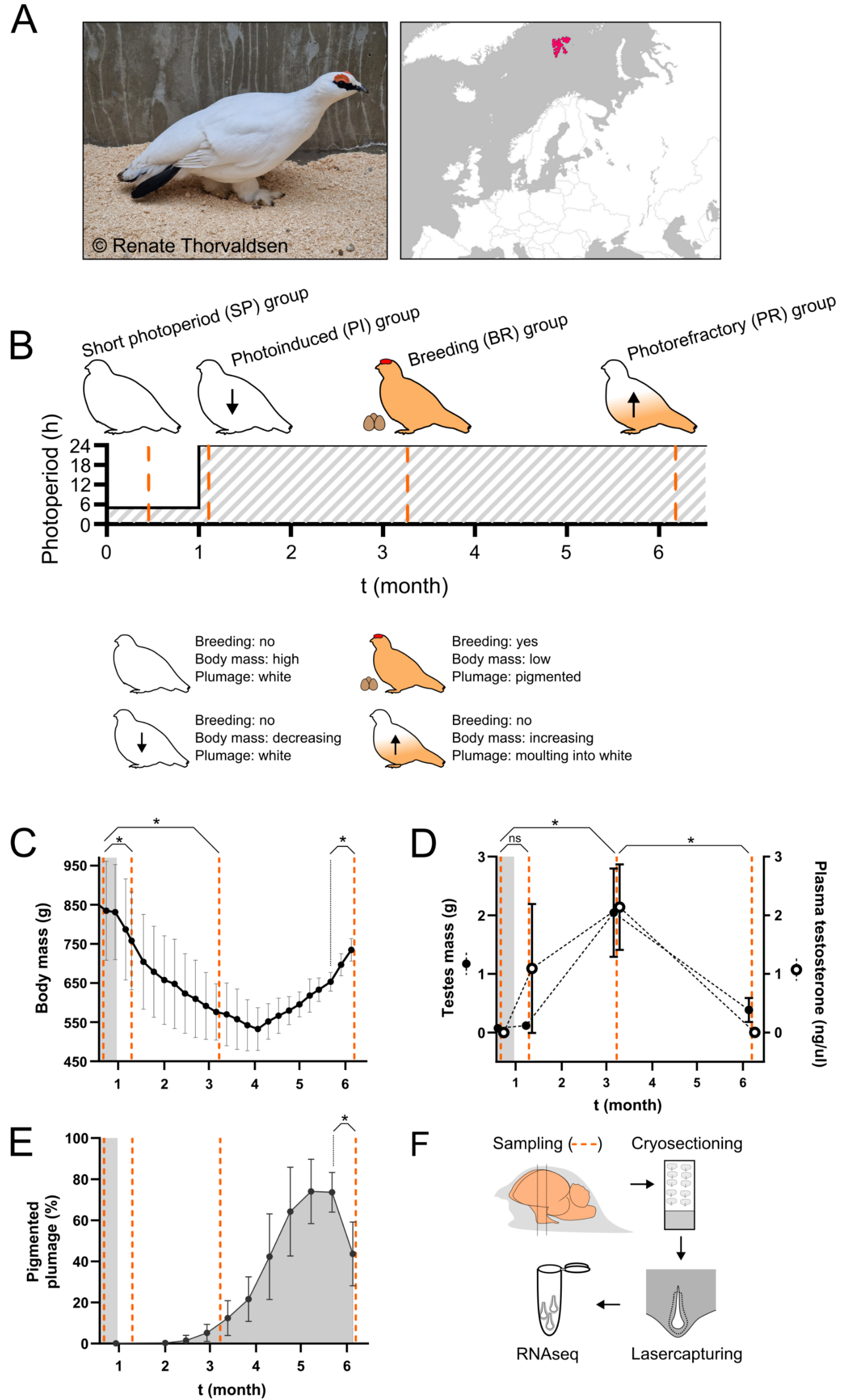

Fig. 1 The Svalbard ptarmigan, experimental design and physiological data. (**A**) The Svalbard ptarmigan (*Lagopus muta hyperborea*) is a High Arctic bird population permanently inhabiting the archipelago of Svalbard. Pictures show a breeding male bird kept at the ptarmigan facility of the University of Tromsø (picture taken by Renate Thorvaldsen) and its natural habitat of Svalbard (indicated in red). (**B**) Experimental male birds were kept under short photoperiod under which they express a winter phenotype. The birds were directly transferred into constant light which triggers physiological and metabolic responses which includes initiation of breeding. After prolonged exposure to constant light, the birds became insensitive to the stimulating photoperiod and reversed back into their winter phenotype. This is an endogenous timing process called photorefractoriness. (**C-E**) Throughout the experiment we collected physiological data including body mass, plumage score, testes mass and testosterone levels. All data is displayed as mean ± SD. Statistical significance (*) indicates p-values < 0.05 of Tukey's tests between the indicated points; ns indicates p-values > 0.05. (**F**) Four male birds were sampled at four occasions as indicated by the orange line in panels B-E. Brains were collected, cryo-sectioned, LASER-dissected for tanycytes and an RNAseq was performed.

summer into white winter plumage and an increase in body mass, effectively reverting to the winter phenotype (Lindgård and Stokkan 1989).

Using laboratory-based photoperiod manipulation and the innate development of photorefractoriness we generated four different seasonal metabolic/reproductive states in male Svalbard ptarmigan. We then sampled the ependymal region of the 3rd ventricle of the hypothalamus, using LASER capture microdissection, to generate tanycyte-enriched samples for RNAseq. We confirmed the seasonal modulation of both thyroid hormone and retinoic acid signalling pathways, as described in seasonal mammals, and demonstrated that remodelling of the hypothalamic tanycytic region is likely a conserved response to changing photoperiod.

Materials and methods

Experimental procedure

Svalbard ptarmigan used in this experiment were bred from wild birds caught on the High Arctic archipelago of Svalbard (78 °N) (Fig. 1A) and reared at the ptarmigan breeding facility at the University of Tromsø (69 °N). All animals were kept in accordance with EU directive 201/63/EU and licenses provided by the Norwegian Food Safety Authority (Mattilsynet, permit ID: 14209).

For this experiment, a total of 20 male birds were used. Birds were housed in individual cages in light and temperature-controlled rooms. Throughout the experiment, the birds had *ad libitum* access to food and water and the ambient temperature was kept constant (8 ± 3 °C) within the animal's thermoneutral zone (Mortensen and Blix 1986). Initially, the birds were kept under SP (LD 5:19) under which they kept a high body mass, white plumage and a non-reproductive state (Stokkan et al. 1995). After 7 weeks under SP, all birds were directly transferred into constant light (LL). Under those conditions, Svalbard ptarmigan lost body mass, prepared for breeding and changed into brown summer plumage (males change colour only after mating) (Stokkan et al. 1988, 1995). Birds were kept under LL until they developed photorefractoriness, which is marked by an increase in body mass, a termination of breeding and a moult into white winter plumage despite the fact that the photoperiod is held constant (Lindgård and Stokkan 1989). The SP to LL treatment was chosen to replicate previous experiments which successfully triggered the desired seasonal phenotypes in Svalbard ptarmigan and were known to be handled well by the experimental birds (Lindgård and Stokkan 1989).

Body mass was measured once a week. The plumage score was assessed every second week by taking a dorsal picture of the birds and calculating pigmentation with the threshold tool in the image analysis software Fiji (Schindelin et al. 2012). Birds were euthanized at four sampling points, determined based on the moult score, body mass and reproductive state of the birds (Fig. 1B). After euthanasia, whole brains were removed and snap-frozen on dry ice before being stored at -80 °C. Testes were removed and weighed. Furthermore, blood was taken, centrifuged and blood plasma was frozen. Testosterone concentrations in plasma samples were measured with a Testosterone ELISA kit (MyBioSource.com, MBS9711529) following the manufacturer's manual. Optical densities were measured with a microplate reader set to 450 nm (GM3500, Promega).

Tukey's multiple comparison test was used to analyse changes in phenotypic parameters. Data was fitted with a one-way ANOVA for testes weight and testosterone levels and with a mixed model for body mass and plumage score. Graphs and statistical tests for phenotypic parameters were produced with GraphPad (version 10).

Transcriptomics – ependymal region of the 3rd ventricle

The whole brains of four males from each group were sectioned with a cryostat (CM3050 S, Leica Biosystems) to a thickness of 20 µm. Hypothalamic sections were collected on membrane slides (415190-9081-000, Carl Zeiss) and all slides were frozen at -80 °C until further analysis.

For RNAseq analysis, membrane slides with tissues were thawed for 30 s and stained in a 0.2% Cresyl violet solution containing ProtectRNA™ RNase inhibitor (R7397, Sigma-Aldrich). After staining, slides were dehydrated by dipping them into increasing ethanol solutions containing RNase inhibitor (75%, 96% and 100% ethanol) and air dried. Stained and dehydrated slides were further processed using a LASER capture microdissection microscope (PALM MicroBeam system, Zeiss) and the PALMRobo software (V4.8, Zeiss). The area around the third ventricle, matching *Dio2* in-situ hybridization references from previous studies (Appenroth et al. 2020, 2021), was LASER-dissected (Fig. S1A) and LASER-catapulted onto the adhesive lids of LCM tubes (415190-9211-000, Carl Zeiss). After all samples were taken, RNA was extracted using the RNeasy Micro kit (74004, Qiagen) following the manufacturer's manual. RNA samples stored on dry ice were sent to BGI Hong Kong for RNA library prep, sequencing (DNBSEQ PE100, BGI), trimming and data filtering.

The data was further processed with the SAGA supercomputer (HPE Apollo 2000/6500 Gen10, Norwegian research infrastructure services). Raw sequences were mapped to the Rock ptarmigan genome (GCA_023344045.1) (Squires et al. 2023) using the alignment tool bowtie2 (Langmead

and Salzberg 2012). Reads for each gene were summarised using the rock ptarmigan annotation provided by NCBI RefSeq in combination with the read summarization tool featureCounts (Liao et al. 2014).

Raw counts were analysed with EdgeR (Chen et al. 2016) using R implemented in Rstudio (R Core Team 2013) following the published manual (Chen et al. 2014). In brief, the data was divided into groups and filtered by setting a threshold of a minimum of 15 counts per million (cpm) in total across all samples and 10 cpm in at least four samples (Chen et al. 2014). Library sizes were normalized using the trimmed mean of M-values (TMM) method and cpm (counts-per-million) values were calculated.

To analyse cell type enrichment in the samples an analysis outlined in Melum et al. (2024) was conducted utilising a hypothalamic single-cell RNAseq dataset (Campbell et al. 2017). Campbell et al. (2017) characterized different cell types in the mural mediobasal hypothalamus by single-cell transcriptomics (Drop-Seq), including tanycytes. Key gene IDs for each specific cluster identified by Campbell et al. (FC $>$ 2) were retrieved and matched to the Svalbard ptarmigan expression data. The cpm data was summed gene-wise across all samples and was plotted as mean $\pm$ SEM across all genes from each cluster. Hence high values within a certain cluster would indicate enrichment of the respective cell type.

TMM-adjusted cpm data was then fitted into a quasi-likelihood negative binomial log-linear model. Next, ANOVA-like testing was conducted by creating a contrast matrix specifying all pairwise contrasts for all 4 groups (6 combinations) and a QL F-test was run to identify differently expressed genes (DEGs). Hence, 6 possible pairwise comparisons were summarized into a single F-statistic. False discovery rate (FDR) values under 0.01 were considered significant. A heatmap for all significant DEGs was constructed with the heatmap.2 R package based on the Z-score of log2 cpm values. Genes and samples were clustered based on Euclidean distance and the heatmap was sub-grouped into 6 separate groups based on gene expression changes (West et al. 2021). We then normalized gene expression for each significant gene between 0 and 1 and plotted the mean $\pm$ SD for each subgroup, hence obtaining the gene expression profile for each subgroup. For each subgroup, we performed a Gene Ontology (GO) enrichment analysis (Wu et al. 2021) and assigned the 10 highest hits (in terms of FDR values) to the subgroups. When appropriate the parent GO term was stated rather than the specific term as many genes showed overlaps in closely related GO terms. For each subgroup, several genes of interest were listed.

Last, differential gene expression between the winter phenotype (SP group) and the summer breeding phenotype (BR group) was tested by conducting a quasi-likelihood F-test. Subsequently, significant genes (FDR $<$ 0.05) were matched to significant genes (FDR $<$ 0.05) of an identical DEG analysis from Melum et al. (2024) between SP and LP-adapted male Siberian hamsters. Common genes, i.e. differently expressed in both species were used in GO enrichment analyses. Detailed results of GO enrichment analyses can be found at: https://doi.org/10.18710/M82D10.

An R-markdown document, including a further description of the EdgeR transcriptomic analysis, can likewise be found at https://doi.org/10.18710/M82D10.

Results

Photoperiod manipulation results in altered seasonal metabolic and reproductive phenotype

We triggered four distinct seasonal phenotypes in male Svalbard ptarmigan (Fig. 1A) by utilising a photoperiodic extension protocol, i.e. transfer from SP to LL (Fig. 1B) and the ptarmigan's innate development of photorefractoriness. Phenotypic measurements included body mass (Fig. 1C), testes mass and testosterone plasma levels (Fig. 1D), and plumage pigmentation (Fig. 1E).

Birds under SP (LD 5:19) displayed a clear winter phenotype: white plumage, high body mass and reproductive inactivity (SP group). 10 days after the transfer from SP to LL birds showed photoinduced responses (PI group), including a drop in body mass ($p < 0.01$ compared to SP birds, Tukey's test) and increasing but non-significantly elevated plasma testosterone levels ($p = 0.12$ compared to SP birds, Tukey's test). After 10 weeks under LL, all experimental birds were reproductive active (BR group). In males, this was characterised by increased testes mass ($p < 0.01$ compared to SP birds, Tukey's test) and increased testosterone plasma concentration ($p < 0.01$ compared to SP birds, Tukey's test). Photorefractoriness developed after 23 weeks in LL (PR group). This was displayed by a return from brown summer plumage into white winter plumage ($p = 0.02$ for comparison between the two last moult scores, Tukey's test), a steady increase in body mass ($p = 0.02$ for comparison between last body mass and body mass 2 weeks before, Tukey's test) and a drop in gonad mass and testosterone back to SP levels ($p < 0.01$ compared to breeding levels, $p > 0.05$ compared to SP bird, Tukey's test).

Changes in developmental processes, thyroid hormone conversion and retinoic acid metabolism define the response to photoperiod in Svalbard ptarmigan tanycytes

Brains from four males from each group were used for RNAseq analysis (Fig. 1F and Fig. S1A). RNA samples from the tanycytic region were sequenced, aligned and reads summarized. A total of 19 879 unique genes were identified, and 14 399 genes were above the filtering threshold. Our in-silico enrichment analysis based on the Campbell et al. (2017) dataset indicates the enrichment of tanycytes in our samples (Fig. S1B). EdgeR analysis using the ANOVA-like QL F-test identified 618 genes as differently expressed genes (DEGs) (FDR < 0.01) across the four sampling points.

We used unsupervised, hierarchical clustering to sort the DEGs into 6 clusters based on their gene expression dynamics over the experiment. This generated four clusters characterised by intermediate to high expression in the SP state and a decline during LL exposure, and two in which the inverse effect was observed. Within the former group, cluster 3 was noteworthy for showing resurgent expression with the onset of photorefractoriness (PR birds), while in the latter group, cluster 6 was noteworthy for showing a spontaneous downturn in expression in PR birds. Jointly these two clusters of 'refractory-linked' genes (20 in total) contributed only 3% of the photoperiod-sensitive transcriptome, making statistically-based GO analysis infeasible, but genes in these clusters have a variety of annotated functions as outlined in Table S1. Here, Sestrin 3 (*Sesn3*) and Pitchfork (*Pifo*) are of note. The former, increased in the SP and PR group, is reported to function in glucose and lipid metabolism (Lee et al. 2012b) and the latter, decreased in SP and PR state, is involved in ciliation and hence potentially cellular sensitivity (Kinzel et al. 2010).

Clusters 1, 2, 4, and 5 showed no transcriptomic reversal in the PR state back to SP levels, hence can be described as 'photoperiod-driven' rather than driven by the endogenous timing mechanism which is presumed to underlie photorefractoriness. We conducted GO enrichment analyses on those 'photoperiod-driven' clusters which are summarised in Fig. 2 and Fig. S2.

Some of the genes expressed in cluster 1 (288 genes) are involved in cell migration, cell differentiation and developmental processes. Notably, this cluster also contains genes involved in thyroid hormone conversion and transport (*Dio1* and *Slc16a2* respectively) and tanycyte-derived neurogenesis (*Fgf10*) (Haan et al. 2013). Cluster 5 (262 genes) also contains a thyroid hormone converting enzyme, *Dio2* and genes involved in the retinoic acid signalling pathway (*Aldh1a1*, *Stra6*, *Rarb*), supporting the enrichment of GO terms relating to involvement in structure development, reproductive processes, regulation of growth and gliogenesis. The remaining clusters (2 & 4) are related to metabolic processes.

In summary, the 'photoperiod-driven' clusters form the majority of the expressional response in Svalbard ptarmigan tanycytes and their surrounding cells. This includes genes involved in thyroid hormone conversion, retinoic acid signalling and various developmental processes, including cell differentiation, cell migration and gliogenesis.

Developmental processes and structural remodelling are conserved features in response to photoperiod

We wished to further explore the conservation of the photoperiodic response in tanycytes between birds and mammals, so we utilised a dataset from the Siberian hamster (*Phodopus sungorus*), which also used the LASER capture technique to enrich for tanycytes from animals kept under SP and LP (equivalent to our SP and BR group, respectively) (Melum et al. 2024). The Siberian hamster also displays strong photoperiodic cycles in breeding, coat colour, body mass regulation and energy metabolism making it a good comparison to the Svalbard ptarmigan.

We identified 1 232 DEGs in Svalbard ptarmigan between the SP and BR group (FDR < 0.05, 646 genes up in SP, 586 genes up in BR) and compared these to the DEGs identified between SP and LP adapted hamsters (FDR < 0.05, 3 078 genes up in SP, 2 950 genes up in LP). Homologues of 42% of ptarmigan DEGs were also DEGs in the hamster in response to photoperiod (Fig. 3A). We divided those common DEGs based on the overlaps under which photoperiod they were upregulated (Fig. 3B). Hence, we had four different categories: DEGs upregulated under SP in both species (144 genes), DEGs upregulated under LP/LL in both species (142 genes), DEGs upregulated in SP hamster and BR ptarmigan (93 genes) and DEGs upregulated in LP hamsters and SP ptarmigan (137 genes). We then ran GO enrichment analyses on the different categories (Fig. 3B and Fig. S3) and found enrichment amongst developmental cell processes, response to growth factors, reproduction, circulatory processes, transport (incl. across the blood-brain barrier), regulation of hormone levels and photoperiodism as well as enrichment in pathways known to be active in tanycytes such as vascular endothelial growth factor (VEGF) signalling and the mitogen-activated protein kinase (MAPK) cascade (Langlet 2019). Detailed results of the GO enrichment analyses can be found under https://doi.org/10.18710/M82D10.

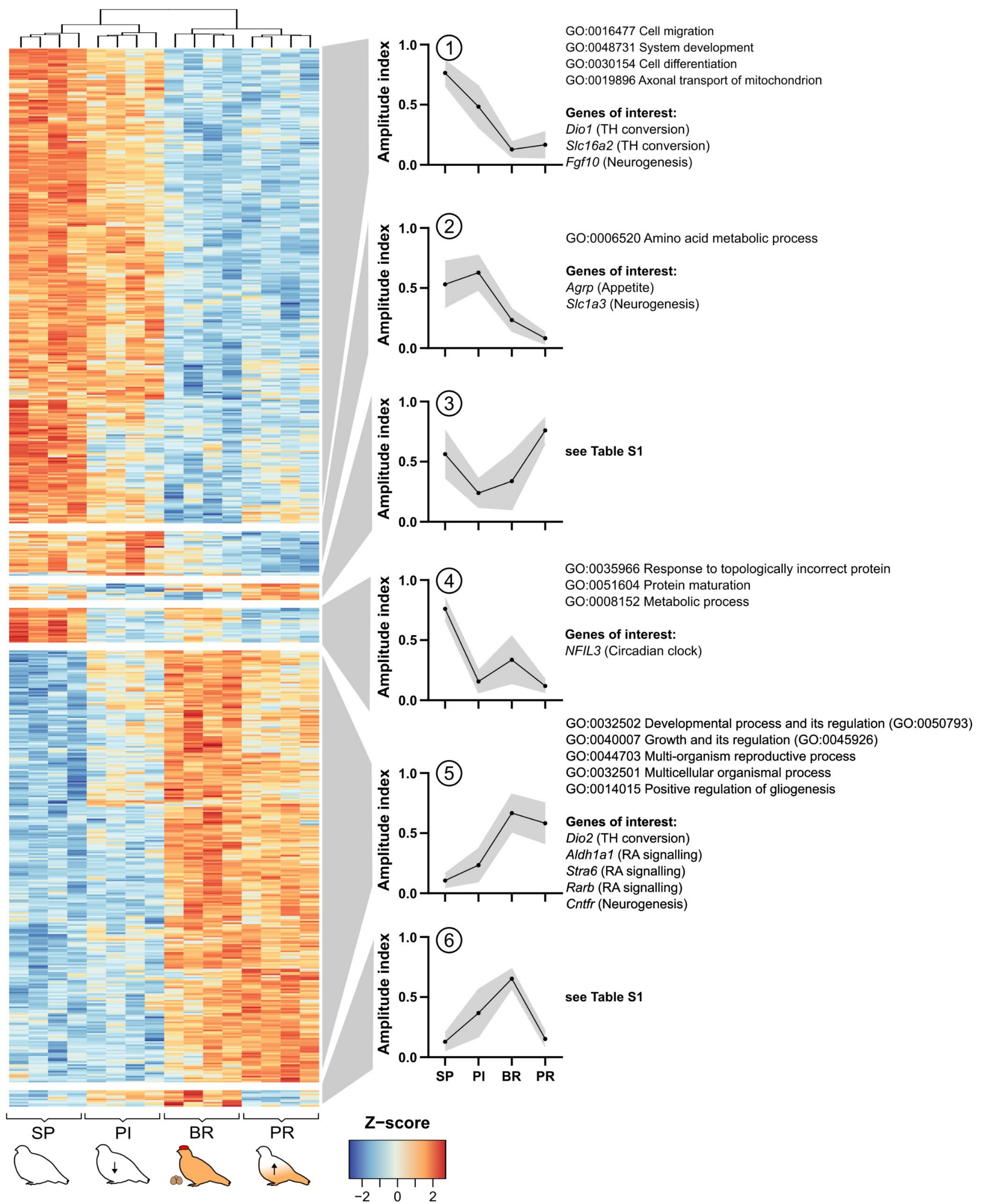

Fig. 2 Gene expression profile across the four sampling groups. The heatmap shows all differently expressed genes according to the ANOVA-like QL F-test (FDR < 0.01, 618 genes). The heatmap was divided into six clusters and the expression profile for each cluster is displayed as mean ± SD for all genes of the cluster. GO analysis terms and genes of interest are stated beside each cluster profile. (SP: short photoperiod, PI: photoinduced, BR: breeding, PR: photorefractory, TH: thyroid hormone, RA: retinoic acid)

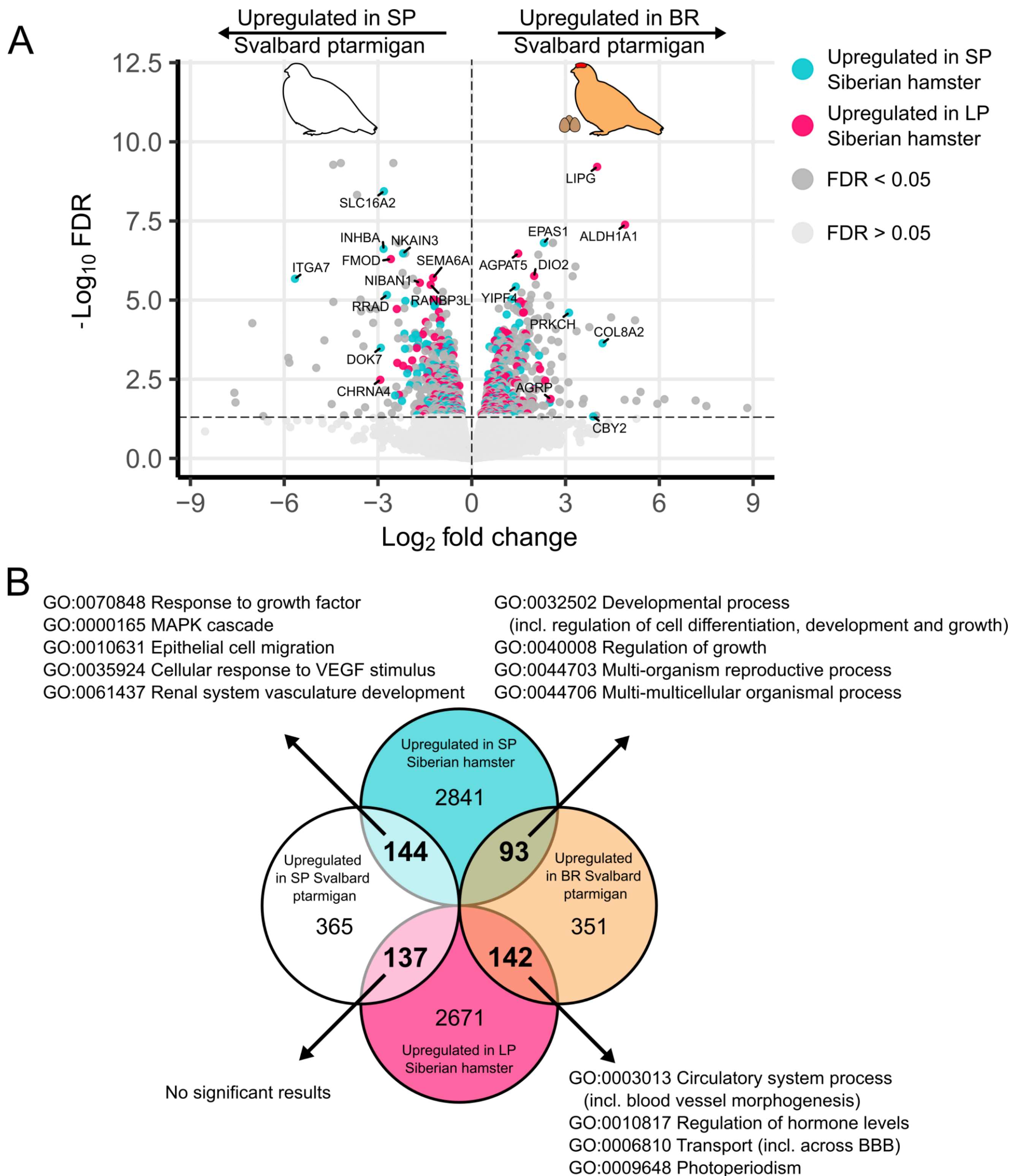

Fig. 3 Comparison of photoperiodically controlled tanycytic genes between Svalbard ptarmigan and Siberian hamster. (**A**) 1 232 genes are differently expressed between the winter (SP group) and summer phenotype (BR group) in Svalbard ptarmigan (FDR < 0.05). Of those photoperiodically controlled genes 516 genes are also differently expressed in the tanycytic area of Siberian Hamster (FDR < 0.05) (Melum et al. 2024). The coloured dots indicate genes which are common in hamster and ptarmigan. Common genes with log2 fold changes higher than 2.5 or -log10 FDR values above 5 are indicated with their respective names. (**B**) GO analyses were performed on all common genes differently expressed in ptarmigan and hamster based on their photoperiodic overlap. The top 10 hits were summarized when appropriate. (SP: short photoperiod, LP: long photoperiod, BR: breeding, FDR: false discovery rate, MAPK: Mitogen-activated protein kinase, VEGF: vascular endothelial growth factor, BBB: blood-brain barrier)

Discussion

In this experiment, we sought to characterize the tanycytes of an avian species undergoing profound changes in seasonal metabolic state. For this purpose, we used a LASER capture microdissection RNAseq approach in the High Arctic Svalbard ptarmigan.

Our analysis confirms our earlier description of the photoperiodic regulation of hypothalamic thyroid hormone signalling in this species (Appenroth et al. 2020, 2021). Yet, besides *Dio2*, the present study also identified type I iodothyronine deiodinase (*Dio1*) as a DEG. This finding is intriguing, since transcriptomic datasets in the hypothalamus of the Japanese quail, another Galliformes bird species, do not identify *Dio1* as seasonal DEG (Morris et al. 2020; Majumdar et al. 2023) and while expression of *Dio1* has been reported in the mediobasal hypothalamus of sheep and goats (Dardente et al. 2022, 2024) we are not aware of any study reporting *Dio1* to be under photoperiodic control in the mediobasal hypothalamus in either birds or mammals. DIO1 can catalyse outer and inner ring deionisation, hence is capable of activating and deactivating thyroid hormone (Bianco et al. 2002; Lechan and Fekete 2005). Whether seasonal variation in the expression of *Dio1* affects the ratio of thyroid hormones in the hypothalamus of Svalbard ptarmigan remains to be determined.

Besides thyroid hormone conversion our study considered wider aspects of tanycyte biology in a photoperiodic bird. Retinoic acid signalling in and around tanycytes is known to be under photoperiodic control in a range of mammals such as Siberian hamsters and photoperiodic F344 rats (Ross et al. 2004; Shearer et al. 2012; Melum et al. 2024), while absent in other species like sheep (Lomet et al. 2018; Helfer et al. 2019). In the present study, several elements of the retinoic acid pathway were upregulated by the exposure to LL, i.e. *Stra6*, *Aldh1a1* and *Rarb*, which are involved in retinol uptake, conversion of retinol to retinoic acid, and the nuclear receptor-mediated response to retinoic acid, respectively. In the F344 rat, high expression levels of these genes correlated with retinoic acid levels in the hypothalamus (Helfer et al. 2012), and it therefore is likely that a corresponding increase in retinoic acid activity also occurs in the Svalbard ptarmigan under LL.

Retinoic acid signalling is thought to be involved in stem cell regulation and tanycytes are a known stem cell niche in the mammalian brain (Lee et al. 2012a; Haan et al. 2013; Helfer et al. 2019; Yoo et al. 2021). Accordingly, GO enrichment analyses of significant DEGs across the four experimental groups indicated roles in cell migration and cell differentiation. We also identified that *Fgf10* was regulated by photoperiod, in tanycytes *Fgf10* has been reported as a marker of tanycytes showing neural stem/progenitor markers in adulthood which then populate the arcuate nucleus (Haan et al. 2013), therefore having potential roles in appetite and seasonal energy balance (Helfer et al. 2019).

In order to further investigate conserved features of tanycyte biology which are relevant for seasonal energy balance we compared our present dataset with data from published work on the Siberian Hamster (Melum et al. 2024), a species with a strong body mass cycle. This comparison reveals a range of common photoperiod-responsive genes and GO enrichment analysis reveals those common genes to be involved in developmental processes including response to growth factors, the regulation of cell differentiation, cell development and cell growth and notably in epithelial cell migration, suggesting a conservation of remodelling in response to photoperiod of the tanycytic region.

Common genes were furthermore involved in responses to vascular endothelial growth factor (VEGF) stimuli and circulatory processes, including blood vessel morphogenesis and transport across the blood-brain barrier. In mammals, modulation of blood vessel fenestration via the tanycytes is hypothesized to act as a gateway of peripheral signals into the hypothalamic parenchyma and is possibly mediated via VEGF signalling (Langlet et al. 2013a; Langlet 2014). These changes can alter the metabolic feedback to the hypothalamus, presumably an important factor in seasonal changes in body mass and voluntary food intake (Appenroth and Cázarez-Márquez 2024). Hence, our data may be reflective of similar dynamic changes in hypothalamic blood vessels associated with tanycytes as a seasonal response in a photoperiodic bird species.

Despite inferred commonalities, the photoperiodic direction of change seems less conserved between Svalbard ptarmigan and Siberian hamster. For example, while *Dio2* and *Aldh1a1* are upregulated under LP/ LL in both species, *Epas1* (aka *Hif1*) is upregulated in Svalbard ptarmigan under LL but in Siberian hamster under SP. Notably, *Epas1* is hypothesized to be involved in hypothalamic glucose sensing, regulation of VEGF expression and vascular modulation making it an interesting factor for the above-outlined tanycyte functions (Carmeliet et al. 1998; Zhang et al. 2011; Langlet 2019). The opposite direction pattern can be observed for 45% of all commonly expressed genes and it is tempting to associate this with the opposed fattening cycles of the two species, i.e. Svalbard ptarmigan fatten under SP while Siberian hamsters fatten under LP (Masuda and Oishi 1988; Stokkan et al. 1995; Ebling et al. 1998; Knopper and Boily 2000; Warner et al. 2010; Melum et al. 2024). Hence, further comparative work is an opportunity to identify tanycytic genes crucial for seasonal energy metabolism.

Besides investigating biological functions of tanycytes our data set also offers a discussion on the temporal dynamics of tanycytes including their role in endogenous timing

mechanisms. Photorefractoriness describes the reversal of a seasonal phenotype by becoming unresponsive to the prevailing photoperiod. In our experiment a surprisingly small number of genes showed expression changes during the transition from the breeding (BR) to photorefractory (PR) state, despite there being a very pronounced shift in the seasonal phenotype (increasing body mass and moulting to white). Interestingly, Lomet et al. (2018) also reported minor transcriptional changes in the mediobasal hypothalamus of ewes during the transition to the LP refractory state.

This paradox might be explained in two ways. One, photorefractoriness is mediated outside or downstream of the tanycytes (García-Fernández et al. 2015) or two, only a small subset of genes (clusters 3 and 6 of Fig. 2) are responsible for driving the reproductive and metabolic changes. In the present study, we identified two photorefractory genes of note, Sestrin 3 (*Sesn3*) and Pitchfork (*Pifo*) (Kinzel et al. 2010).

Both genes are involved in glucose metabolism and altered cellular sensitivity to signals. In particular, SESN3 has a role in the sensitization to insulin (Tao et al. 2015) and changes in the number of primary cilia, potentially mediated by PIFO, can alter signalling through a reduction in G-protein coupled receptor signalling (Schou et al. 2015). Recently, changes in the number of cilia in response to gestational and post-weaning photoperiod were reported in Siberian hamsters of different seasonal metabolic states (Melum et al. 2024). This is of particular interest since ciliopathologies can cause severe metabolic phenotypes (Loktev and Jackson 2013; Volta and Gerdes 2017). While more research is required, it is attractive to speculate that changes in sensitivity to downstream hypothalamic circuits through altered cilia-based cellular signalling in tanycytes could drive photorefractoriness.

In summary, our data strongly suggest a conserved seasonal role of tanycytes between birds and mammals, besides hypothalamic thyroid hormone conversion we found markers of retinoic acid signalling and cell developmental processes. Comparison between Svalbard ptarmigan and Siberian hamster further suggests evolutionary conservation of photoperiodic genes regulating cellular differentiation and dynamic changes in vascularization. However, the modest transcriptome changes seen during the development of photorefractoriness suggest that rather subtle changes in tanycytes occur during the reversal of the summer seasonal state.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00359-024-01716-3.

Acknowledgements We thank our animal technicians; without their dedication and efforts this research would not be possible. Special thanks also to Vebjørn Melum for his help with the RNAseq analysis and to Fredrik Markussen for his help with the sampling. The authors also thank Linn Aspelund for her help with sampling and ptarmigan upkeep.

Author contributions All authors designed the experiment D.A. and A.C.W. acquired samples D.A. and S.H.W. did transcriptomic analyses D.A., A.C.W. and S.H.W. prepared figures D.A., S.H.W. and D.G.H wrote the main manuscript. All authors reviewed the manuscript.

Funding S.H.W. is supported by the Tromsø Forskningsstiftelse (TFS) starter grant TFS2016SW and the TFS infrastructure grant (IS3_17_SW). D.A., A.C.W., S.H.W. and D.G.H. are supported by the Arctic Seasonal Timekeeping Initiative (ASTI), awarded by UiT: The Arctic University of Norway. The authors would like to acknowledge the National Infrastructure for Research Data (NIRD) project number nn8093k and nn9893k for use of the computing cluster SAGA to complete the bioinformatic analysis.
Open access funding provided by UiT The Arctic University of Norway (incl University Hospital of North Norway)

Data availability R-script, physiological measurements and gene expression data have been deposited in DataverseNO: https://doi.org/10.18710/M82D10.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Anderson GM, Barrell GK (1998) Effects of thyroidectomy and thyroxine replacement on seasonal reproduction in the red deer Hind. Reproduction 113:239–250

Anderson GM, Hardy SL, Valent M, Billings HJ, Connors JM, Goodman RL (2003) Evidence that thyroid hormones act in the ventromedial preoptic area and the premammillary region of the brain to allow the termination of the breeding season in the ewe. Endocrinology 144:2892–2901

Appenroth D, Cázarez-Márquez F (2024) Seasonal food intake and energy balance: neuronal and non-neuronal control mechanisms. Neuropharmacology 110050

Appenroth D, Melum VJ, West AC, Dardente H, Hazlerigg DG, Wagner GC (2020) Photoperiodic induction without light-mediated circadian entrainment in a high arctic resident bird. J Exp Biol 223(16):jeb220699

Appenroth D, Wagner GC, Hazlerigg DG, West AC (2021) Evidence for circadian-based photoperiodic timekeeping in Svalbard Ptarmigan, the northernmost resident bird. Curr Biol 31(12):2720–2727

Balland E, Dam J, Langlet F, Caron E, Steculorum S, Messina A, Rasika S, Falluel-Morel A, Anouar Y, Dehouck B (2014) Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. Cell Metab 19:293–301

Barrett P, Ebling FJP, Schuhler S, Wilson D, Ross AW, Warner A, Jethwa P, Boelen A, Visser TJ, Ozanne DM (2007) Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. Endocrinology 148:3608–3617

Benford H, Bolborea M, Pollatzek E, Lossow K, Hermans-Borgmeyer I, Liu B, Meyerhof W, Kasparov S, Dale N (2017) A sweet taste receptor-dependent mechanism of glucosensing in hypothalamic tanycytes. Glia 65:773–789

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38–89

Billings HJ, Viguié C, Karsch FJ, Goodman RL, Connors JM, Anderson GM (2002) Temporal requirements of thyroid hormones for seasonal changes in LH secretion. Endocrinology 143:2618–2625

Bolborea M, Dale N (2013) Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. Trends Neurosci 36:91–100

Campbell JN, Macosko EZ, Fenselau H, Pers TH, Lyubetskaya A, Tenen D, Goldman M, Verstegen AMJ, Resch JM, McCarroll SA (2017) A molecular census of arcuate hypothalamus and median eminence cell types. Nat Neurosci 20:484–496

Carmeliet P, Dor Y, Herbert J-M, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P (1998) Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394:485–490

Chen Y, McCarthy D, Robinson M, Smyth GK (2014) edgeR: differential expression analysis of digital gene expression data user's guide. Bioconductor User's Guide

Chen Y, Lun ATL, Smyth GK (2016) From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using rsubread and the edgeR quasi-likelihood pipeline. F1000Research 5

R Core Team R (2013) R: A language and environment for statistical computing

Dale N (2011) Purinergic signaling in hypothalamic tanycytes: potential roles in chemosensing. Seminars in Cell & Developmental Biology. Elsevier, pp 237–244

Dali R, Estrada-Meza J, Langlet F (2023) Tanycyte, the neuron whisperer. Physiol Behav 263:114108

Dardente H, Simonneaux V (2022) GnRH and the photoperiodic control of seasonal reproduction: delegating the task to kisspeptin and RFRP-3. J Neuroendocrinol 34:e13124

Dardente H, Wyse CA, Birnie MJ, Dupré SM, Loudon ASI, Lincoln GA, Hazlerigg DG (2010) A molecular switch for photoperiod responsiveness in mammals. Curr Biol 20:2193–2198

Dardente H, Hazlerigg DG, Ebling FJP (2014) Thyroid hormone and seasonal rhythmicity. Front Endocrinol (Lausanne) 5:19

Dardente H, Lomet D, Desmarchais A, Téteau O, Lasserre O, Gonzalez A, Dubois E, Beltramo M, Elis S (2022) Impact of food restriction on the medio-basal hypothalamus of intact ewes as revealed by a large-scale transcriptomics study. J Neuroendocrinol 34:e13198

Dardente H, Lomet D, Robert V, Lasserre O, Gonzalez A-A, Mialhe X, Beltramo M (2024) Photoperiod, but not progesterone, has a strong impact upon the transcriptome of the medio-basal hypothalamus in female goats and ewes. Mol Cell Endocrinol 588:112216

Dawson A, King VM, Bentley GE, Ball GF (2001) Photoperiodic control of seasonality in birds. J Biol Rhythms 16:365–380

de Sáenz C, Hanon EA, Dardente H, Birnie M, Simonneaux V, Lincoln GA, Hazlerigg DG (2013) Circannual variation in thyroid hormone deiodinases in a short-day breeder. J Neuroendocrinol 25:412–421

Ebling FJP (2014) On the value of seasonal mammals for identifying mechanisms underlying the control of food intake and body weight. Horm Behav 66:56–65

Ebling FJP (2015) Hypothalamic control of seasonal changes in food intake and body weight. Front Neuroendocrinol 37:97–107

Ebling FJP, Lewis JE (2018) Tanycytes and hypothalamic control of energy metabolism. Glia 66:1176–1184

Ebling FP, Arthurs OJ, Turney BW, Cronin AS (1998) Seasonal neuroendocrine rhythms in the male siberian hamster persist after monosodium glutamate-induced lesions of the arcuate nucleus in the neonatal period. J Neuroendocrinol 10:701–712

Elizondo-Vega RJ, Recabal A, Oyarce K (2019) Nutrient sensing by hypothalamic tanycytes. Front Endocrinol (Lausanne) 10:244

Follett BK, Nicholls TJ (1988) Acute effect of thyroid hormones in mimicking photoperiodically induced release of gonadotropins in Japanese quail. J Comp Physiol B 157:837–843

Frayling C, Britton R, Dale N (2011) ATP-mediated glucosensing by hypothalamic tanycytes. J Physiol 589:2275–2286

Fuglei E, Blanchet M-A, Unander S, Ims RA, Pedersen ÅØ (2017) Hidden in the darkness of the Polar night: a first glimpse into winter migration of the Svalbard rock ptarmigan. Wildlife Biol 2017

García-Fernández JM, Cernuda-Cernuda R, Davies WIL, Rodgers J, Turton M, Peirson SN, Follett BK, Halford S, Hughes S, Hankins MW, Foster RG (2015) The hypothalamic photoreceptors regulating seasonal reproduction in birds: a prime role for VA opsin. Front Neuroendocrinol 37:13–28

Haan N, Goodman T, Najdi-Samiei A, Stratford CM, Rice R, El Agha E, Bellusci S, Hajihosseini MK (2013) Fgf10-expressing tanycytes add new neurons to the appetite/energy-balance regulating centers of the postnatal and adult hypothalamus. J Neurosci 33:6170–6180

Halford S, Pires SS, Turton M, Zheng L, González-Menéndez I, Davies WL, Peirson SN, García-Fernández JM, Hankins MW, Foster RG (2009) VA opsin-based photoreceptors in the hypothalamus of birds. Curr Biol 19:1396–1402

Hanon EA, Lincoln GA, Fustin J-M, Dardente H, Masson-Pévet M, Morgan PJ, Hazlerigg DG (2008) Ancestral TSH mechanism signals summer in a photoperiodic mammal. Curr Biol 18:1147–1152

Hazlerigg D, Loudon A (2008) New insights into ancient seasonal life timers. Curr Biol 18:R795–R804

Helfer G, Ross AW, Russell L, Thomson LM, Shearer KD, Goodman TH, McCaffery PJ, Morgan PJ (2012) Photoperiod regulates vitamin A and Wnt/β-catenin signaling in F344 rats. Endocrinology 153:815–824

Helfer G, Barrett P, Morgan PJ (2019) A unifying hypothesis for control of body weight and reproduction in seasonally breeding mammals. J Neuroendocrinol 31:e12680

Henson JR, Carter SN, Freeman DA (2013) Exogenous T3 elicits long day–like alterations in Testis size and the RFamides Kisspeptin and Gonadotropin-Inhibitory hormone in short-day siberian hamsters. J Biol Rhythms 28:193–200

Horstmann E (1954) Die faserglia des selachiergehirns. Z für Zellforsch und Mikroskopische Anat 39:588–617

Hut RA, Dardente H, Riede SJ (2014) Seasonal timing: how does a hibernator know when to stop hibernating? Curr Biol 24:R602–R605

Kinzel D, Boldt K, Davis EE, Burtscher I, Trümbach D, Diplas B, Attié-Bitach T, Wurst W, Katsanis N, Ueffing M (2010) Pitchfork regulates primary cilia disassembly and left-right asymmetry. Dev Cell 19:66–77

Klosen P, Sébert M, Rasri K, Laran-Chich M, Simonneaux V (2013) TSH restores a summer phenotype in photoinhibited mammals via the RF-amides RFRP3 and kisspeptin. FASEB J 27:2677–2686

Knopper LD, Boily P (2000) The energy budget of captive siberian hamsters, Phodopus sungorus, exposed to photoperiod changes: mass loss is caused by a voluntary decrease in food intake. Physiol Biochem Zool 73:517–522

Langlet F (2014) Tanycytes: a gateway to the metabolic hypothalamus. J Neuroendocrinol 26:753–760

Langlet F (2019) Tanycyte gene expression dynamics in the regulation of energy homeostasis. Front Endocrinol (Lausanne) 10:286

Langlet F, Levin BE, Luquet S, Mazzone M, Messina A, Dunn-Meynell AA, Balland E, Lacombe A, Mazur D, Carmeliet P (2013a) Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting. Cell Metab 17:607–617

Langlet F, Mullier A, Bouret SG, Prevot V, Dehouck B (2013b) Tanycyte-like cells form a blood–cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. J Comp Neurol 521:3389–3405

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359

Lechan RM, Fekete C (2005) Role of thyroid hormone deiodination in the hypothalamus. Thyroid 15:883–897

Lee DA, Bedont JL, Pak T, Wang H, Song J, Miranda-Angulo A, Takiar V, Charubhumi V, Balordi F, Takebayashi H (2012a) Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche. Nat Neurosci 15:700–702

Lee JH, Budanov AV, Talukdar S, Park EJ, Park HL, Park H-W, Bandyopadhyay G, Li N, Aghajan M, Jang I (2012b) Maintenance of metabolic homeostasis by Sestrin2 and Sestrin3. Cell Metab 16:311–321

Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930

Lindgård K, Stokkan K-A (1989) Daylength control of food intake and body weight in Svalbard Ptarmigan Lagopus mutus hyperboreus. Ornis Scand 176–180

Lindgård K, Stokkan KA, Näslund S (1995) Annual changes in body mass in captive Svalbard ptarmigan: role of changes in locomotor activity and food intake. J Comp Physiol B 165:445–449

Loktev AV, Jackson PK (2013) Neuropeptide Y family receptors traffic via the Bardet-Biedl syndrome pathway to signal in neuronal primary cilia. Cell Rep 5:1316–1329

Lomet D, Cognié J, Chesneau D, Dubois E, Hazlerigg D, Dardente H (2018) The impact of thyroid hormone in seasonal breeding has a restricted transcriptional signature. Cell Mol life Sci 75:905–919

Majumdar G, Liddle TA, Stewart C, Marshall CJ, Bain M, Stevenson T (2023) FSHβ links photoperiodic signaling to seasonal reproduction in Japanese quail. Elife 12:RP87751

Masuda A, Oishi T (1988) Effects of photoperiod and temperature on body weight, food intake, food storage, and pelage color in the djungarian hamster, Phodopus sungorus. J Exp Zool 248:133–139

Masumoto K, Ukai-Tadenuma M, Kasukawa T, Nagano M, Uno KD, Tsujino K, Horikawa K, Shigeyoshi Y, Ueda HR (2010) Acute induction of Eya3 by late-night light stimulation triggers TSHβ expression in photoperiodism. Curr Biol 20:2199–2206

Melum VJ, de Miera CS, Markussen FAF, Cázarez-Márquez F, Jaeger C, Sandve SR, Simonneaux V, Hazlerigg DG, Wood SH (2024) Hypothalamic tanycytes as mediators of maternally programmed seasonal plasticity. Curr Biol 34(3):632–640

Milesi S, Simonneaux V, Klosen P (2017) Downregulation of deiodinase 3 is the earliest event in photoperiodic and photorefractory activation of the gonadotropic axis in seasonal hamsters. Sci Rep 7:17739

Morris KM, Hindle MM, Boitard S, Burt DW, Danner AF, Eory L, Forrest HL, Gourichon D, Gros J, Hillier LW (2020) The quail genome: insights into social behaviour, seasonal biology and infectious disease response. BMC Biol 18:1–18

Mortensen A, Blix AS (1986) Seasonal changes in resting metabolic rate and mass-specific conductance in Svalbard Ptarmigan, Norwegian rock ptarmigan and Norwegian willow ptarmigan. Ornis Scand 8–13

Mortensen A, Unander S, Kolstad M, Blix AS (1983) Seasonal changes in body composition and crop content of Spitzbergen Ptarmigan Lagopus mutus hyperboreus. Ornis Scand 144–148

Murphy M, Jethwa PH, Warner A, Barrett P, Nilaweera KN, Brameld JM, Ebling FJP (2012) Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in siberian hamsters. Endocrinology 153:101–112

Nakane Y, Yoshimura T (2019) Photoperiodic regulation of reproduction in vertebrates. Annu Rev Anim Biosci 7:173–194

Nakane Y, Ikegami K, Ono H, Yamamoto N, Yoshida S, Hirunagi K, Ebihara S, Kubo Y, Yoshimura T (2010) A mammalian neural tissue opsin (opsin 5) is a deep brain photoreceptor in birds. Proc Natl Acad Sci 107:15264–15268

Nakane Y, Ikegami K, Iigo M, Ono H, Takeda K, Takahashi D, Uesaka M, Kimijima M, Hashimoto R, Arai N (2013) The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nat Commun 4:2108

Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, Yasuo S, Katou Y, Kageyama S, Uno Y (2008a) Thyrotrophin in the pars tuberalis triggers photoperiodic response. Nature 452:317–322

Nakao N, Ono H, Yoshimura T (2008b) Thyroid hormones and seasonal reproductive neuroendocrine interactions. Reproduction 136:1

Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, Ebihara S, Korf H-W, Yoshimura T (2008) Involvement of thyrotropin in photoperiodic signal transduction in mice. Proc Natl Acad Sci 105:18238–18242

Orellana JA, Sáez PJ, Cortés-campos C, Elizondo RJ, Shoji KF, Contreras-Duarte S, Figueroa V, Velarde V, Jiang JX, Nualart F (2012) Glucose increases intracellular free Ca2 + in tanycytes via ATP released through connexin 43 hemichannels. Glia 60:53–68

Pasquettaz R, Kolotuev I, Rohrbach A, Gouelle C, Pellerin L, Langlet F (2021) Peculiar protrusions along tanycyte processes face diverse neural and nonneural cell types in the hypothalamic parenchyma. J Comp Neurol 529:553–575

Pérez JH, Tolla E, Dunn IC, Meddle SL, Stevenson TJ (2019) A comparative perspective on extra-retinal photoreception. Trends Endocrinol Metab 30:39–53

Quignon C, Beymer M, Gauthier K, Gauer F, Simonneaux V (2020) Thyroid hormone receptors are required for the melatonin-dependent control of rfrp gene expression in mice. FASEB J 34:12072–12082

Ross AW, Webster CA, Mercer JG, Moar KM, Ebling FJ, Schuhler S, Barrett P, Morgan PJ (2004) Photoperiodic regulation of hypothalamic retinoid signaling: association of retinoid X receptor γ with body weight. Endocrinology 145:13–20

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682

Schou KB, Pedersen LB, Christensen ST (2015) Ins and outs of GPCR signaling in primary cilia. EMBO Rep 16:1099–1113

Shearer KD, Stoney PN, Nanescu SE, Helfer G, Barrett P, Ross AW, Morgan PJ, McCaffery P (2012) Photoperiodic expression of two RALDH enzymes and the regulation of cell proliferation by retinoic acid in the rat hypothalamus. J Neurochem 122:789–799

Squires TE, Rödin-Mörch P, Formenti G, Tracey A, Abueg L, Brajuka N, Jarvis E, Halapi EC, Melsted P, Höglund J (2023) A chromosome-level genome assembly for the Rock Ptarmigan (Lagopus muta). G3 Genes, Genomes, Genet 13:jkad099

Steen JB, Unander S (1985) Breeding biology of the Svalbard Rock Ptarmigan Lagopus mutus hyperboreus. Ornis Scand 191–197

Stokkan K-A, Sharp PJ, Unander S (1986a) The annual breeding cycle of the high-arctic Svalbard ptarmigan (Lagopus mutus hyperboreus). Gen Comp Endocrinol 61:446–451

Stokkan KA, Mortensen A, Blix AS (1986b) Food intake, feeding rhythm, and body mass regulation in Svalbard rock ptarmigan. Am J Physiol 251:R264–R267

Stokkan K-A, Sharp PJ, Dunn IC, Lea RW (1988) Endocrine changes in photostimulated willow ptarmigan (Lagopus lagopus lagopus) and Svalbard ptarmigan (Lagopus mutus hyperboreus). Gen Comp Endocrinol 70:169–177

Stokkan K-A, Lindgård K, Reierth E (1995) Photoperiodic and ambient temperature control of the annual body mass cycle in Svalbard Ptarmigan. J Comp Physiol B 165:359–365

Tao R, Xiong X, Liangpunsakul S, Dong XC (2015) Sestrin 3 protein enhances hepatic insulin sensitivity by direct activation of the mTORC2-Akt signaling. Diabetes 64:1211–1223

Volta F, Gerdes JM (2017) The role of primary cilia in obesity and diabetes. Ann N Y Acad Sci 1391:71–84

Warner A, Jethwa PH, Wyse CA, I'anson H, Brameld JM, Ebling FJP (2010) Effects of photoperiod on daily locomotor activity, energy expenditure, and feeding behavior in a seasonal mammal. Am J Physiol Integr Comp Physiol 298:R1409–R1416

Watanabe T, Yamamura T, Watanabe M, Yasuo S, Nakao N, Dawson A, Ebihara S, Yoshimura T (2007) Hypothalamic expression of thyroid hormone-activating and-inactivating enzyme genes in relation to photorefractoriness in birds and mammals. Am J Physiol Integr Comp Physiol 292:R568–R572

West AC, Wood SH (2018) Seasonal physiology: making the future a thing of the past. Curr Opin Physiol 5:1–8

West AC, Mizoro Y, Wood SH, Ince LM, Iversen M, Jørgensen EH, Nome T, Sandve SR, Martin SAM, Loudon ASI (2021) Immunologic profiling of the Atlantic salmon gill by single nuclei transcriptomics. Front Immunol 12:669889

Woitkewitsch AA (1940) Dependence of seasonal periodicity in gonadal changes on the thyroid gland in Sturnus vulgaris L. In: CR (Doklady) Acad Sci URSS. pp 741–745

Wood SH, Christian HC, Miedzinska K, Saer BRC, Johnson M, Paton B, Yu L, McNeilly J, Davis JRE, McNeilly AS (2015) Binary switching of calendar cells in the pituitary defines the phase of the circannual cycle in mammals. Curr Biol 25:2651–2662

Wood SH, Hindle MM, Mizoro Y, Cheng Y, Saer BRC, Miedzinska K, Christian HC, Begley N, McNeilly J, McNeilly AS, Meddle SL, Burt DW, Loudon ASI (2020) Circadian clock mechanism driving mammalian photoperiodism. Nat Commun 11:4291

Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan LI (2021) clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innov 2

Yamamura T, Hirunagi K, Ebihara S, Yoshimura T (2004) Seasonal morphological changes in the neuro-glial interaction between gonadotropin-releasing hormone nerve terminals and glial endfeet in Japanese quail. Endocrinology 145:4264–4267

Yamamura T, Yasuo S, Hirunagi K, Ebihara S, Yoshimura T (2006) T3 implantation mimics photoperiodically reduced encasement of nerve terminals by glial processes in the median eminence of Japanese quail. Cell Tissue Res 324:175–179

Yasuo S, Watanabe M, Nakao N, Takagi T, Follett BK, Ebihara S, Yoshimura T (2005) The reciprocal switching of two thyroid hormone-activating and-inactivating enzyme genes is involved in the photoperiodic gonadal response of Japanese quail. Endocrinology 146:2551–2554

Yoo S, Kim J, Lyu P, Hoang TV, Ma A, Trinh V, Dai W, Jiang L, Leavey P, Duncan L (2021) Control of neurogenic competence in mammalian hypothalamic tanycytes. Sci Adv 7:eabg3777

Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S (2003) Light-induced hormone conversion of T 4 to T 3 regulates photoperiodic response of gonads in birds. Nature 426:178

Zhang H, Zhang G, Gonzalez FJ, Park S, Cai D (2011) Hypoxia-inducible factor directs POMC gene to mediate hypothalamic glucose sensing and energy balance regulation. PLoS Biol 9:e1001112

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.