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Integration of photoperiod and time-restricted feeding on the circadian gene rhythms in juvenile salmon

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The circadian clock has evolved to synchronize animal behaviour and physiology with the external environment. Present in almost all cells, the clock is made up of a transcription-translation feedback loop that is responsive to cues such as light/dark cycles (photoperiod) and the time of feeding. Chinook salmon (*Oncorhynchus tshawytscha*) is a fish species whose clock is thought to be adapted in natural populations according to their latitude, where photoperiod variation can be extreme in northern spring/summer conditions. Here, we probed for the expression of circadian clock genes in four tissues of juvenile Chinook salmon under different environmental conditions. We find that the circadian clock is optimal when photoperiod is coupled with regular feeding during daylight hours. We further tested the effects of constant light and time-restricted feeding, environmental factors that are known to affect daily gene expression rhythms, on the expression of clock genes, appetite-regulating hormones, and metabolic regulators in the intestine of juvenile Chinook. We find that overall constant light is chrono-disruptive irrespective of the timing of food. The resulting disruption in gene expression produces aberrant rhythms, and affects glucose homeostasis, despite an increase in growth. Our data suggests photoperiod and time-restricted feeding could be optimized in Chinook aquaculture and raise the question of whether and how photoperiod changes are compensated in northern-adapted populations.

Keywords Circadian rhythms, Salmon, Photoperiod, Circadian entrainment

The 24-hour planetary cycles of Earth's rotation have played a significant role in the evolution of animal physiology. Daily cycles of light and dark photoperiod on the planet's surface have led to the adaptation of circadian rhythms that enable animals to anticipate daily timing, and synchronize their physiological activity accordingly to optimize fitness[1,2]. The loss of rhythms has been linked to a large number of chronic illnesses in both animal models and clinical studies[2,3]. At a fundamental level, circadian rhythms are 24-hour oscillations in molecular activity that are driven by a system, known as the circadian clock, that resides in nearly all the cells of the body in diverse animal species[4–6]. This clock system is a highly conserved daily cycle that maintains 24-hour periodicity to generate circadian rhythms.

The clock that underlies circadian rhythms consists of a transcription-translation feedback loop; transcription factors of the two positive regulators *circadian locomotor output cycles kaput* (*clock*) and *brain and muscle aryl-hydrocarbon receptor nuclear translocator-like* (*bmal*) heterodimerize to drive the expression of the negative regulators *period* (*per*) and *cryptochrome* (*cry*) by binding to E-box elements in their promoters[6]. The translated Per and Cry proteins dimerize and translocate into the nucleus, where they inhibit Clock/Bmal transcriptional activation, causing a new cycle to begin. Per/Cry themselves are then targeted for degradation, resetting the system for another cycle of transcription/translation. In vertebrates, an additional feedback loop gives stability to the core clock by directing the rhythmic expression of *bmal* through the transcriptional regulators *rar-related orphan receptor alpha* (*rorα*) and *nuclear receptor subfamily 1 group d member 1* (*nr1d1*, also known as *rev-erbα*). Rorα and Nr1d1 proteins activate and repress the transcription of *bmal*, respectively. In fish, *clock* may also be under the control of Rorα and Nr1d1. For this system to function efficiently, the expression rhythms of positive regulators, *clock* and *bmal1*, occur at different phases compared to the regulators *per*, *cry*, *rorα*, and

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nr1d1. Essentially, these regulators are co-expressed at opposite times to strengthen rhythmic expression of clock components: when *clock*/*bmal1* are high, their repressors *per*/*cry* are low and vice versa. In this manner, the circadian clock is a robust timekeeper that accurately tracks daily time within cells and matches this to that of the external 24-hour environment.

While the circadian clock is conserved in all animals, fish possess intriguing features that highlight unique evolutionary adaptations. First, clocks in the different cells of the body are directly entrained by light[7,8], meaning that photoperiod (24-hour light/dark cycle) is a Zeitgeber, a clock entrainment factor, in the fish body. Second, fish possess extra copies of many of the clock genes due to a genome duplication that took place during the evolution of teleosts[6]. Salmonids experienced an additional whole genome duplication[9]. These extra copies may evolve redundant functions or may be adapted to support additional cellular and molecular interactions[10]. While studying duplicated genes is challenging, the circadian system in fish offers new opportunities for investigating the evolution of circadian function.

For example, Chinook salmon (*Oncorhynchus tshawytscha*) is a species where circadian clock function has been linked to local adaptation. Chinook range in the Pacific from central California to Alaska, and from Northern Japan to northern Siberia. Juvenile salmon reside in freshwater for approximately one year, then migrate into the sea and do not return to their natal streams until reproduction[11]. Salmon smoltification – the physiological adaptation of juvenile salmon from freshwater to saltwater environments, is known to be regulated by photoperiod changes[12,13]. Chinook migration patterns are thought to be influenced by circadian clock timing which, entrained by photoperiod light cues, is used to separate early- from late-migrating fish populations according to seasonal change[14,15]. In support of this notion, migration timing is correlated with genetic variance in circadian clock genes present in northern versus southern populations[16,17]. Specifically, the *clock1b* gene possesses a 1,200 bp non-coding segment, termed the polyQ domain, that displays a length polymorphism that segregates between populations found at northern versus southern latitudes[16]. These clock gene adaptations are not restricted to Chinook salmon *clock1b*, other salmon species[14,18], and additional circadian clock components[15] also exhibit northern versus southern differences in sequence and migration timing. Chinook salmon are thus a good model species to provide insight into how circadian clock function may be playing a role in their evolution. Our previous study tested clock gene expression in five different tissues of juvenile Chinook, revealing tissue-dependent timing in daily gene expression rhythms[19]. Characterizing the specific gene expression cycles in this species is key to understanding how changes in clock gene polymorphisms can drive adaptive outcomes.

An unanswered question is how circadian clock function is altered under different environmental circumstances. Photoperiod entrains the clock to drive behavioural and physiological rhythms throughout the body[1]. Additionally, the timing of daily food intake can also entrain rhythmicity in digestive system tissues[20,21]. Indeed a form of nutrition, called Time-Restricted Feeding (TRF), is able to entrain peripheral clocks tissues such as the intestine and liver directly, apart from the photoperiod-responding clock in the brain[20–22]. The mechanisms underlying this phenomenon are not clear but may involve nutrients themselves, such as glucose[23], and/or nutrition-dependent hormones such as glucocorticoids[24–26]. In natural circumstances, photoperiod dictates behavioural activity which then affects food consumption, synergizing the effect of these two environmental cues to produce strong diurnal rhythms in the body. Yet photoperiod varies considerably across the north-south range of Chinook salmon, affecting juvenile exposure to light and their subsequent daily food intake patterns. Do Chinook salmon tissue clocks respond specifically to photoperiod and/or feeding cues? It is possible that different populations of Chinook have evolved a tissue-specific clock response driven by these different cues consistent with local adaptation? Of note, Chinook salmon are an important fish in the aquaculture industry, where efforts to provide photoperiod and food according to species' natural schedules can produce healthier outcomes[27].

We asked how the circadian clock in Chinook salmon is impacted by changes in photoperiod and feeding. We tested clock gene expression in four peripheral tissues of juvenile Chinook reared in different conditions and found that these display environment specific daily timing. Gene expression patterns in certain tissues, such as muscle and heart are less robust than ones such as the liver and intestine. We found overall that constant light is chrono-disruptive, negatively impacting the diurnal rhythmicity of clock genes in the body. We focused on the intestine as the primary organ responsible for the uptake of nutrients and found that it is resistant to TRF but is susceptible to photoperiod. Constant light drives profound changes in the daily rhythms of genes involved in metabolism and digestion, resulting in alterations of glucose metabolism despite increased growth in fish reared under these conditions. Our work underscores that constant light causes the loss of rhythms in Chinook salmon and could have a negative impact on their health. These results have implications for how salmonids are reared, highlighting that while constant light (or extended light) might foster higher growth, the negative effects associated might negate any possible gains. Furthermore, our work contributes to our understanding of local adaptations among wild populations of Chinook salmon which are experiencing alarming declines.

Materials and methods

Experimental setup

For experiments in Figs. 1, 2, 3, 4, 5 and 6, Juvenile Chinook salmon (6 months old, mixed sex) from a genetically homogenous stock derived from fish native to British Columbia were reared at Yellow Island Aquaculture Ltd. (Quadra Island, BC). The fish were divided into groups housed in opaque 200 L tanks with open tops, each containing about ~ 200 fish in the same flow-through water system equipped with UV filters from a common header pond, to make sure that experimental fish experience the same water chemistry, oxygen levels and temperature. Fish were reared under LD photoperiod (ambient conditions for June, 16 h light : 8 h dark) or under LL constant light (24 h light), for a duration of 14 days. The light used consisted of fluorescent lights (CFL Fluorescent Lighting, full daylight spectrum 6500 K) on a timed photoelectric cell control of 32 Watts. This light level was ~ 200 lx measured at the top of the tank. Fish were presented with food either in the morning only

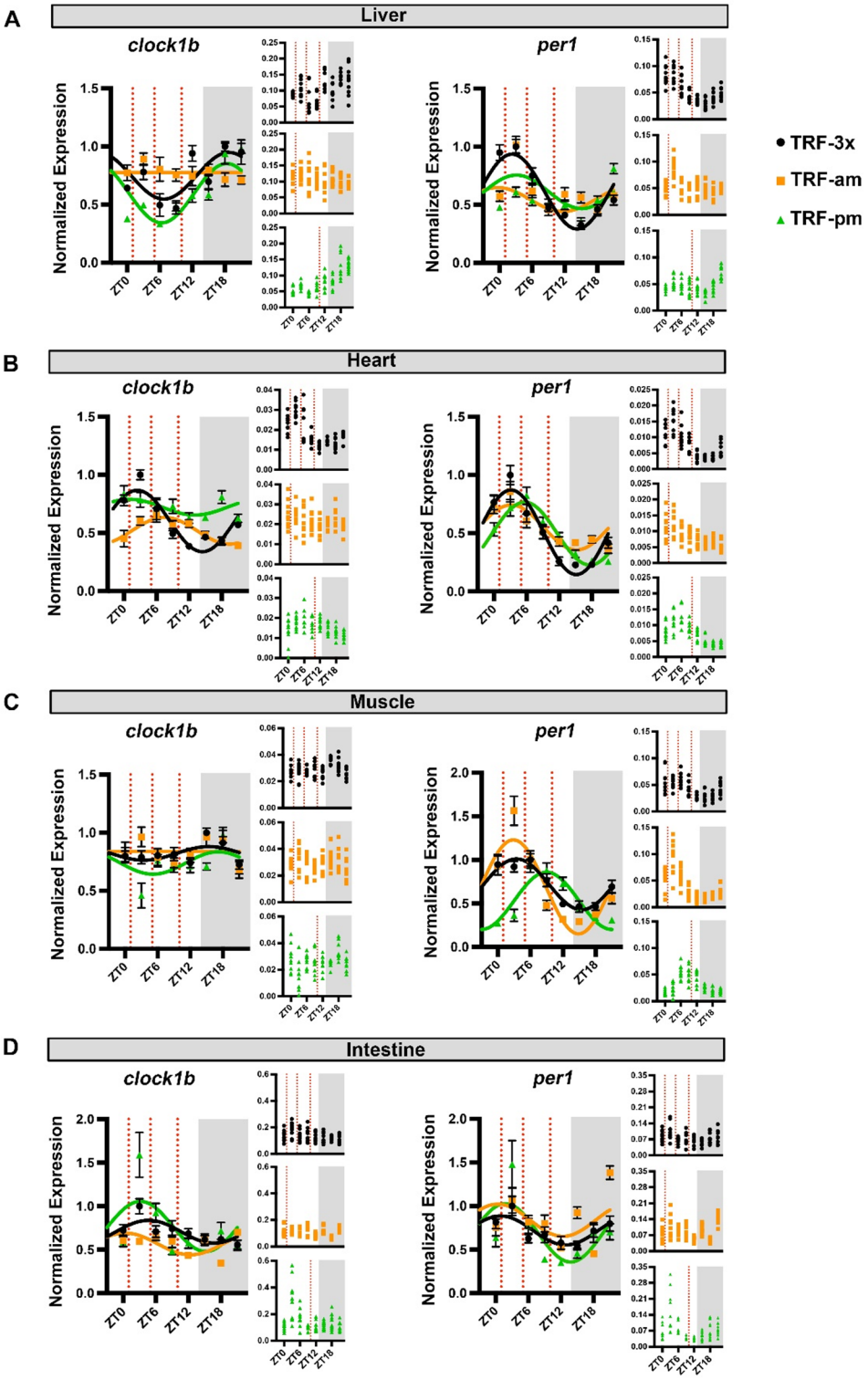

Fig. 1. Rhythmic expression of the clock genes *clock1b* and *per1* is tissue-specific in Chinook salmon. Gene expression was assessed through quantitative PCR. The white and black shaded regions on the plots represent light and dark photoperiod, 16-h light & 8-h dark (LD). The dotted red lines represent feeding time TRF-am (8 a.m.), TRF-pm (5 p.m.), and TRF-3 × (8 a.m., 12 p.m., and 5 p.m.). Data are shown normalized to the peak expression point of the 3x-TRF condition as cosinor curves from CircaCompare (mean ± SEM). Non-significant rhythmicity is indicated as a flat line at the mesor level. Rhythmic expression is seen in most tissues including (**A**) liver (TRF-3x $p = 2.81E-06$, TRF-pm $p = 4.89E-11$), (**B**) heart (TRF-3x $p = 2.08E-14$, TRF-am $p = 2.35E-02$, TRF-pm $p = 1.34E-06$), (**C**) muscle (TRF-3x $p = 2.21E-02$, TRF-pm $p = 1.97E-02$), and (**D**) intestine (TRF-3x $p = 3.65E-04$, TRF-am $p = 4.46E-03$, TRF-pm $p = 1.23E-04$). The scatter graphs shown to the left indicate raw gene expression levels before CircaCompare analysis was carried out. Full statistical details are available in Supplementary Tables 1 and 8.

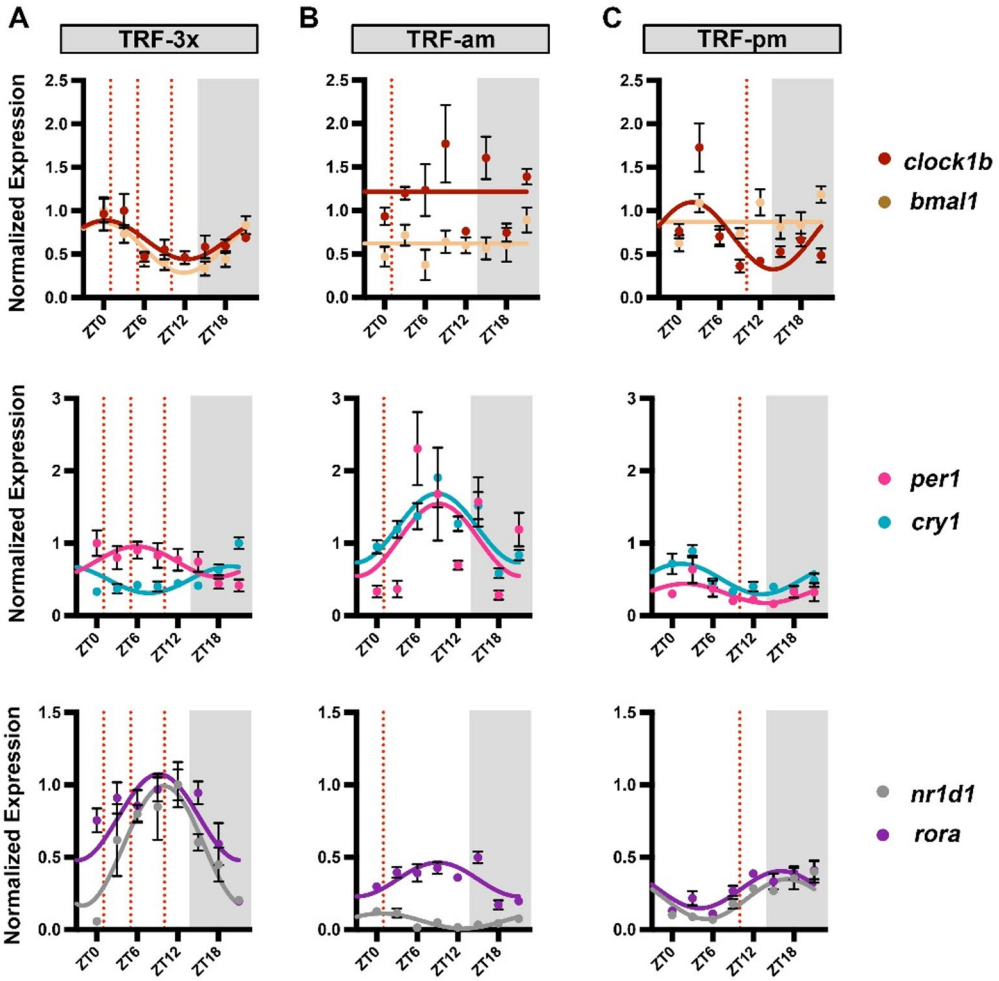

Fig. 2. Altered diurnal rhythmicity of intestinal circadian clock genes in response to alternative feeding. Taqman™ OpenArray™ gene expression chips were used to measure expression of six core clock genes simultaneously. Gene expression data were analyzed as in Fig. 1. (**A**) In TRF-3x the transactivators (*clock1b* and *bmal1*) ($p = 6.93E\text{-}04$ and $p = 5.61E-07$), their negative regulators (*per1* and *cry1*) ($p = 6.88E-03$ and $p = 4.03E-05$), and co-regulators (*nr1d1* and *rorα*) ($p = 1.15E-07$ and $p = 6.99E-07$) are rhythmic. (**B**) Shows the same for some genes under TRF-am (*per1p* = 1.50E − 02, *cry1p* = 5.38E − 06, *nr1d1p* = 6.55E-06, *rorαp* = 2.43E − 06), and (**C**) shows TRF-pm (*clock1bp* = 2.49E − 05, *per1p* = 5.34E − 03, *cry1p* = 6.97E − 06, *nr1d1p* = 7.55E − 08, *rorαp* = 3.03E − 07). TRF-3x produces the most optimal daily expression rhythms: all six genes are rhythmic. The white and black shaded regions represent LD photoperiod. Data are shown normalized to the peak expression point of the 3x-TRF condition as cosinor curves from CircaCompare (mean ± SEM). Non-significant rhythmicity shown as flat line at the mesor level. Full statistical details are available in Supplementary Tables 2 and 9.

(TRF-am, morning = ZT3), evening only (TRF-pm, evening = ZT12), or three times per day at equal intervals relative to the 16 h photoperiod (TRF-3x: morning = ZT3, afternoon = ZT7, evening ZT12; ZT is Zeitgeber Time that is relative to photoperiod, with lights on at ZT0 and off at ZT16). Chinook salmon have been shown to feed in the morning and evening primarily[28], and this diet regimen mimics this timing with an additional meal provided at mid-day to test the effect of regular daily feeding on gene rhythms. The total amount of feed provided to each feeding group in one day was identical (isocaloric). The food (Taplow, Organic Chinook Grower feed) used was 50 g per tank containing 200 fish (1–2% of body weight. Fish were fed to satiation. Following 4 weeks acclimation, tissues were dissected from juveniles and subjected to transcriptional analysis by qPCR (Figs. 1, 2, 3, 4, 5 and 6). For experiments in Fig. 7, juvenile Chinook salmon native to Ontario were reared from fertilized eggs to the fry life stage at the Freshwater Restoration Ecology Centre (an experimental research fish hatchery) in Lasalle, Ontario, to allow for the conditions required to experimentally manipulate the rearing environment and feeding routine of the fish. All experimental fish were then divided between tanks on the same recirculating water system equipped with UV filters and aerators, to make sure that experimental fish experience the same water chemistry, oxygen levels and temperature. In each tank, water levels were held at ~ 500 L and a stocking density of ~ 10 g/L for consistency across treatment groups. Fish were reared in 16/8 LD or constant light (LL) and feeding was done three times per day. Juvenile salmon were raised in these tanks for 4 months after the

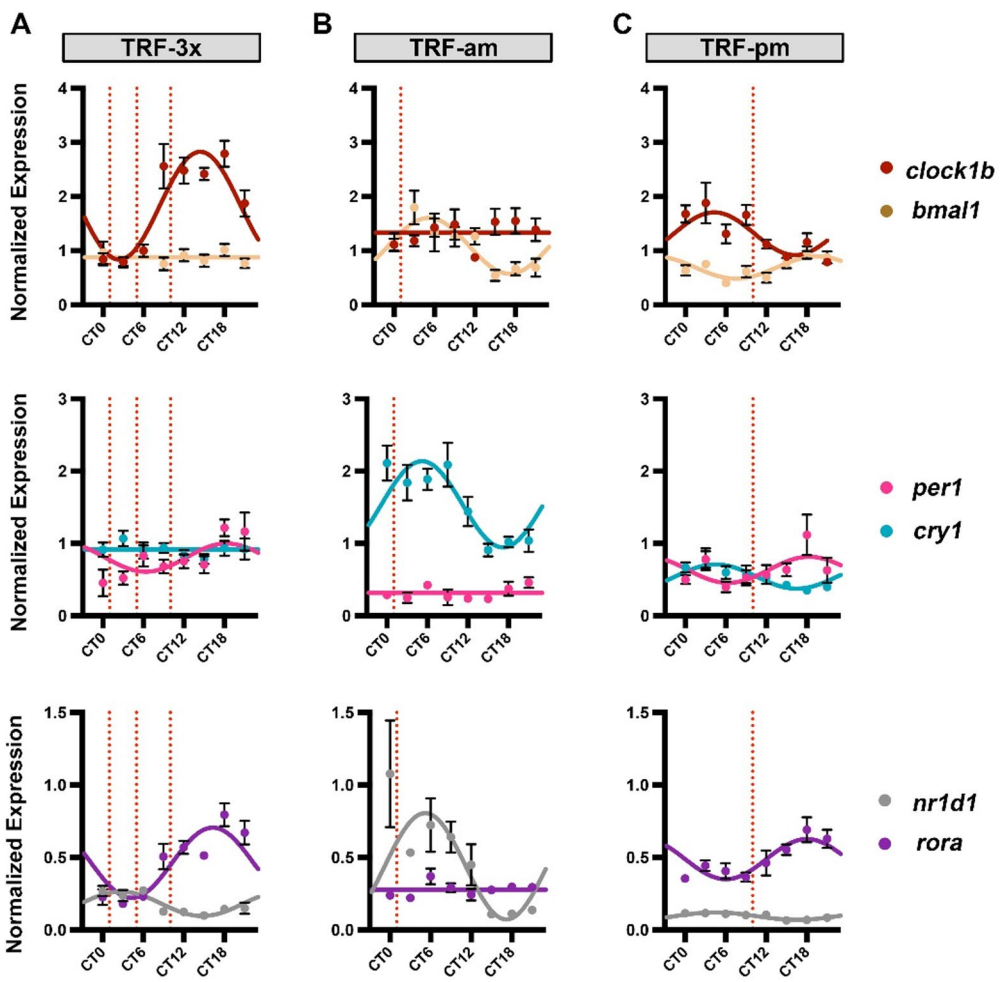

Fig. 3. Decreased intestinal clock gene rhythmicity is observed under constant light. Chinook intestine, under 24 h of constant light (LL), have worsened daily rhythms compared to LD conditions (Fig. 2). (**A**) TRF-3x transactivators *clock1b* ($p = 5.36E-12$) and *bmal1*, negative regulators *per1* ($p = 1.14E-02$) and *cry1*, and co-regulators *nr1d1* ($p = 4.31E-07$) and *rorα* ($p = 6.64E-10$). (**B**) TRF-am (*bmal1p* = 6.16E − 06, *cry1p* = 1.33E − 06, *nr1d1p* = 1.31E − 06), and (**C**) TRF-pm (*clock1bp* = 1.38E − 04, *bmal1p* = 7.79E − 05, *per1p* = 2.65E − 02, *cry1p* = 1.26E − 05, *nr1d1p* = 4.01E − 06, *rorαp* = 1.96E − 05). Under constant light, the intestine exhibits either a loss in the rhythm of clock genes, or shows that both positive and negative components are co-expressed with the same daily timing. Data are shown normalized to the peak expression point of the 3x-TRF condition as cosinor curves from CircaCompare (mean ± SEM). Non-significant rhythmicity shown as flat line at the mesor level. Full statistical details are available in Supplementary Tables 3 and 9.

larval stage then tested. All experimental protocols were approved by the University of Windsor Animal Care Committee, and performed in accordance with Canadian Council on Animal Care regulations, and ARRIVE guidelines.

Blood glucose and lactate analysis

Samples were collected at ZT8-9. Fish were individually netted from their rearing tanks and immediately placed into an aerated 19 L bucket with 1.25 g of Tricaine Methanesulfonate (MS-222) anesthetic, 2.5 g of Sodium Bicarbonate buffer, and 10 L of fresh water from the same source as the rearing tank water. Fish were left in the anesthetic for ~ 1 min 45 s before being placed into a trough (60 × 25 × 20 cm) that was filled with ~ 2 L of water. To minimize air exposure, the trough was filled so that each fish could have their gills fully submerged during the sampling procedure. Approximately 0.2 ml of blood was then collected non-lethally from the caudal vein using a heparinized syringe. Time was recorded for each blood collection procedure, and all blood related data (i.e., glucose and lactate levels) were excluded from the analyses if it took longer than 5 min to collect, including total elapsed time in anesthetic[29]. Whole blood samples were immediately placed on ice in a cooler where they remained until all blood samples were collected from each treatment group. Upon completion of the blood sample collection, ~ 50µL of whole blood was taken from each stored sample for glucose (Contour Next Glucose Meter) and lactate (EDGE Lactate Meter) analysis using real-time handheld meters within one hour of sample collection.

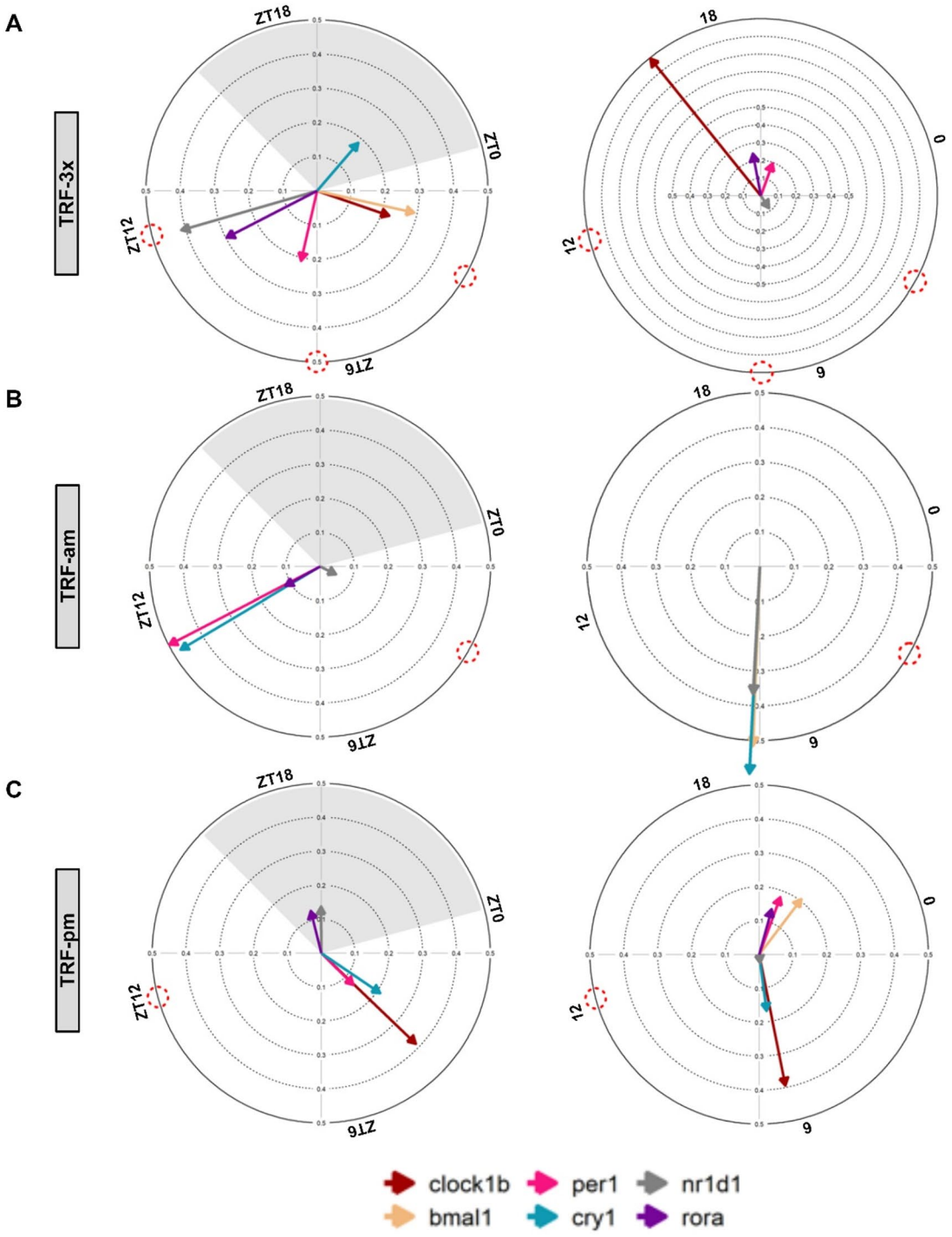

Fig. 4. Constant light and food timing affect the daily timing of intestinal clock gene expression. Circular plots show phase shift differences among clock components. The angle of each gene arrow represents the maximum rhythmic expression of each gene graphed as a cosinor curve from Figs. 2 and 3. (**A**) comparison between LD and LL under TRF-3x, (**B**) the same under TRF-am, (**C**) TRF-p.m. The time of day, ZT0/ZT6/ZT12/ZT18 are indicated on the graphs and dotted red circles indicate feeding times. The length of the arrow represents amplitude of the expression curve, and the non-significant rhythms do not have arrows shown. The effects of constant light (LL) and once a day feeding (TRF-a.m./p.m.) shows changes in the six clock genes tested, when compared to TRF-3x LD where all six gene are rhythmic. These plots also demonstrate that positive and negative arms of the clock are often aberrantly co-expressed in LL. Full statistical details are available in Supplementary Table 4.

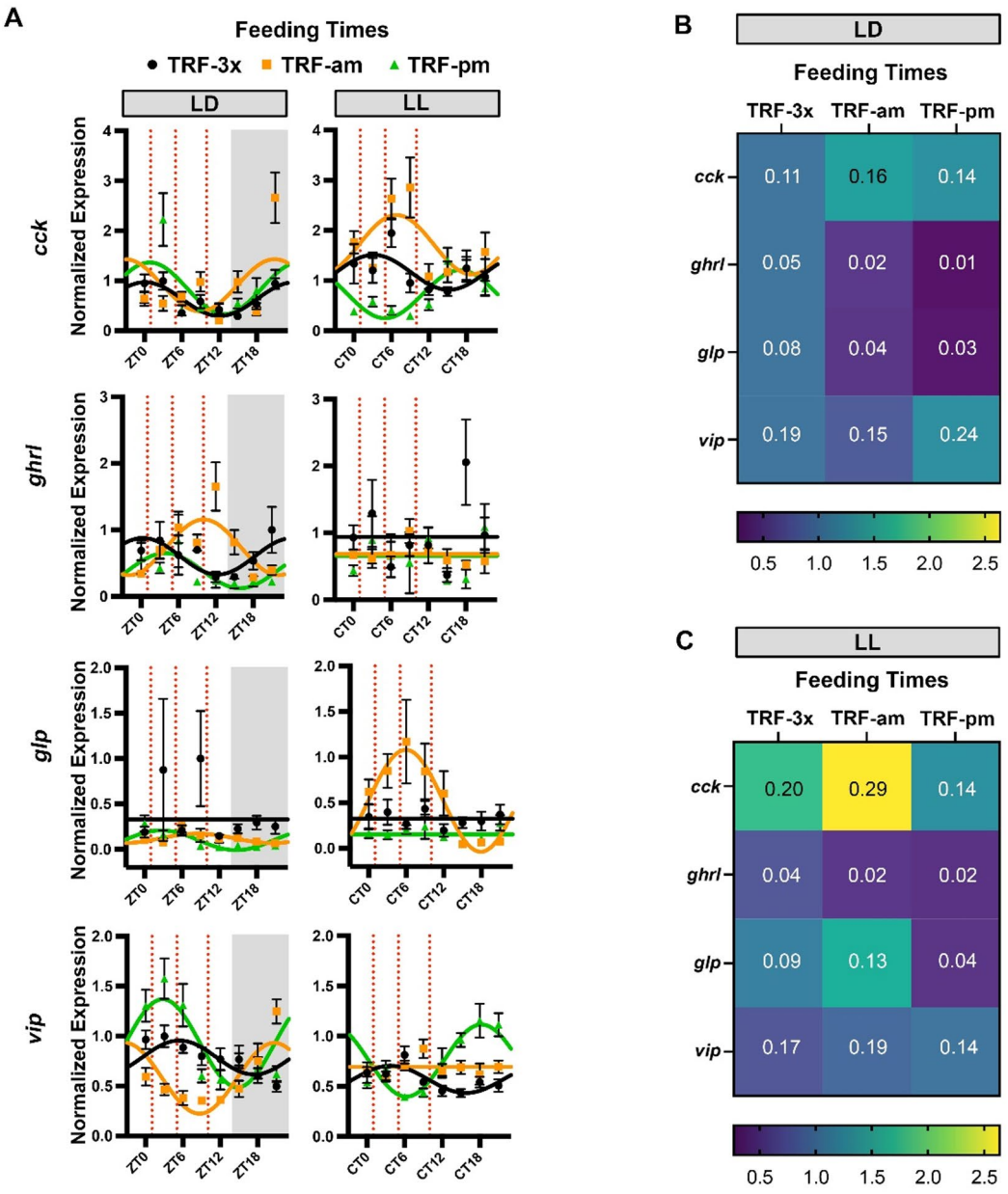

Fig. 5. Altered timing of appetite regulation genes occurs under constant light. (**A**) Taqman™ chip assays were used to measure rhythmicity of the intestinal hormone genes *cck* (TRF-3x LD $p = 2.76E-06$, LL $p = 7.40E-03$), *ghrl* (TRF-3x LD $p = 3.12E-02$, LL $p = 9.59E-02$), *glp* (TRF-3x LD $p = 1.55E--01$, LL $p = 2.76E-01$), and *vip* (TRF-3x LD $p = 2.28E-04$, LL $p = 7.31E-05$). Data are shown normalized to the peak expression point of the 3x-TRF condition as cosinor curves from CircaCompare (mean ± SEM). Non-significant rhythmicity shown as flat line at the mesor level. We find overall that constant light affects the rhythmicity and/or daily timing of hormone gene expression. (**B**) Shows total average daily expression, which is essentially equivalent in both LD and (**C**) LL, with the exception of *cck*, a gene that while rhythmic under both is elevated in LL. Full statistical details are available in Supplementary Tables 5 and 9.

RNA sample collection and isolation

For tissue sampling, ten fish were tested 8 times over a 24-hour timespan by collecting 10 individuals every 3 h over 24 h. Fish were humanely euthanized in 0.04% clove oil (New Directions Aromatics Inc.), then decapitated. Intestine, liver, and a sample of skeletal muscle directly anterior to the dorsal fin were preserved in 7 mL of a concentrated salt buffer consisting of 0.5 M EDTA disodium dehydrate (Fisher Scientific, CAS: 6381-92-6) at pH 8.0, 1 M sodium citrate (Produits Chimiques ACP Chemicals Inc, Catalog #: S-2990), ammonium sulfate (Alpha chem, Catalog #: AM7210), sulfuric acid (Fisher chemical, Catalog #: A300) at pH 5.2. Tissues were stored at -80°C for later RNA extraction and qPCR. Between 5 and 30 mg of tissue were used for RNA extraction using the RNeasy® Plus Mini kit (QIAGEN, Catalog #: 74136) following the manufacturer's protocol. The recommendation

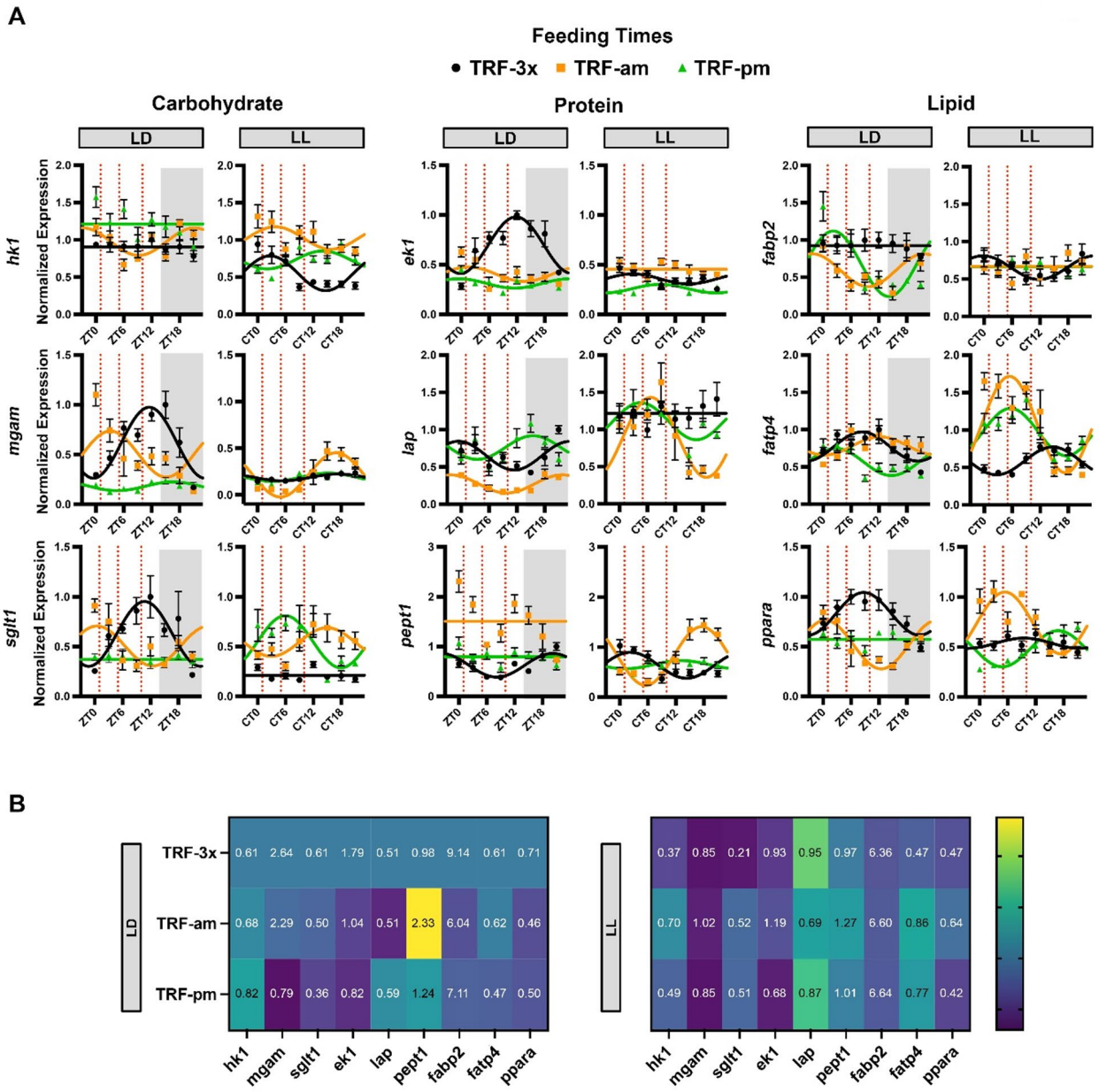

Fig. 6. Constant light and food timing affect the daily rhythms of intestinal genes involved in digestion and metabolism. (**A**) Carbohydrate metabolic genes indicate that constant light affects the timing and rhythmicity of gene expression. Protein metabolism is also affected as well as lipid metabolism. In nearly all genes tested, changes in expression patterns are evident under TRF-am/pm feeding schedules, and 3x-TRF (black lines) most consistently yielded strong rhythmicity. These data indicate that both photoperiod and food timing are integrated in the expression of intestinal genes. Data are shown normalized to the peak expression point of the 3x-TRF condition as cosinor curves from CircaCompare (mean ± SEM). Non-significant rhythmicity shown as flat line at the mesor level. (**B**) Shows total average daily expression, which is essentially equivalent with the exception of *pept1* that decreases in constant light conditions. Full statistical details are available in Supplementary Tables 6 and 9.

to use 50% ethanol instead of 70% ethanol for liver tissue was followed. 1 µg of cDNA was prepared using the iScript cDNA Synthesis Kit (BIO-RAD, (Cat. No. 170–8891) according to manufacturer's protocol.

Primer design

Chinook salmon gene sequences were obtained from the NCBI database, and genes of interest were confirmed by running a BLAST to known zebrafish transcript sequences. In the cases where multiple isoforms of a transcript were found, the isoforms were aligned using Geneious software (Geneious Biologics) to find a region of homology in which to develop primers and/or probes. Primers in Fig. 1 were designed using NCBI's primer

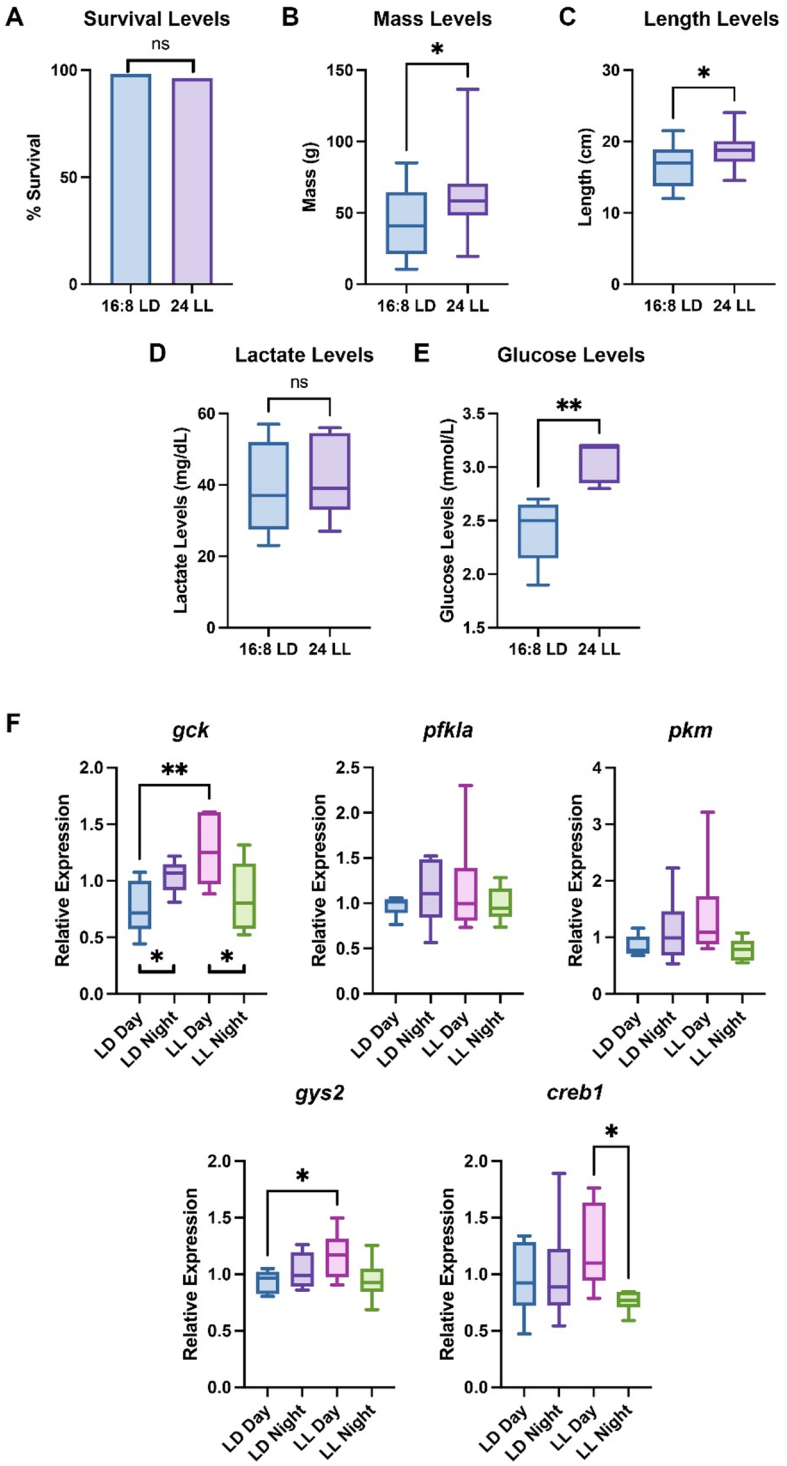

Fig. 7. Glucose metabolism is altered under constant light. While survival (**A**) is not affected, both (**B**) body mass ($p = 2.54E-02$) and (**C**) body length ($p = 1.99E-02$) of juvenile Chinook salmon are significantly higher in LL compared to LD. (**D**) In the blood, the levels of lactate are equivalent, but (**E**) the levels of glucose from juvenile Chinook raised in constant light are significantly higher indicating changes in glucose homeostasis ($p = 4.60E-02$). (**F**) Gene expression of carbohydrate enzymes in the liver were assessed to compare daytime (ZT3-ZT9) vs. nighttime (ZT15-ZT21) glucose homeostasis. *gck* ($p = 9.20E-03$), *gys2* ($p = 3.72E-02$), and *creb1* ($p = 1.53E-02$) show differences in their expression under constant light. Asterisks indicate significance, full statistical details are available in Supplementary Table 7.

designing tool (Table 1), and all other primers used in the Taqman Arrays (Figs. 2, 3, 4, 5 and 6, Table 2) were designed using Primer Express Software 3.0 (Applied Biosystems). Target amplicon size was 70–90 bp, primer length 18–22 bp, primer Tm 58–60°C, specificity check database was Chinook salmon (taxid: 74940) with specificity check stringency of 2 mismatches within the last 5 bps at the 3' end, GC content 30–80%, avoid a G in the second position of the 5' end, and < 4 consecutive G residues in the primers. All primers (Eurofins Genomics) were tested with the iTaq™ Universal SYBR® Green Supermix (BIO-RAD, Catalog #: 1725125) on the ViiA™ 7 qPCR system (Applied Biosystems). Gel electrophoresis was used to verify the absence of multiple bands to ensure primer specificity. To ensure that the results achieved with SYBR® green qPCR predict successful assays when using Taqman™ chemistry later, assays were also validated with the Taqman™ Fast Advanced Master Mix (Applied Biosystems, Catalog #: 4444553) using the ViiA™ 7 qPCR system (Applied Biosystems).

The following (5'-->3') primer sequences were used:

Sybr green qPCR

Validated primers were used in qPCR to quantify the relative abundance of genes of interest compared to the two control genes, *gapdh* and *tubα*. 10 µL reactions: 5 µL SYBR® green, 3.2 µL nuclease-free H_2O, 0.4 µL of each of forward and reverse primers) were run in duplicates using MicroAmpTM Optical 384-Well Reaction Plate (Applied Biosystems, Catalog #: 4309849) incubated in the ViiA7 qPCR machine at 95 °C for 20 s, followed by 40 cycles of denaturation at 95 °C for 3 s and annealing and extension at 56 °C for 30 s. This was followed by a final step consisting of 15 s at 95 °C and 60 s at 65 °C. If the standard deviation between the duplicates exceeded 0.5 threshold cycles (Ct), the reactions were repeated to ensure accurate readings.

Taqman™ openarray™

OpenArray gene expression chips (Applied Biosystems) were used to quantify the transcription of genes, run in duplicate reactions. The chips were preloaded with primer and FAM-MGB probe sequences for a total of 479 samples. The QuantStudioTM 12 K Flex Real-Time PCR System (ThermoFisher Scientific) was used for expression quantification according to the manufacturer's run method. The master mix was prepared in 384 well plates and then transferred onto the chips using the OpenArray™ AccuFill™ system. Each reaction was composed of 2.5 µL of Taqman™ OpenArray™ Real-Time PCR Master Mix (Applied Biosystems, Catalog #: 4462156), 1.2 µL of cDNA and 1.3 µL of nuclease-free H2O.

Gene expression, rhythmicity analysis, and statistics

Based on our prior work testing housekeeping gene transcripts (*18 S*, *g6pd*, *gapdh*, *tubα*, *b-act*, *hprt*, and *ef1a*) using geNorm analysis in qbase+ (Biogazelle, version 3.2), we selected two genes with the lowest geNorm M value (*gapdh*, *tuba*) as control genes for normalization. The expression of each clock gene was normalized by calculating the geometric mean for the Ct values of *gapdh* and *tuba* using the following equation: $Geometric\ mean = SQRT(POWER(2, Ct\mathrm{Gapdh} - Ctgene) \times POWER(2Ct\mathrm{Tub}\alpha - Ctgene))$. qPCR data across the 24-hour timespan from each feeding and photoperiod condition was considered to be only potentially rhythmic if $p < 0.05$ using a one-way ANOVA or Kruskal-Wallis tests depending on the parametric vs. non-parametric distribution of the datasets (Graphpad Prism Version 7.0c for Mac OS X), shown in Supplementary Tables 8–9. Data was then processed using the CircaCompare R package version 0.1.1. that utilizes circular-linear regression analysis to construct cosinor curves and assess rhythmicity [30]. Cosinor curves were deemed rhythmic if $p < 0.05$ by CircaCompare. If the daily expression of a gene was determined to be rhythmic, the mesor, phase, shift, and period were used to create a trendline on Graphpad Prism. This analysis is

gapdh	Forward primer	TGTCAGTGGTGGACCTAACC
	Reverse primer	GCCTTCTTGACAGCCTCCTT
tubα	Forward primer	AGACGACTCCTTCAACACCTT
	Reverse primer	CAGTGGGCTCCAGATCCA
clock1b	Forward primer	CAGCAGCACACGGTTCAA
	Reverse primer	AGGACAGAGCTGGTGTCTTG
per1	Forward primer	GAGTGGGAAGCACCAATGAA
	Reverse primer	ATTGGCTGCCATGGTTGTTG
gck	Forward primer	TAGGTCCAGAGCAAAAGCCA
	Reverse primer	GCGTTAGCCTAGCAGTGAAT
pfkla	Forward primer	CTGGGGACTAAACGAACCCT
	Reverse primer	CGATCAGCAGAGCCTGGATA
gys2	Forward primer	AGTTAACCGACGAGCTTGGA
	Reverse primer	GAGAATAAGTCCTGCCCCGA
pkm	Forward primer	TAGCGATGGCATCATGGTTG
	Reverse primer	TCCGGTCATCATCTTCTGGG
creb1	Forward primer	AAATCGGAGGAGGACACAGC
	Reverse primer	ACTGCTGGTCTGGTAGATGG

Table 1. Sybr green qPCR (Fig. 1).

b-act	Forward primer	GGCCGTACCACCGGTATC
	Reverse primer	AGCCCTCGTAGATGGGTACT
	Taqman Probe	TCCGGTGACGGCGTGA
gapdh	Forward primer	TGTCAGTGGTGGACCTAACC
	Reverse primer	GCCTTCTTGACAGCCTCCTT
	Taqman Probe	CCGGCAGCTACGCT
tubα	Forward primer	AGACGACTCCTTCAACACCTT
	Reverse primer	CAGTGGGCTCCAGATCCA
	Taqman Probe	CTGGCAAGCACGTCC
bmal1	Forward primer	GACCAGGCCTCGGTATCC
	Reverse primer	CCCAGGTTGGCATCAGTCT
	Taqman Probe	AACGACGAGGCTGCCAT
clock1b	Forward primer	CAGCAGCACACGGTTCAA
	Reverse primer	AGGACAGAGCTGGTGTCTTG
	Taqman Probe	CAACCTCAGCAGCAGG
cry1	Forward primer	GCAGGGTGCATCGTAGGTAA
	Reverse primer	TCCTCTGGATGTTCTTCTTGCT
	Taqman Probe	CCCATAGTGGAGCACGA
per1	Forward primer	GAGTGGGAAGCACCAATGAA
	Reverse primer	ATTGGCTGCCATGGTTGTTG
	Taqman Probe	AAGAGGAGGAGGCGGGA
nr1d1	Forward primer	ATCGCGTCTGGCTTCCATT
	Reverse primer	TGGATGTTCTGCTGGATGCT
	Taqman Probe	ACGGCGTTCACGCC
rorα	Forward primer	AAGACACTGTCGGGAAAATGG
	Reverse primer	GGGTGAGGGACAGAGGAGATA
	Taqman Probe	ACGGGCGGTGGTG
cck	Forward primer	ACACGACAGGATGCAAAAGC
	Reverse primer	GGTGCAGGACTGACCCTTAT
	Taqman Probe	ACCGGCCCTCCCA
ghrl	Forward primer	ACCACAGGTAAGACAGGGTAAA
	Reverse primer	AGCTCAGCAAAGCTCTCAATG
	Taqman Probe	CCTCGGGTTGGTCG
glp-1	Forward primer	GCAGAGATCTACCTACGCACA
	Reverse primer	TGACCTCCTCTGTTCCCTCT
	Taqman Probe	CAGCCAGGCACAGAC
vip	Forward primer	ACGCAGACGGTCTCTTTACA
	Reverse primer	CGCTTTCCAATCAAGGATTCCA
	Taqman Probe	ACAGCTGTCGGCGCG
sglt1	Forward primer	CCCTCTTTGCGAGCAACATT
	Reverse primer	AATCCGCCAATGGCAAGTC
	Taqman Probe	ATCGCAGGGACTGCA
mgam	Forward primer	GAGGTAGAGCTCACACACAAC
	Reverse primer	ACCCATTGTGGTGTCAAACAG
	Taqman Probe	TTCCAAGTACGCAGGGC
hk1	Forward primer	CACATCCACAGTACCCCAAGA
	Reverse primer	ACTCTCAGACAGCACAAAGC
	Taqman Probe	CCAGTTGGTGCCAGAG
pept1	Forward primer	GGTATCCACTCCCAACAGAAGT
	Reverse primer	GCCCATAATGAACACGATGAGA
	Taqman Probe	CTTCGGCGTCCCTG
lap	Forward primer	TCAACAACGTCGGCAAATACAG
	Reverse primer	CAATGAGGAGCCGTCACAAAC
	Taqman Probe	CTGCACGGCGGCAG
ek	Forward primer	GTGGACAGTACGAGTGTCAGA
	Reverse primer	TCCAGTGTTGACCACAGGTT
	Taqman Probe	CAGCTCCAGGTCCAGA

Continued

fatp4	Forward primer	AGGGTAAACACAAGGTCCGTAT
	Reverse primer	CGTTGAAGCGAGAGGTGAAC
	Taqman Probe	TCGGTAACGGCCTGC
fabpi	Forward primer	GGAAAGACAACAGTAAGGTGCT
	Reverse primer	GCATCCACCCCATCGTAGTTA
	Taqman Probe	CACCACTCGGGCCGT
ppara	Forward primer	GTGACCTGGCTCTGTTTGTG
	Reverse primer	TCGATGTGGGTCACGTTCA
	Taqman Probe	CTGTGGAGACCGCC

Table 2. Taqman™ openarray™ qPCR (Figs. 2, 3, 4, 5 and 6).

shown in Supplementary Tables 1–3, and 4–5. Comparisons of the same gene was carried out by CircaCompare to assess when curves were significantly altered from each other ($p < 0.05$). Circular plots were developed in RStudio Desktop for Mac OS X Version 2023.03.0 to show phase relationships between the genes that displayed rhythmicity. Statistical analysis of the circular plots is shown in Supplementary Table 4. Statistical analysis of graphs shown in Fig. 7 included T-test and one-way ANOVA as appropriate (Graphpad Prism Version 7.0c for Mac OS X), and this analysis is shown in Supplementary Table 7.

Results

Time-restricted feeding alters circadian clock gene rhythms

We first tested how Time Restricted Feeding (TRF) affects circadian clock gene rhythmicity, predicting that if food timing was an entrainment factor it would shift the timing of circadian genes by the same difference in food timing. We tested the liver, heart, skeletal muscle, and intestine to gain insight into physiological changes as a result of feeding at different times.

In the liver, *clock1b* exhibits daily changes in TRF-3x and TRF-pm where it displays a significant Cosinor rhythm with similar amplitude and phase (Fig. 1A, and Supplementary Table 1). In contrast, TRF-am could not be analyzed by Cosinor fit as its expression shows no daily rhythms. Cosinor analysis reveals that *per1* exhibits rhythms in all three conditions with the highest amplitude rhythms present in the TRF-3x, and the lowest amplitude present in TRF-am (Fig. 1A). As expected, *clock1b* and *per1* are anti-phasic in TRF-3x and TRF-pm, indicating the presence of a functional circadian clock where the higher expression of the repressor (*per1*) is linked to lower *clock1b* expression and vice versa. When fish were fed only in the morning (TRF-am) gene expression was chrono-disruptive. Of note, neither morning nor evening feeding show a clear shift in the timing of diurnal gene expression that matches the timing of feeding (Fig. 1A).

Under TRF-3x, *clock1b* and *per1* expression have robust Cosinor rhythms in the heart that, unlike the liver, are in-phase with both genes showing a peak during the day and a trough at night (Fig. 1B, and Supplementary Table 1). Like the liver, under TRF-am, *clock1b* and *per1* expression have lower amplitude and *clock1b* exhibits a phase-delay relative to TRF-3x (Fig. 1B). Similarly, under TRF-pm, *clock1b* has low amplitude and *per1* exhibits a phase-delay (Fig. 1B). This suggests feeding once a day in the morning or evening is chrono-disruptive compared to TRF-3x.

In the skeletal muscle, low amplitude rhythms of *clock1b* are observed in TRF-3x and TRF-pm conditions and TRF-am is arrhythmic (Fig. 1C, and Supplementary Table 1). *per1* exhibits rhythms in both TRF-3x and TRF-am and a relative phase-delay in TRF-pm (Fig. 1C). Of note, under TRF-3x, *clock1b* and *per1* are anti-phasic indicating normal clock gene rhythmicity in juvenile salmon are present under TRF-3x (Fig. 1C). Similar to heart and liver, TRF-am/pm is chrono-disruptive.

In the intestine, significant daily changes in *clock1b* and *per1* were observed under all conditions tested (Fig. 1D, and Supplementary Table 1). Cosinor analysis revealed that *clock1b* rhythms peak slightly later than *per1* in TRF-3x. The juvenile Chinook salmon intestine also does not exhibit antiphasic rhythms in clock gene expression: *clock1b* and *per1* show essentially the same phase rather than clearly opposite phases in the timing of their expression (Fig. 1D). Of note, the intestine appears to be more robust than the other tissues examined in that it is rhythmic under all three different feeding conditions.

Constant light is chrono-disruptive in the salmon intestine

In northern latitude habitats, light of long duration in the spring/summer is an environmental factor for juvenile fish. These conditions might disrupt clock function, and thereby daily expression rhythms to negatively affect health. Because it was rhythmic under different food timing regimens, we explored the intestinal clock under increased light and tested the clock genes *clock1b*, *per1*, *bmal1*, *cry1*, *nr1d1*, and *rorα* by TaqMan Arrays to gain a better understanding of clock function.

Under LD photoperiod and TRF-3x, gene expression for all clock genes tested was rhythmic (Fig. 2A, and Supplementary Table 2). In contrast to the Sybr-based qPCR (Fig. 1D), Taqman qPCR revealed that *per1* peaks at ZT7 and *cry1* at ZT20, in contrast to *clock1b* and *bmal1*, which both peak at ZT0 in-phase with each other as would be expected in a functioning circadian oscillator (Fig. 2A). Additionally, *nr1d1* and *rorα* were also in-phase and show peaks of expression at different times than the other clock genes (Fig. 2A). TRF-am under LD photoperiod exhibits transcriptional oscillations suggestive of chrono-disruption: neither *clock1b* or *bmal1* are rhythmic, *per1*/*cry1* both peak at ZT8, *nr1d1*/ *rorα* are of low amplitude and *nr1d1* is also phase-shifted

(Fig. 2B). Under TRF-pm, *bmal1* is arrhythmic and *per1*, *cry1*, *nr1d1*, *rorα* are of low amplitude and phase-shifted compared to TRF-3x, only *clock1b* rhythms are similar (Fig. 2C).

Under constant light (LL), fed TRF-3x, juvenile salmon intestines are chrono-disrupted (Fig. 3A, and Supplementary Table 3). *bmal1* and *cry1* are arrhythmic, *nr1d1* is low amplitude, and the remaining genes, *clock1b* and *rorα* are rhythmic but peak and trough at different times than when photoperiod is present. Similarly, TRF-am and TRF-pm both exhibit dysfunctional circadian clock gene expression rhythms in LL. TRF-am shows no rhythms in *clock1b*, *per1*, or *rorα*, and the rhythmic genes such as *bmal1* and *cry1* are phase-shifted compared to LD conditions (Fig. 3B). TRF-pm shows rhythms, however, pairs of genes that should be approximately co-phasic are anti-phasic, such as *clock1b* and *bmal1*, *per1* and *cry1*, as well as *nr1d1* and *rorα* (Fig. 3C). The relationships between clock gene phasing are further evident when these are plotted to show the peak of maximum daily clock gene expression. LD TRF-3x shows all six genes peak at different times over 24 h, thereby exhibiting stereotypical clock oscillation in which positive and negative transcriptional regulators are phase-shifted (Fig. 4A and Supplementary Table 4). This is altered in LL TRF-3x, where four genes show rhythms with different timing. LD TRF-am shows four rhythmic genes (Fig. 4B), while LD TRF-pm shows five (Fig. 4C). LD TRF-pm positive and negative components are co-phasic (i.e. *clock1b* and *per1*/*cry1* peak at the same time). LL TRF-am and TRF-pm are changed: each shows rhythmic gene expression in a subset of components, but when compared with LD TRF-3x, positive and negative components do not peak at the same time and fewer genes are detected as rhythmic with significantly separated phase relationships (Fig. 4B-C).

Constant photoperiod alters rhythms in intestinal hormones and digestion/metabolism

We next asked whether photoperiod and timed feeding would affect daily transcript rhythms in peptide hormones expressed by the intestine. These included *cholecystokinin* (*cck*), a stimulator of digestive enzymes following meals, *ghrelin* (*ghrl*), a promoter of hunger and feeding, *glucagon like peptide* (*glp*), a promoter of insulin secretion, and *vasoactive intestinal peptide* (*vip*), a regulator of intestinal motility and secretion. We predicted that again, LD TRF-3x conditions would display the greatest daily rhythmicity as rhythms in circadian clock gene rhythms are present. Rhythms in *cck* and *vip* are evident in all LD conditions, but *glp* is not rhythmic in TRF-3x (Fig. 5A, and Supplementary Table 5). In LL, hormone expression rhythms change: *cck* is phase-shifted in TRF-am and TRF-pm, *grhl* is arrhythmic, and a loss of rhythmicity in *glp* and *vip* occurs in TRF-pm and TRF-am, respectively (Fig. 5A). A stronger rhythm in *glp* was noted in TRF-am under constant light compared to LD photoperiod (Fig. 5A). We further tested the mean levels of these genes across a 24-hour timespan and noted increases in *cck* under LL, but no other major alterations in the overall level of hormone gene expression (Fig. 5B-C). These tests indicate that hormone transcript rhythms do not appear follow specific morning vs. evening feeding times nor correlate with clock gene rhythms.

We next profiled intestinal genes involved in digestion/metabolism or nutrient uptake. These included: carbohydrate regulators *hexokinase 1* (*hk1*), a glucose phosphatase, *maltase-glucoamylase* (*mgam*), an enzyme that breaks down complex carbohydrates, *sodium glucose co-transporter* (*sglt*), that promotes glucose uptake; protein regulators *enterokinase 1* (*ek1*), an activator of the trypsin digestive enzyme, *leucine aminopeptidase* (*lap*), a protein digestive enzyme, *h-coupled peptide transporter 1* (*pept1*), that promotes peptide uptake; fatty acid regulators *intestinal fatty acid binding protein* (*fabp2*), that enables cells to store lipids, *long-chain fatty acid transport protein 4* (*fatp4*), that promotes fatty acid uptake, *peroxisome proliferator activated receptor alpha* (*ppara*), a transcriptional regulator of lipid metabolism. Consistent with our previous results, TRF-3x exhibits higher amplitude rhythms than TRF-am or TRF-pm in most of these genes, notably *mgam*, *sglt*, *ek1*, and *ppara* (Fig. 6A, and Supplementary Table 6). However in some cases, TRF-am or TRF-pm elicit the presence of rhythms that are absent in TRF-3x (for instance *hk1* and *fabp2* in Fig. 6A). Changes in the daily timing and rhythmicity show that food consumption regulates digestion/absorption/metabolism genes. Gene expression over a 24-hour timespan was averaged and compared between conditions but no significant changes in the levels of carbohydrate, protein, or fat digestion genes were noted, with the exception of *pept1* that is higher in TRF-am, and *mgam* and *ek1* that are lower in TRF-pm (Fig. 6B).

Constant light alters the diurnal expression of these digestive system genes. First, the amplitude of rhythms in LL TRF-3x is generally lower compared to LD TRF-3x, and in many cases the timing of rhythms is altered in constant light or becomes arrhythmic (Fig. 6A). Second, when fed only once a day, the rhythmicity of many TRF-am and TRF-pm genes is sometimes increased under constant light, for instance *lap*, *pept1*, *fatp4*, and *ppara* all have higher amplitude when photoperiod is absent and food is provided at different times, although the timing of their rhythms is changed (Fig. 6A). Third, genes involved in nutrient digestion and absorption which could be driven by the timing of food intake, do not show consistent changes in timing relative to feeding. For instance, *lap* and *fatp4* peak at the same time in both TRF-am and TRF-pm in constant light even though animals were fed 9 h earlier in the former (Fig. 6A). We also compared the average of gene expression levels over 24-hours but found that LL conditions did not significantly differ from LD conditions (Fig. 6B), the rhythms of these genes is not associated with increases or decreases in expression.

Continuous light impacts juvenile salmon physiology

Our results suggest that daily gene expression rhythms are impacted by constant light. Does the deregulation of these affect growth or physiological outcomes? To address this question, we tested salmon reared in LD vs. LL under TRF-3x feeding. The survival of fish in LD conditions and LL conditions does not differ (Fig. 7A and Supplementary Table 7), indicating mortality is unaffected by constant light. However, the length (Fig. 7B) and mass (Fig. 7C) of LL fish is significantly higher than LD fish, even though feeding regimens were the same in both conditions, indicating that constant light increases growth. To further examine metabolism, glucose and lactate were measured. Lactate levels showed no changes (Fig. 7D), however, glucose levels in LL fish were significantly higher (Fig. 7E).

To further test glucose metabolism, we examined the liver of the fish under constant light and analyzed the expression of carbohydrate metabolic components. We tested *glucokinase* (*gck*), *phosphofructokinase* (*pfkla*), and *pyruvate kinase* (*pkm*), enzymes that initiate the utilization of glucose, *glycogen synthase* (*gys1*), an enzyme that stores glucose in liver cells, and *cyclic AMP-responsive element-binding protein* (*creb*), a downstream transcription factor that regulates glucose metabolism. We tested liver samples early in the day, before meals were consumed, and late in the day, after meals were consumed. *gck* is elevated at night compared to daytime under LD, however this relationship is lost in LL where *gck* is elevated and shows inverted expression timing (Fig. 7F). We found no changes in *pfkla* and *pkm*, but *gys2* is elevated during the day under LL conditions, and *creb1* shows reduced expression at night (Fig. 7F).

Discussion

The circadian clock has been studied in different fish species, where its function as a transcriptional regulator in peripheral tissues is well-established[31–41]. Our previous work determined that circadian clock genes have distinct 24 h rhythms in different tissues of Chinook salmon[19], and we now report that both photoperiod and the time of feeding can affect diurnal rhythms. Overall, we find that TRF-3x provides the best conditions for clock gene rhythmicity in the liver, heart, muscle, and intestine of juvenile salmon parr. Liver and muscle show the characteristics of a normal anti-phasic clock system in these conditions, while heart and intestine exhibit rhythms that appear to be co-phasic (same daily timing). Our data reveal the regulatory effects of a changing food regimen on the daily rhythms of circadian clock genes in juvenile Chinook salmon.

Photoperiod emerges as a potent entrainment factor in juvenile Chinook salmon tissues. *per1* expression in multiple tissues shows a peak during the day, while *clock1b* peaks more variably: during the day in the heart, midday in the intestine, and evening/night in the liver and muscle (Fig. 1). Similar daily rhythms have been found in trout and tilapia liver[37,42], cod muscle[41], and zebrafish intestine[31]. *per1* in zebrafish possesses light-inducible elements in its promoter[6], hence it is possible that light penetration in the body of the juvenile salmon may play a role in the timing of clock gene expression even in tissues such as the intestine. Of note, not all the juvenile salmon tissues we tested display typical clock gene rhythms where positive vs. negative regulators increase and decrease at different times of day. We can only speculate why this is the case, perhaps the clock is still developing its rhythmicity at these early stages of the life cycle. Our analysis of transcript levels provide a baseline of investigation in juvenile Chinook. It would be informative to repeat tests at the protein level, to confirm the gene product of these transcripts oscillates and displays rhythms characteristic of the circadian clock. In addition, we tested rhythms under light rather than in constant darkness where circadian and diurnal (daily) rhythms can be discriminated. Future work in constant darkness would confirm that the gene rhythms we detect are truly circadian in nature.

In our work we tested the effect of constant light after 14 days on rhythms, however, shifting Chinook salmon from a LD environment, where clock genes are initially rhythmic, to a constant light or constant darkness might result in the temporary persistence of rhythms in the absence of photoperiod. For instance, this was noted in the tilapia liver when fish are transferred short-term to constant light[37]. Although clock rhythm persistence may have occurred in our experiments, we nonetheless observed that constant light was chrono-disruptive in our experiments, resulting in the loss or change of most clock gene rhythms (Figs. 2, 3, 4 and 5). Our longer tests of constant light on juvenile Chinook physiology also show that size and weight increase, and glucose metabolism is affected (Fig. 7). These increases in growth are consistent with certain aquaculture practices where fish are raised in constant light to increase yield[43]. Because circadian rhythms are thought to be so important in optimal health, it is counterintuitive that disruption of these rhythms results in increased growth. It is important to note that this seems to be coupled with a trade-off in other aspects of healthy physiological function, such as glucose metabolism. Glucose levels are higher in constant light suggesting that either glucose uptake is reduced, or glucose mobilization is increased. Our data also suggest that glucose processing genes are altered in expression in the liver at these times. These results suggest that the Chinook salmon genotype we tested would be increased in size in northern latitude regions during a spring/summer when photoperiod is essentially constant light. Alternatively, Chinook salmon in these regions have evolved mechanisms of sustaining robust circadian clock function independent of photoperiod regulation, perhaps via genetic elements such as the PolyQ domain of *clock1b*, and/or other clock gene regulators. Future tests of growth and glucose metabolism changes over the course of a day would provide insight into how genotype and metabolism interact rhythmically.

An ongoing question in the field is the relative contribution of time of feeding vs. photoperiod to the entrainment of clock gene expression. Feeding entrainment has been observed in the liver of zebrafish, goldfish, sea bream, trout, and tilapia and mouse[34–38]. Although we did not observe a consistent entrainment whereby food phase-shifts clock gene expression according to the morning or evening, we do find evidence that food timing can alter daily gene rhythms (Figs. 2, 3 and 4). An example of this is the gene, *bmal1*, which in the intestine has disrupted rhythms in LD photoperiod when food is provided once a day but has rhythms in constant light under these same conditions (Figs. 2 and 3). This indicates that food can time *bmal1* when photoperiod is absent. Indeed, under constant light the genes *bmal1*, *cry1*, and *nr1d1* peak ~ 6 h after morning feeding, while *bmal1*, *per1*, and *rorα* peak ~ 6 h after evening feeding. Thus, our data indicates that food can entrain clock genes. A lack of a straightforward effect of feeding on daily gene transcription may occur due to competition between feeding and photoperiod cues in setting the daily timing of tissue cells. However, it is important to note that because constant light disrupts many clock genes, photoperiod is a strong cue in entraining Chinook salmon tissue clocks.

Rhythmic daily changes have been documented in gastrointestinal functions such as gastric emptying, nutrient uptake, digestion, colonic motility, and epithelial cell regeneration[44,45]. This rhythmicity allows for the optimization of organism fitness by anticipating events and synchronizing physiological function to the most advantageous time of the day. We tested intestinal hormones involved in appetite and digestion, and several

factors involved in the digestion and absorption of nutrients. Overall, we find that many of these display rhythms that are perturbed with either photoperiod or feeding (Figs. 5 and 6). Daily rhythms in the hormones we tested have been previously shown in rodents[46–49]. We find that many of these are rhythmic even in constant light combined with TRF-am/pm conditions when the clock is dysfunctional (Fig. 5). This suggest these are not under clock control but rather a diurnal rhythm that emerges from a response to other factors.

Similar to digestive hormones, the regulators of nutrient uptake and digestion that we tested exhibit daily rhythms in many vertebrates. These include those regulating carbohydrate[50–52], protein[53–55], and lipid[56–59] nutrition. The timing of feeding affects digestive tract gene timing but does not simply phase-shift daily rhythms by the difference in time of morning vs. evening. Similar to the other genes we tested, photoperiod was a strong cue in affecting the daily rhythm of digestive tract genes. However, certain genes such as *cck*, *hk1*, and *ppara* respond to morning vs. evening feeding under constant light conditions. We therefore suggest that photoperiod and food regulate the expression timing of these genes in a complex manner (Fig. 6). Of note one gene that we studied, *ppara*, is thought to be transactivated by clock components, and itself can entrain diurnal gene expression rhythms[60–62]. Because juvenile Chinook salmon predominantly feed during the day[28,63,64], it seems reasonable to infer that daily feeding impacts the expression of many genes in the intestine in natural Chinook populations.

Our results demonstrate that clock rhythmicity and nutrient absorption/digestion are interconnected in the juvenile Chinook salmon intestine. These findings shed light on the daily tissue-specific rhythms emerging in juvenile salmon under different environmental conditions. Our data will inform captive rearing programs (either government or commercial) on the costs versus benefits of modifying photoperiod to improve growth. We identify regular photoperiod and food provision as optimal for regular daily timing of gene expression and our study raises questions about how photoperiod is compensated in sub-arctic populations of juvenile salmon under spring/summer conditions.

Data availability

All data generated or analysed during this study are included in this published article and its Supplementary Tables 1-9.

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Author contributions

M.T., A.P. lead the study, performed experiments, prepared figures and text. A.P, K.S., H.A-A., H.C., D.R. performed experiments. D.H., T.E.P., V. C-A., and P.K. provided research support, conceptual guidance, and wrote the main manuscript. M.T., D.H., V. C-A., T.E.P., and P.K. conceived and initiated the study.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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