Fish and Shellfish Immunology 165 (2025) 110525

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Viral infections affect the transcription of biological clock components in lymphoid organs and lymphoid-associated tissues of common carp (*Cyprinus carpio* L.)

Mikolaj Mazur [a,b], Lukasz Pijanowski [a], Mikolaj Adamek [c], Marek Matras [d], Krzysztof Rakus [a], Magdalena Chadzinska [a,*]

[a] Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387, Krakow, Poland
[b] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Lojasiewicza 11, 30-348, Krakow, Poland
[c] Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Bunteweg 17, 30559, Hannover, Germany
[d] Department of Parasitology and Invasive Diseases, Bee Diseases and Aquatic Animal Diseases, National Veterinary Research Institute, Aleja Partyzantow 57, 24-100, Pulawy, Poland

ARTICLE INFO

Keywords:
Common carp
Circadian clock
Lymphoid organs
SVCV
CyHV-3
Viral infection

ABSTRACT

The circadian clock is a mechanism that allows organisms to adapt to the changing environment in a diurnal manner. It receives external cues (time givers), which synchronize the internal circadian clock with the external environment. A crucial aspect of the circadian clock involves the positive and negative feedback loops of clock genes.

In the present study, we examined the diurnal changes in the expression of clock genes in the lymphoid organs (thymus, spleen, head kidney, trunk kidney), lymphoid-associated tissues (gills and gut), and peripheral blood leukocytes (PBLs) of common carp. Moreover, we investigated how the expression of these clock genes changes in these tissues/organs upon viral infections.

Our findings showed that clock genes are constitutively expressed in the lymphoid organs/tissues, gills, gut and PBLs of common carp, and that different light regimes (LD, DD, and LL) altered their expression patterns. We also demonstrated that the two studied viruses, cyprinid herpesvirus 3 (CyHV-3) and spring viremia of carp virus (SVCV), affect the expression of clock genes (*per1, per2*, *cry1,2*, *clock* and *bmal1,2*) in the head kidney, trunk kidney, gill, and skin of common carp under the LD regime.

Clock gene expression was negatively correlated with viral copy numbers.

Overall, this study shows that the immune system and biological clock are interconnected and suggests that proper lighting conditions are crucial for the well-being and functionality of the circadian clock in fish. This consideration is important both in experimental settings and especially in aquaculture.

1. Introduction

The circadian clock is a mechanism that enables organisms to adapt to the changing environment in a diurnal manner, and it is present in almost every cell of all organisms [1]. However, the hierarchy of its structures varies among different groups of animals (reviewed in Ref. [2]). In mammals, the master/central clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and it receives external cues, known as time givers (German: Zeitgeber), such as light and temperature [3], food intake [4], and social interactions [5]. Time givers synchronize the internal circadian clock and the external environment. A crucial mechanism of the circadian clock consists of the positive and negative feedback loops of the clock genes. In mammals there are six core clock genes: *Period 1* and *2* (*Per1*, and *2*), *Cryptochrome 1* and *2* (*Cry1*, and *2*), *Brain and muscle ARNT-like 1* (*Bmal1*) and *Circadian locomotor output cycles kaput* (*Clock*) encoding PER1 and PER2, CRY1 and CRY2, BMAL1 and CLOCK proteins. Heterodimer of BMAL1 and CLOCK bind to an E-box promoter element and activates transcription of *Per* and *Cry*. Heterodimer of PER and CRY translocates from cytoplasm to the nucleus and inhibits *Clock/Bmal1* transcription,

* Corresponding author. Department of Evolutionary Immunology, Jagiellonian University, Gronostajowa 9, PL30-387, Krakow, Poland.
E-mail address: magdalena.chadzinska@uj.edu.pl (M. Chadzinska).

https://doi.org/10.1016/j.fsi.2025.110525
Received 7 May 2025; Received in revised form 13 June 2025; Accepted 26 June 2025
Available online 27 June 2025

allowing the cycle to start over [6]. This loop lasts for about 24 h resulting in an occurrence of a circadian rhythm [1].

Once the master clock receives information from external cues, it transmits signals through the nervous and/or endocrine system to various organs that function as peripheral clocks e.g. lungs, kidneys, and intestines (reviewed in Ref. [7,8]). These peripheral clocks operate using the same transcription-translation feedback loops as the master clock [9]. Peripheral clocks function also in lymphoid organs and leukocytes. Leukocytes express clock genes that regulate their differentiation and maturation, activity, migration, and proliferation cycles [10–12].

The circadian clock is also present in the earliest vertebrates, such as fish. The role and structure of the clock in teleost fish are very similar to those observed in mammals, indicating a strong evolutionary conservation of this mechanism [13]. In fish, the rhythmicity of the clock is also maintained by clock genes. However, due to a genome duplication event that occurred during their evolution, there are multiple copies of the same genes. For example in zebrafish (*Danio rerio*) there are four *per* genes (*per1a*, *per1b*, *per2* and *per3*) [14], six *cry* genes (*cry1a*, *cry1b*, *cry2a*, *cry2b*, *cry3* and *cry4*) [15], three *clock* genes (*clock1a*, *clock1b* and *clock2*) [16] and three *bmal* genes (*bmal1a*, *bmal1b* and *bmal2*) [17]. Zebrafish molecular clock works in a very similar manner as mammalian one, where clock proteins Clock and Bmal form a heterodimer which stimulate transcription of *per* and *cry* (with exception of *cry1a*) genes. After the translation, Per and Cry proteins also form a heterodimer that inhibits transcription of *clock/bmal* [18]. The main difference between mammalian and fish circadian clocks is the hierarchy of its structures. In fish, the central clock was initially believed to be located in the pineal gland [19]. However, later studies suggested its higher complexity, as peripheral organs (e.g. heart and kidney) contain a light-entrainable circadian clocks. This indicates that a single central clock is not necessary for the synchronization of peripheral clocks and the overall mechanism to function [20]. Furthermore, it was found in zebrafish that the expression of the *per2* and *cry1a* genes is stimulated by light [21–24]. In our previous work, we also described a direct effect of light on the expression of clock genes in both common carp and zebrafish, demonstrating that the absence of light stimuli (constant darkness conditions) diminishes all circadian differences in constitutive expression of clock genes [23,24].

The immune system and circadian clock interact with each other to maintain homeostasis. Both in mammals and fish, the immune system exhibits diurnal changes in activity. In mammals, circadian oscillations in the immune system are well-documented. For instance, it has been found that in macrophages, the expression of inflammatory cytokines/chemokines, and pathogen recognition receptors (PRRs) follows a circadian rhythm [25,26]. In the case of neutrophils, the circadian clock affects their circulation, tissue infiltration, ability to produce neutrophil extracellular traps (NETs), and expression of chemokine receptors [27–29]. Also, dendritic cells migrate to lymph nodes following a diurnal pattern, and their maturity markers, such as major histocompatibility complex II (MHC II) and CD86, are also expressed rhythmically [27,30]. The diurnal pattern is moreover observed in lymphocytes. For example, in T cells, it affects cell recruitment to lymph nodes and cytokine expression [31,32], while in B cells, it influences circulation and activation of the B-cell receptor (BCR) signaling pathway [32,33]. The circulation of natural killer (NK) cells and the release of granzyme B, perforin, and proinflammatory cytokines also follow a circadian pattern [34,35]. Although data is limited, there is evidence of rhythmicity in the immune system of fish. For instance, zebrafish myeloid cells phagocytize bacteria at different rates throughout the day, with a peak during the light phase [36]. Additionally, elements of the innate immune response, including serum alkaline phosphatase, lysozyme, peroxidase, and protease, exhibit circadian rhythmicity in their activity in Nile tilapia (*Oreochromis niloticus)* [37]. Furthermore, in Japanese medaka *(Oryzias latipes*), it has been shown that the *tlr9* gene, which encodes the pattern recognition receptor Tlr-9, is expressed in a circadian manner with peak expression during the light phase [38].

Our recent study indicated that in the model of Tilapia lake virus (TiLV) infection in zebrafish, the time of virus injection did not only affect the clock genes expression, but also virus replication and expression of type-I interferon (IFN) pathway related genes [24]. However, studies of bidirectional interaction of circadian clock and immune response in fish are very limited.

In the present study we examined the diurnal changes of clock genes expression in lymphoid organs and lymphoid-associated tissues of common carp. Moreover, we verified how this expression changes during infections with two viruses: cyprinid herpesvirus 3 (CyHV-3) and spring viremia of carp virus (SVCV). This research is particularly important in view of the increasing phenomenon of light pollution in recent years and given the high risk of viral diseases in aquaculture.

2. Materials and methods

2.1. Animals

The experiments were conducted on sexually immature individuals of common carp (*Cyprinus carpio* L.) obtained from the Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences, Golysz, Poland or from the University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, Vodnany, Czech Republic. Upon their arrival to the animal facility, fish were quarantined for 4 weeks in order to acclimate them to the new environment. They were maintained in tanks with recirculating tap water at 21 °C (volume 375 L, flow rate 4 L/min, density 45 fish/tank, and 60 g/L) at 21 °C. Fish were fed pelleted dry food (Aller Master, Aller Aqua, Poland) daily at a rate of 1 % of their estimated body weight (at different times in order not to entrain the circadian clock to feeding schedule). Fish were kept under a 12L:12D (LD) photoperiod (12 h of light and 12 h of darkness) with lights switching on at ZT0 for 4 weeks.

The light was set to imitate a sunrise by gradually increasing the intensity (every 5 min for the first 30 min), and a sunset by gradually decreasing the intensity (every 5 min for the last 30 min). During the light phase, light intensity at the water surface level was 400 lux, while during dark phase it was 0 lux.

2.2. Light regimes experiment and sample collection

After the acclimation, fish were divided into 3 groups (n = 24–32 fish *per* group) and transferred at once to different lighting conditions: (i) 12 h of light and 12 h of darkness (LD), (ii) constant darkness (DD, 0 h of light and 24 h of darkness) and (iii) constant light (LL, 24 h of light and 0 h of darkness). Fish were adapted to LD, DD, LL conditions for next 3 weeks. Samples were collected at four different time points: Zeitgeber/Circadian Time (ZT/CT) 2, 10, 14 and 22 (n = 6–8 *per* time point) as described previously [23]. Fish were euthanized with tricaine methanesulfonate (TMS; Sigma-Aldrich, St. Louis, MO, USA) buffered with NaHCO3 (POCH, Gliwice, Poland) in a 0.2:0.4 g/L ratio. At time points when the light was switched off and in DD conditions fish were anesthetized in the darkness.

Fish were bled and PBLs were collected as described before (Mazur et al., 2022). PBL layer was homogenized in RL buffer (EURx, Gdansk, Poland) with addition of 1 % β2-mercaptoethanol, and placed at −80 °C. Lymphoid organs (head kidney, trunk kidney, spleen and thymus) and lymphoid-associated tissues (gills, foregut and hindgut) were isolated and quickly transferred to the FIX RNA solution (EURx, Gdansk, Poland) and placed at −80 °C until RNA purification was performed.

All procedures were approved by the local ethical committee (1st Local Institutional Animal Care and Use Committee (IACUC) in Krakow, Poland, license number 884/2024).

2.3. Viral infections experiments and sample collection

Fish from LD regime were divided into tanks (volume of 200 L) and

acclimated to the water temperature depending on the viral infection model used: (i) 21 °C for CyHV-3 and (ii) 12 °C for SVCV. The viral infections were performed between ZT10 and ZT12 and the viral load was measured as described previously [39]. In both viral infection experiments a mock-infected control group was included. Animals were euthanized between ZT2 and ZT4. Samples (head kidney, trunk kidney, gills and skin) were collected from control and infected fish (n = 5 fish *per* time point) at 0, 12, 36, 84, 156 and 324 h post infection (hpi). Samples were transferred to RNA*later* (Invitrogen, Waltham, MA, USA) and placed at −80 °C. Experiments were performed in accordance with national and international regulations for experimentation with animals and under approval of the Local Ethical Committee in Lublin, Poland (no 19/2013).

2.4. Gene expression studies

2.4.1. RNA purification, cDNA synthesis

RNA from the tissues was purified, according to the manufacturer's protocol, using either a GeneMATRIX Universal RNA Purification Kit (EURx, Gdansk, Poland) or TRI Reagent® (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. To enhance the process of purification a DNase treatment using RNase-free DNase I (EURx, Gdansk, Poland) was done according to the manufacturer's protocol. The RNA samples were stored at −80 °C until a cDNA synthesis reaction was performed.

The synthesis of cDNA was done using either a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Waltham, MA, USA) or Maxima™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. After the reaction, samples were diluted five times and the cDNA samples were stored at −20 °C.

2.4.2. Real-time quantitative polymerase chain reaction (RT-qPCR)

Clock gene primers were used for gene expression measurement (Supplementary Table 1) and *40S ribosomal protein s11* gene served as a housekeeping gene. RT-qPCR reactions were done using the same reaction design as described previously [23].

Results were calculated on the Pfaffl method [40].

2.5. Statistical analysis

All statistical analyses were performed using GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA). Every data set was analyzed with Grubb's test to remove outliers. When the outliers were removed from the data set, the differences between each time point and light regime were calculated using the two-way analysis of variance (ANOVA) test with significance set at $p \leq 0.05$. The n values indicate the number of individuals analyzed. Data were presented as means ± SEM.

In case of viral infection results, after the outliers were removed from the data set, the Shapiro-Wilk normality test was applied. Significant differences in the gene expression between the control fish at day 0 and infected fish in following sampling points were assessed using one-way ANOVA followed by Dunnett's multiple comparisons test in cases when the data were normally distributed, or by the non-parametric Kruskal–Wallis test followed by Dunn's multiple comparison test when the data were not normally distributed. The results are presented as heatmaps created using an online heatmap generator site (http://www.heatmapper.ca/). The correlation between viral load and clock genes expression level was calculated using Spearman correlation test.

3. Results

3.1. Diurnal changes of the expression of biological clock genes in lymphoid organs and peripheral blood leukocytes

Carp lymphoid organs show constitutive expression of clock genes, and the profile of this expression depends on the time of day and light conditions in which the animals are kept (Fig. S1-2 and S4-5). In fish kept under the LD regime a significantly higher expression of *per1* and *per2* (in head kidney, trunk kidney, thymus and spleen) was observed at ZT2 than at ZT10 and ZT14. Moreover, in the trunk kidney and thymus expression of *bmal1* and *bmal2* was higher at ZT2 than at ZT10, ZT14 and ZT 22, while significant differences in the expression of cry1 were found in the head kidney (between ZT2 and ZT14) and in the thymus (between ZT2 and ZT14, ZT22) (Fig. 1A, B, D, E). In the peripheral blood leukocytes from fish kept under LD regime the expression of clock genes did not differ between time points (Fig. 1C and S3). In lymphoid organs of fish kept under DD regime, the highest expression of clock genes was demonstrated at CT14. At this sampling point, the expression of *cry1* (in trunk kidney and thymus), *clock* (in head kidney, trunk kidney, thymus and spleen), and *bmal1* (in head kidney and spleen) was significantly higher than in most of the other time points (Fig. 1A, B, D, E). In PBLs of fish kept under DD regime the highest expression of studied genes was observed at CT10 and CT14. Expression of *per2*, *cry1*, and *bmal1* was significantly higher at CT14 as compared to CT2 or CT22, while expression of *cry2* and *clock* was significantly higher at CT10 as compared to CT22 (in case of *cry2*) or to CT2, CT14 and CT22 (in case of *clock*) (Fig. 1C). Finally, in the head kidney of fish kept under LL regime expression of *per1*, *per2* and *clock* was the highest at CT2 (Fig. 1A). A similar pattern of expression was also found for *per2* and *cry2* in the trunk kidney (Fig. 1B) and thymus (Fig. 1D). In the spleen of fish from LL regime expression of *per2* was the highest at CT2 while expression of *bmal2* was the highest at CT22 (Fig. 1E). Similarly, in the PBLs from fish kept under LL regime, *cry2* and *bmal1* showed the highest expression at CT22 (Fig. 1C).

At specific time points of the day, statistically significant differences in the expression of clock genes in lymphoid organs from fish kept under LD and DD regimes were observed. In the head kidney of fish from LD regime *per1* expression at ZT22 was significantly higher than in fish from DD regime at CT22. In turn, expression of *cry2*, *clock* and *bmal1* was higher at CT14 in the head kidney of fish from DD regime than in fish kept under LD regime at ZT14 (Fig. 1A). Both, in the trunk kidney and thymus of fish kept under DD regime, expression of all clock genes, except *cry2* and *clock*, was lower at CT2 as compared to fish from LD regime at ZT2 (Fig. 1B and D). The expression of *cry1* in TK and thymus and *clock* and *bmal1* in spleen was higher at CT14 in fish from DD regime than in fish kept under LD lighting at ZT14 (Fig. 1B–D and E). Several differences in the clock genes expression were observed in PBLs from fish kept under LD and DD regimes with higher expression of *per1*, *cry2* and *clock* genes in fish from DD regime at CT10 than in fish from LD regime at ZT10. Moreover, expression of *per2*, *cry1*, *cry2* and *bmal1* was higher in PBLs from animals kept under DD regime at CT14 than in fish from LD regime at ZT14 (Fig. 1C).

Furthermore, at specific time points of the day, statistically significant differences in the expression of clock genes were also found between fish kept under LD and LL and between fish kept under DD and LL regimes. In the first case, higher expression of *cry2* and *clock* genes at CT2 in the head kidney of fish kept under LL regime was observed than at ZT2 in fish from LD regime. At this time point expression of *per1*, *per2*, *cry2* and *clock* was higher in the head kidney of fish from LL lighting than in their counterparts from DD regime, while expression of *bmal1* at CT14 was lower in fish from LL regime than this measured at CT14 in fish from DD lighting (Fig. 1A). In the trunk kidney and thymus of fish from LL regime the expression of *bmal1* and *bmal2* was lower at CT2 than expression measured in fish from LD regime at ZT2, while at this time point expression of *cry2* was higher in both organs of fish from LL regime than in animals kept under DD lighting (Fig. 1B and D). At CT2, a higher expression of *per2* was observed in the spleen of fish from LL regime compared to the expression of this gene in the spleen from fish kept under DD lighting. A similar difference was also found for *bmal2* expression at CT22, while at CT14, the expression of *clock* was lower in the spleen of animals from LL regime compared to expression measured

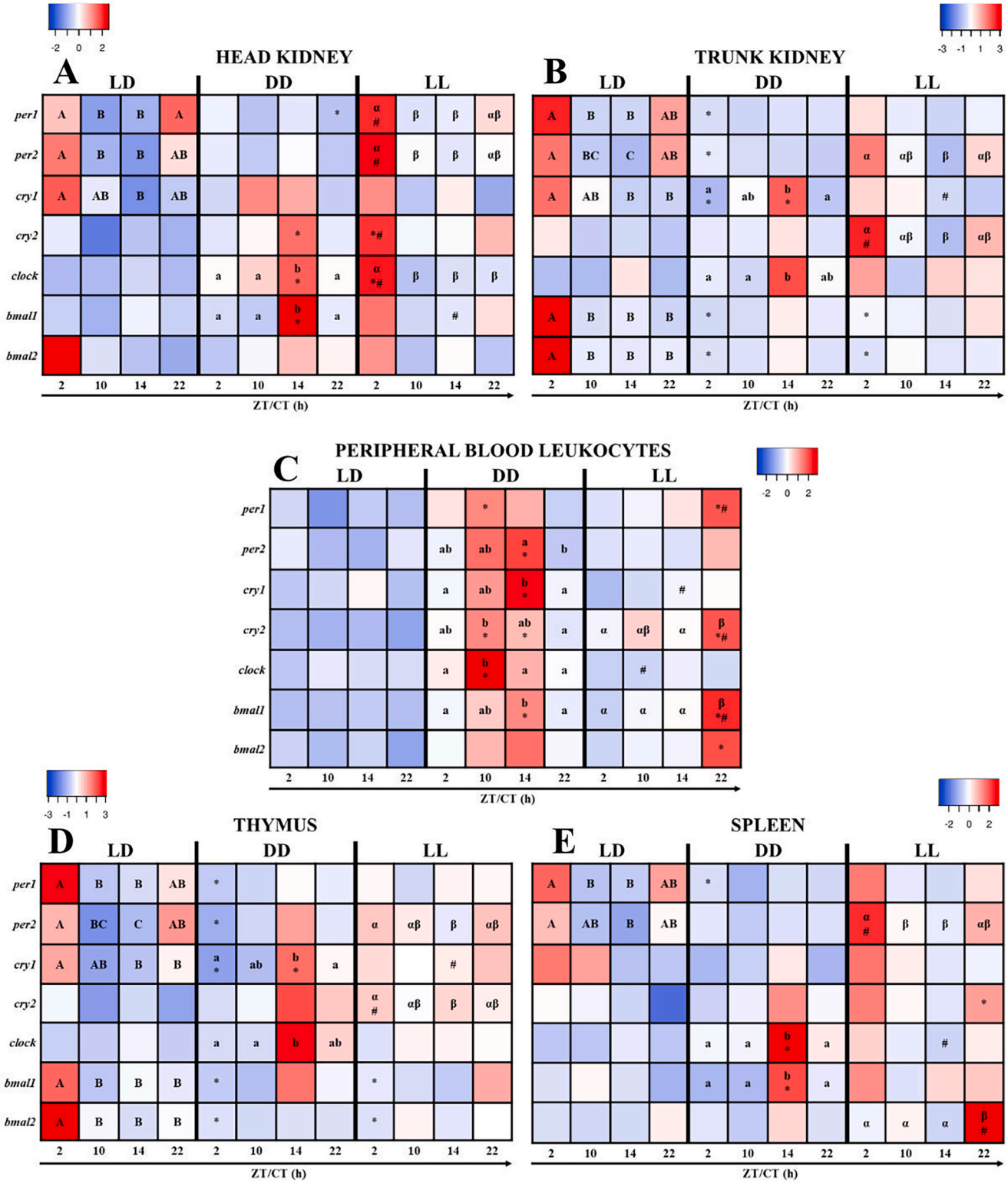


Fig. 1. Diurnal changes in the expression of clock genes in the lymphoid organs and peripheral blood leukocytes of fish kept under LD (12L:12D), DD (0L:24D) and LL (24L:0D) light regimes (n = 4–11). Gene expression was measured at different time points of the day. Data obtained from RT-qPCR analysis are shown as shown as the heatmap, where the colors indicate the normalized mean gene expression values (calculated as $z = (x-\mu)/\sigma$, where x is the mean gene expression value for the given group, μ is the mean of the dataset (the average of all the scores), and σ is the standard deviation) on the Heatmapper website [66]. Within each gene, all of the z-score values were compared and presented as different colors: blue indicates the lower than the mean values (down-regulation; $z < 0$), white indicates the mean values (no change; $z = 0$) and red indicates the higher than the mean values (up-regulation; $z > 0$). The *40S* ribosomal protein s11 gene served as the reference housekeeping gene. Significant differences (two-way ANOVA, $p < 0.05$) between time points within single light regime are indicated by different letters (e.g. A, B for LD; a, b for DD and α, β for LL) and differences between time points within different light regimes are indicated by different symbols (e.g. asterisk (*) differences between DD and LL vs LD regime, hash (#) difference between DD vs LL). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

at this time point in fish from DD regime (Fig. 1E). Higher expression of *per1, cry2*, *bmal1* and *bmal2* was also observed in PBLs of animals kept under LL regime at CT22 than in cells retrieved from fish kept under LD lighting at ZT22. At this time point expression of *per1*, *cry2* and *bmal1* was also higher in PBLs of fish from LL compared to DD regimes (Fig. 1C).

3.2. Diurnal changes of the expression of biological clock genes in lymphoid-associated organs/tissues

We also measured clock gene expression in lymphoid-associated organs such as gills, foregut and hindgut (Fig. S6–8). We found that in the gills of fish kept under LD regime, the only clock gene which expression changed during the day was *per2*. Its expression was at ZT2 higher at ZT2 than at ZT14 (Fig. 2A). More diurnal changes in the clock gene expression in animals from LD regime were observed in the foregut and hindgut. In the foregut, the highest expression of *cry1* and *bmal2* was measured at ZT2, while *per1*, *per2* and *cry2* expression was the highest at ZT22 (Fig. 2B). In turn, in the hindgut at ZT2 the highest expression showed *per1* and *bmal2*, while expression of *per2* was lowest at ZT14 (Fig. 2C).

Upon acclimation to DD and LL regimes several changes in the expression of clock genes were observed in both gills and gut. In the gills of fish from DD regime the expression of *clock* was the highest at CT14 (Fig. 2A). At this time point in fish kept under DD regime, we also observed the highest expression of *cry1*, *clock* and *bmal1* in the foregut (Fig. 2B) and *clock*, *bmal1* and *bmal2* in the hindgut (Fig. 2C).

In gills of fish kept under constant lighting (LL regime) we observed significant diurnal changes in the expression of *per2* and *bmal2* (the highest expression at CT10) as well as *bmal1* (the highest expression at CT2) (Fig. 2A). In foregut from fish kept under LL regime expression of *per2* was the highest at CT2, while expression of cry2 was higher at CT2 and CT22 than at CT19 and CT14 (Fig. 2B). In the hindgut of fish from LL regime we observed that only *cry1* expression differs between the time points and it was the higher at CT2 than at CT22 (Fig. 2C).

Furthermore, at specific time points of the day, statistically significant differences in the expression of clock genes were also found between fish kept under LD and LL and between fish kept under DD and LL regimes. In gills of fish kept under DD regime expression of *clock* was significantly higher at CT14 than at this time point in fish kept under LD and LL regimes. Moreover in fish kept under DD regime expression of *per1* was lower at CT2 than in fish from LD regime at ZT2. At CT2 expression of *bmal1* in the gills of fish from LL regime was higher than this measured at ZT2 and CT2 in fish from LD and DD regime, respectively. Such differences were also observed at CT10 for *per2* and *bmal2* expression. Moreover, at CT10 expression of *per1* and *bmal1* was significantly higher in fish from LL regime than in fish from DD regime. Similar differences were also found at CT22 for *cry2* expression (Fig. 2A). At this time point we also found that expression of *cry2* was higher in the foregut of fish kept in constant light than in fish from LD and DD regimes. Moreover, at CT2 expression of *bmal2* was lower in fish from LL regime than this measured at ZT2 in foregut of fish kept under LD regime, while expression of *cry1* was lower in fish from LL regime than in fish kept under constant darkness (Fig. 2B).

In turn, at CT2 *cry1* expression was higher in the hindgut of fish from LL regime than this observed at the corresponding time point in fish kept under DD regime. Furthermore, at CT2 expression of *per2* was significantly higher in the hindgut of fish from LL regime than in their counterparts from LD and DD regimes (Fig. 2C).

3.3. Infection-induced changes of the expression of biological clock genes in lymphoid organs and lymphoid-associated tissues

3.3.1. CyHV-3 infection

Upon CyHV-3 infection, the expression of *per1* and *cry1* did not change significantly in the studied organs/tissues. CyHV-3 induced significant up-regulation of the expression of *per2* in the skin at 324 hp and down-regulation of the expression of *cry2* in the trunk kidney (at 12, 36 and 156 hpi), gills (at 12, 36, 85, 156 and 324 hpi) and skin (at 36 and 85 hpi). At the early time points post infection *clock* expression was up-regulated in gills (at 12 and 36 hpi) and skin (at 12 hpi), while it was down-regulated in the skin of infected fish at 85, 156 and 324 hpi. A similar pattern of changes was induced by CyHV-3 infection in case of *bmal1*, which expression was significantly up-regulated in the trunk kidney, gills and skin at 36 hpi and down-regulated at the latter time points post infection. Furthermore, in the gills and skin of CyHV-3-infected fish the expression of *bmal2* was up-regulated at 36 hpi (Fig. 3A and S9-12).

Moreover, in the head kidney, *bmal1* expression showed significant, negative correlation with viral load. Similar phenomena were also observed for *per1* and *per2* expression in the trunk kidney, *clock*, *bmal1* and *bmal2* expression in the gills as well as *cry1*, *clock* and *bmal1* expression in the skin (Fig. 3B and S17-20).

3.3.2. SVCV infection

SVCV infection induced significant down-regulation of the expression of *per1* in the skin at 85 and 156 hpi. Expression of *per2* was up-regulated in the head kidney, trunk kidney and gills at 36 hpi, but down-regulated in the head kidney at 324 hpi in SVCV-infected fish. The expression of *cry1* was significantly up-regulated in the gills (at 12 hpi) and head kidney, trunk and skin (at 36 hpi), but further down-regulated in the skin of SVCV-infected fish at 85 and 156 hpi. Furthermore, *cry2* in the skin and *bmal2* in the head kidney of infected fish showed down-regulated expression at 156 hpi. In the gills of SVCV-infected fish significant up-regulation of *clock* expression was observed at 36 and 85 hpi. The expression of *bmal1* was up-regulated in the head kidney (at 36 hpi), but down-regulated at 156 hpi in the trunk kidney and at both 85 and 156 hpi in the gills and skin (Fig. 4A and S13-16).

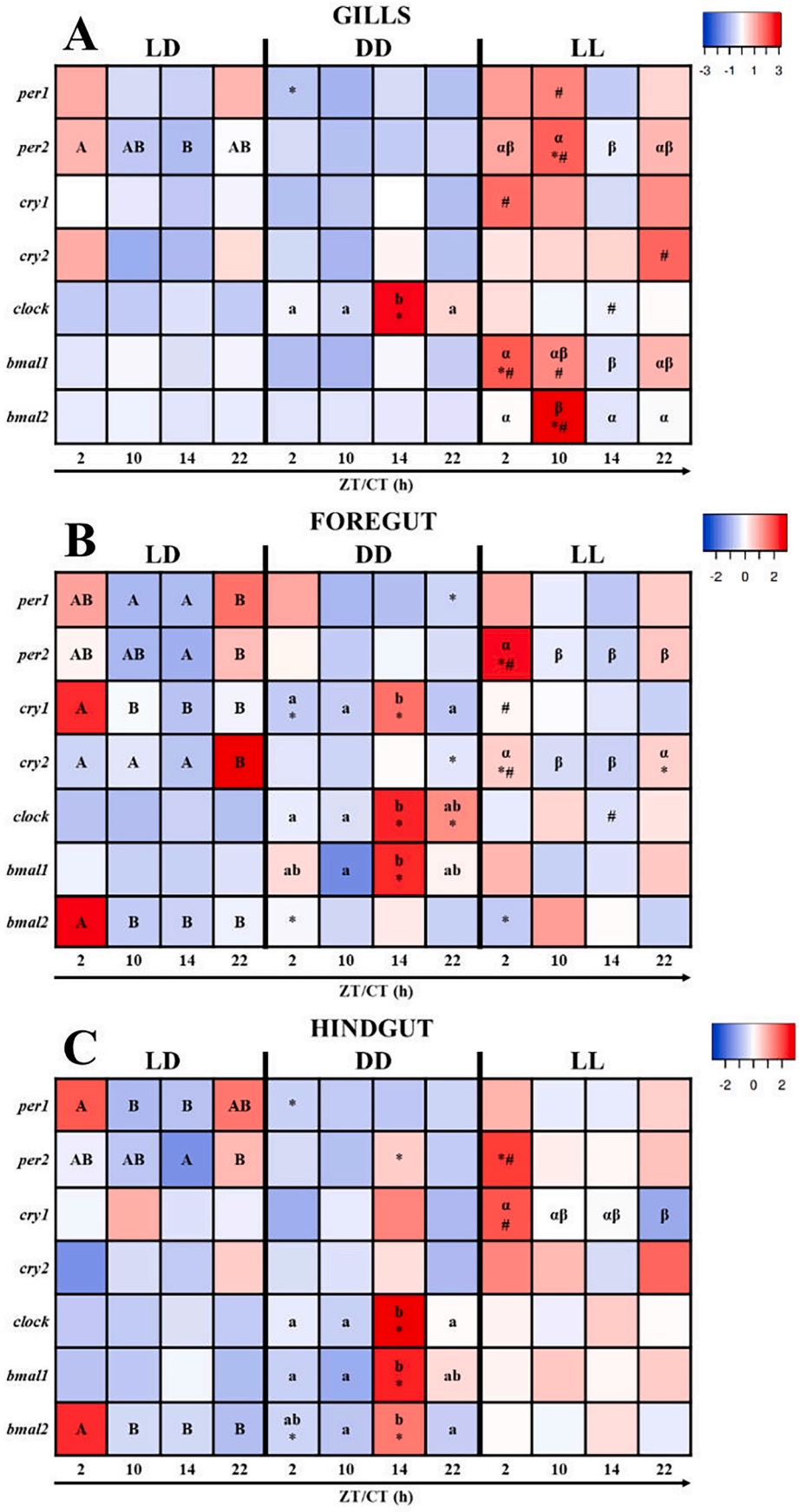
Moreover, in the head kidney, a significant, negative correlation of all examined clock genes (except *bmal2*) expression with viral load was observed. Similar occurrences were also observed for *per1*and *bmal1* in the trunk kidney, for all examined clock genes in the gill and for the *cry1*, *cry2* and *bmal1* in the skin (Fig. 4B and S21-24).

4. Discussion

In the present study, we examined the diurnal changes in the expression of clock genes in various lymphoid organs, lymphoid-associated tissues, and peripheral blood leukocytes (PBLs) of common carp. Additionally, we investigated how the expression of the clock genes changes in these tissues/cells upon viral infections. Our findings showed that clock genes are constitutively expressed in the lymphoid organs/tissues and PBLs of common carp, and the expression of some clock genes varies depending on the time of day and/or light regime. These observations align with our previous studies focused on the effect of different light regimes on clock genes expression in the brain, pituitary gland, retina, heart, and liver of common carp [23]. Furthermore, we demonstrated that a higher amount of viral copies in the tissue was associated with lower expression of clock genes. The overall expression trend showed an up-regulation of clock genes at the early stages of infection (36 h post-infection), followed by a decrease at later time points (85–324 h post-infection).

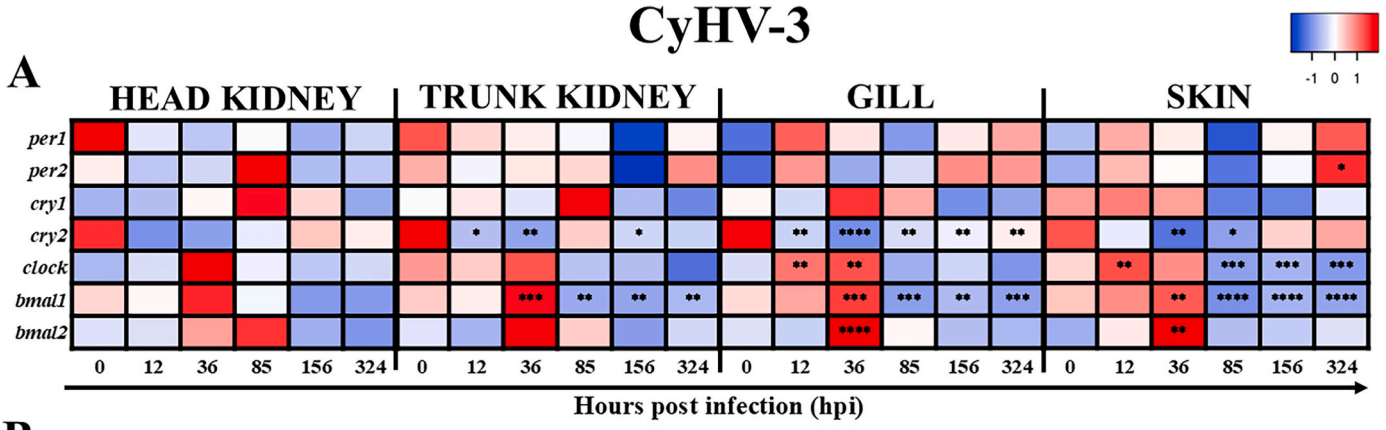
All of these results clearly indicate that in the lymphoid organs, lymphoid-associated tissues, and PBLs of common carp, there are functioning circadian clock genes, and their expression is altered during viral infection.

Moreover, we observed that when diurnal changes were present, the acrophases of the *per1* and *per2* genes occurred either at the beginning of the light phase or at the end of the dark phase in fish kept under LD conditions (or at the same subjective times in fish kept under constant lighting). Similar findings were also described in the head kidney of goldfish (*Carassius auratus*) [41]. Furthermore, in all examined tissues,



(caption on next page)

Fig. 2. Diurnal changes in the expression of clock genes in the lymphoid-associated organs/tissues of fish kept under LD (12L:12D), DD (0L:24D) and LL (24L:0D) light regimes (n = 4–11). Gene expression was measured at different time points of the day. Data obtained from RT-qPCR analysis are shown as shown as the heatmap, where the colors indicate the normalized mean gene expression values (calculated as $z = (x-\mu)/\sigma$, where x is the mean gene expression value for the given group, μ is the mean of the dataset (the average of all the scores), and σ is the standard deviation) on the Heatmapper website [66]. Within each gene, all of the z-score values were compared and presented as different colors: blue indicates the lower than the mean values (down-regulation; $z < 0$), white indicates the mean values (no change; $z = 0$) and red indicates the higher than the mean values (up-regulation; $z > 0$). The *40S* ribosomal protein s11 gene served as the reference housekeeping gene. Significant differences (two-way ANOVA, $p < 0.05$) between time points within single light regime are indicated by different letters (e.g. A, B for LD; a, b for DD and α, β for LL) and differences between time points within different light regimes are indicated by different symbols (e.g. asterisk (*) differences between DD and LL vs LD regime, hash (#) difference between DD vs LL). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



B

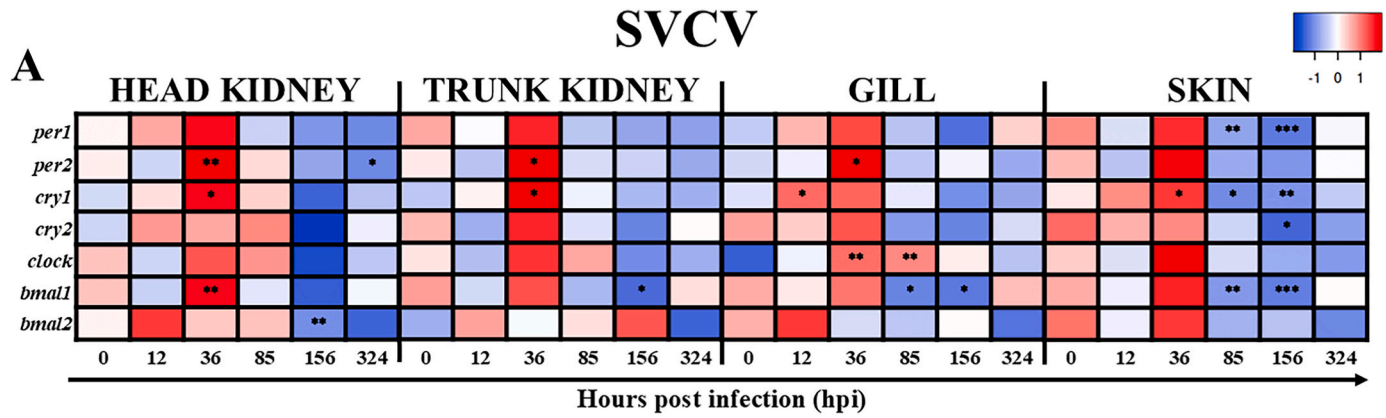
	HEAD KIDNEY		TRUNK KIDNEY		GILL		SKIN	
per1	p = 0.0059	r = -0.5792 (**)	p = 0.0171	r = -0.5263 (*)	p = 0.0305	r = -0.4842 (*)	p = 0.3931	r = -0.1826
per2	p = 0.0122	r = -0.5364 (*)	p = 0.1213	r = -0.3579	p = 0.0191	r = -0.5188 (*)	p = 0.1823	r = -0.2817
cry1	p = 0.0181	r = -0.5104 (*)	p = 0.0733	r = -0.4090	p = 0.0001	r = -0.7729 (****)	p = 0.0178	r = -0.4791 (*)
cry2	p = 0.0001	r = -0.7351 (***)	p = 0.0688	r = -0.4150	p = 0.0027	r = -0.6346 (**)	p = 0.0008	r = -0.6391 (***)
clock	p = 0.0009	r = -0.6701 (***)	p = 0.1247	r = -0.3549	p = 0.0160	r = -0.5308 (*)	p = 0.2046	r = -0.2747
bmal1	p = 0.0002	r = -0.7247 (***)	p = 0.0023	r = -0.6406 (**)	p = 0.0042	r = -0.6105 (**)	p = 0.0322	r = -0.4383 (*)
bmal2	p = 0.5985	r = -0.1331	p = 0.9999	r = 0.0000	p = 0.0408	r = -0.4861 (*)	p = 0.5144	r = -0.1549

Fig. 3. Infection-related changes in the expression of the clock genes in the head kidney, trunk kidney, gills and skin. Gene expression was measured at different time points of CyHV-3 infection (n = 5) and data obtained from RT-qPCR analysis are shown as the heatmap, where the colors indicate the normalized mean gene expression values (calculated as $z = (x-\mu)/\sigma$, where x is the mean gene expression value for the given group, μ is the mean of the dataset (the average of all the scores), and σ is the standard deviation) on the Heatmapper website [66]. Within each gene, all of the z-score values were compared and presented as different colors: blue indicates the lower than the mean values (down-regulation; $z < 0$), white indicates the mean values (no change; $z = 0$) and red indicates the higher than the mean values (up-regulation; $z > 0$). The *40S ribosomal protein s11* gene served as the reference housekeeping gene. When significant (ordinary one-way ANOVA with Dunnett's multiple comparison test, Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparison test or Kruskal-Wallis test, $p < 0.05$), differences between each time point and control uninfected fish (time point 0) within single examined tissue are indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$) (A). Correlation test parameters (p and r) measured between examined genes expression level and viral load using the correlation Spearman test ($p < 0.05$), when significant, the correlation is indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$), when the value of the r parameter is negative it indicates a negative correlation (B). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

there were no diurnal changes in the expression of both *per1* and *per2* genes in fish kept under constant darkness. These results align with our previous observations, with the exception of *per1* expression in the liver and *per2* expression in the brain and pituitary gland [23], indicating that light input is necessary for the proper functioning of these genes.

Interestingly, it was found that the expression pattern of *per2* in the head kidney, trunk kidney, thymus, spleen, and foregut of fish kept under constant lighting (with the highest expression at CT2) was similar to what we found in the liver of common carp [23]. This suggests that there may be a unified expression pattern across the peripheral clocks in common carp.

Furthermore, expression of *cry1* in the head kidney, trunk kidney, thymus and foregut of fish kept under LD conditions peaked at ZT2 similarly to our previous findings in the heart and liver [23]. Expression of *cry1aa* in the eyes of zebrafish, goldfish and Japanese medaka showed similar pattern of peak expression in the early morning hours [42]. Interestingly, *cry1* and *cry2* showed opposite expression patterns in the foregut of fish maintained under LD conditions, suggesting possible subfunctionalization of both *cry* genes. Study focusing on the expression pattern of *cry* genes in the gut of goldfish showed lack of circadian differences in expression for *cry1*, and peaks of expression of *cry2* and *cry3* genes in the middle of the light phase and at night, respectively [43]. It was also shown that *Cry* genes in mice are related to food anticipation activity and their knock-out disrupt and delay the development of food anticipation [44]. These findings together with our results may suggest that in fish, *cry* genes are interacting with food related processes, thus



B

	HEAD KIDNEY		TRUNK KIDNEY		GILL		SKIN	
per1	p = 0.0059	r = -0.5792 (**)	p = 0.0171	r = -0.5263 (*)	p = 0.0305	r = -0.4842 (*)	p = 0.3931	r = -0.1826
per2	p = 0.0122	r = -0.5364 (*)	p = 0.1213	r = -0.3579	p = 0.0191	r = -0.5188 (*)	p = 0.1823	r = -0.2817
cry1	p = 0.0181	r = -0.5104 (*)	p = 0.0733	r = -0.4090	p = 0.0001	r = -0.7729 (****)	p = 0.0178	r = -0.4791 (*)
cry2	p = 0.0001	r = -0.7351 (***)	p = 0.0688	r = -0.4150	p = 0.0027	r = -0.6346 (**)	p = 0.0008	r = -0.6391 (***)
clock	p = 0.0009	r = -0.6701 (***)	p = 0.1247	r = -0.3549	p = 0.0160	r = -0.5308 (*)	p = 0.2046	r = -0.2747
bmal1	p = 0.0002	r = -0.7247 (***)	p = 0.0023	r = -0.6406 (**)	p = 0.0042	r = -0.6105 (**)	p = 0.0322	r = -0.4383 (*)
bmal2	p = 0.5985	r = -0.1331	p = 0.9999	r = 0.0000	p = 0.0408	r = -0.4861 (*)	p = 0.5144	r = -0.1549

Fig. 4. Infection-related changes in the expression of the clock genes in the head kidney, trunk kidney, gills and skin. Gene expression was measured at different time points of SVCV infection and data obtained from RT-qPCR analysis are shown as the heatmap, where the colors indicate the normalized mean gene expression values (calculated as $z = (x-\mu)/\sigma$, where x is the mean gene expression value for the given group, μ is the mean of the dataset (the average of all the scores), and σ is the standard deviation) on the Heatmapper website [66]. Within each gene, all of the z-score values were compared and presented as different colors: blue indicates the lower than the mean values (down-regulation; $z < 0$), white indicates the mean values (no change; $z = 0$) and red indicates the higher than the mean values (up-regulation; $z > 0$). The *40S ribosomal protein s11* gene served as the reference housekeeping gene. When significant (ordinary one-way ANOVA with Dunnett's multiple comparison test, Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparison test or Kruskal-Wallis test, $p < 0.05$), differences between each time point and control uninfected fish (time point 0) within single examined tissue are indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) (A). Correlation test parameters (p and r) measured between examined genes expression level and viral load using the correlation Spearman test ($p < 0.05$), when significant, the correlation is indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$), when the value of the r parameter is negative it indicates a negative correlation (B). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the expression pattern of *cry1* and *cry2* genes in foregut of common carp was inverted.

In the head kidney, spleen and gut of fish kept under DD conditions, expression of *bmal1* and *clock* was similar. This might indicate that, similar to mammals [45] and zebrafish [46], these genes and their protein products share a role in regulating the circadian clock under constant darkness in the peripheral tissues of common carp. An interesting case in our study was the expression of the *clock* gene, which showed diurnal changes in all examined tissues only in fish kept under constant darkness (DD) conditions. Moreover, in our previous study, we found that the *clock* gene does not show diurnal changes in expression in the brain, pituitary gland, or retina, which are organs associated with the central oscillator of common carp [23]. These results suggest that the *clock* gene is a crucial element in the peripheral clock mechanism of common carp in a constant darkness environment.

The expression of *per1* and both *cry* genes in the gills was highest in fish kept under constant lighting. This may suggest that the expression of clock genes in the gills is directly stimulated by light, or that the gills themselves receive the light signal and are able to generate their own pattern of clock gene expression when exposed to light for long periods. It is known that in Atlantic salmon (*Salmo salar*), the smoltification process (the transition from freshwater to seawater) requires prolonged light exposure, achieved by keeping fish under constant light (LL) conditions, to allow the gills to restructure and adapt to the different environment [47]. Although common carp does not undergo smoltification, it is possible that gill sensitivity to light is conserved among many groups of fish.

We also studied expression of clock genes in PBLs. In fish kept under a standard light conditions (LD) there were no diurnal changes of expression of any clock genes in the PBLs unlike in fish kept under DD or LL conditions. The *Clock* gene expression in PBLs of rat [48] or horse [49] also did not show any diurnal changes in expression under LD conditions, however, in the case of rat, DD did not induce the expression change. These results may suggest that PBLs of common carp either generate clock genes rhythmicity themselves, or they are influenced by the circadian clock mechanism upstream, when disrupted lighting conditions occur.

Majority of the results from this study aligned with our previous study focused on the examination of clock gene expression patterns in the brain, pituitary gland, retina as well as in the heart and liver. For example changes in expression of *per1*, *per2* and *cry1* are similar between organs of the immune system and those of the central nervous system, heart and liver, showing a certain level of harmonization between them. Moreover, in both studies, change of the light regime diminishes the diurnal differences of gene expression observed in fish from LD regime. However, the expression profile of *bmal2* differed between nervous system (brain and pituitary gland) and retina and the peripheral organs, what may suggest that some of the peripheral clocks are able to generate a rhythm without involving the "central" clock located in the nervous system [23]. Taken all together, results from both studies show a general clock gene transcription pattern across plethora of various organs and tissues that provide an insight into the circadian clock function in

common carp.

Here, we also examined the interactions between ongoing viral infection and the circadian clock through analysis of the core clock genes expression in the head kidney, trunk kidney, gill and skin. We used two viruses (CyHV-3 and SVCV) which infect common carp and are responsible for high losses in carp aquaculture worldwide. CyHV-3 belongs to the genus *Cyprinivirus*, family *Alloherpesviridae*, order *Herpesvirales* and has linear, double-stranded DNA genome [50], while SVCV is a member of the genus *Spirivivirus*, family *Rhabdoviridae* and its genome is a negative single-stranded RNA [51]. Interestingly, in case of CyHV-3 skin is the major portal of entry for this virus [52]. We discovered that infection with studied viruses causes changes in the core clock genes expression in almost every examined tissue, except for the head kidney in case of CyHV-3 infection. Previous studies [39] found that in common carp both SVCV and CyHV-3 infection upregulates expression of antiviral genes (i*fn a2* and *vig1*). During SVCV infection increased expression of *ifn a2* was observed in all studied organs/tissues (skin, gills, trunk kidney and head kidney) between 85 and 156 hpi, while *vig1* expression was upregulated between 36 and 324 hpi. In case of CyHV-3, elevated expression of *vig1* was found in the skin (85–156 hpi), in the trunk kidney (12–324 hpi) and in the head kidney (36–156 hpi).

Links between viral diseases and circadian clock disruptions have been previously demonstrated in various vertebrates and cell lines. For example, it was found that in the human OR6 cell line, hepatitis C virus (HCV) infection can modulate clock gene machinery, and the circadian protein PER2 counteracts viral replication [53]. Similarly, Jin and colleagues [54] observed up-regulation of CLOCK, PER1, and PER2 in the human embryonic kidney cell line 293T (HEK293T) infected with Coxsackievirus A16 (CVA16) [54]. Furthermore, it has been shown that the course of the antiviral response varies depending on the time of day when the infection occurs. Mice infected with encephalitis virus exhibited significantly lower survival rates when the virus was administered at the end of the active phase [55]. In contrast, during murine herpes simplex virus 2 (HSV-2) infection, the severity was lower when the virus was injected during the rest phase compared to the active phase [56]. This experiment also demonstrated that Nectin1, the entry receptor used by HSV-2, is expressed in a circadian manner, and silencing CLOCK leads to a decrease in receptor expression [56]. Additionally, silencing Bmal1 in the epithelial lung cell line (Calu-3) or treating the cells with either an agonist of the transcription factor REV-ERBβ (a major regulator of the circadian clock) or a cryptochrome stabilizer (KL001) that inhibits BMAL1 activity resulted in strong inhibitory effects on SARS-CoV-2 virus entry and replication, as well as Angiotensin-converting enzyme 2 (ACE2) expression. Therefore, the authors hypothesized a potential role for the circadian clock in regulating SARS-CoV-2 infection and suggested the potential use of circadian-modifying agents in the prevention and/or treatment of COVID-19 [57].

Research focusing on the interaction between diseases and chronobiology in fish, especially in terms of gene expression, is limited. In zebrafish, the rhythmicity of *per3* was diminished by pro-inflammatory factors such as $CuSO_4$, which causes superficial organ inflammation, or dextran sodium sulfate (DSS), which causes gut inflammation. Conversely, it was enhanced by anti-inflammatory factors such as cyclosporin or the non-steroidal anti-inflammatory drug (NSAID) naproxen [58]. Furthermore, down-regulation of clock gene expression was observed in zebrafish infected with *Pseudoloma neurophilia*, a zebrafish microsporidium, likely due to the microsporidium evasion mechanisms [59]. In the skin of rainbow trout (*Oncorhynchus mykiss*) infected with *Argulus foliaceus*, clock gene expression rhythmicity was also altered, with a reduced amplitude of *clock3* and shifted phases of p*er1*, *cry1*, and *bmal1* [60]. Additionally, in Japanese flounder (*Paralichthys olivaceus*), *bmal1* expression was down-regulated after infection with the bacterium *Vibrio harveyi* [7,8].

Our previous study showed that Tilapia lake virus (TiLV) infection in adult zebrafish induced up-regulation of *arntl2* (*bmal2*) expression 6 days post-infection (dpi) in fish maintained under LD conditions, however we did not find the significant differences in the expression of clock genes between animals infected at ZT2 or ZT14 [24] therefore in the present study we decided to infect fish only at one time point. Taken together, these previously described cases and our observations demonstrate a clear interaction between infection and biological clock function in fish. This highlights the importance of maintaining proper environmental conditions and preventing infections in aquaculture, as disruption of one mechanism affects the other.

We observed that higher viral copy number is negatively correlated with clock genes expression. This might suggest that disruption of clock functioning observed upon viral infection can be a mechanism of pathogen-induced manipulation of host response. This is in accordance with studies of Edgar and colleagues [61], showing that infection with either murid herpesvirus 4 or herpes simplex virus 1 was enhanced in the *Bmal1*$^{-/-}$ mice mutants compared to WT mice. Similar results showing enhanced disease symptoms in *Bmal1*$^{-/-}$ mice were also observed in the case of respiratory syncytial virus [62], Sendai virus and Influenza A virus [63] infections.

However, we cannot exclude that disturbance of the expression of clock genes may improve response against a pathogen. Interestingly, studies of Curtis and co-workers [64] indicated that BMAL1 level in the macrophages may regulate the intensity of the immune response. They found that, BMAL1 in the myeloid lineage inhibits activation of NF-κB and proinflammatory microRNA (miR-155) and protects mice from LPS-induced sepsis. It has to be mentioned that Bmal1 has two miR-155–binding sites in its 3′-UTR, and, in response to LPS, miR-155 binds to these two target sites, leading to suppression of Bmal1 mRNA and protein. Moreover, they uncovered that miR-155 induction is greater in the evening than in the morning, providing one rationale as to why our immune response is more active at the transition into night. Previously, BMAL1 has also been shown to attenuate NF-κB activation by sequestering CLOCK, which is required for acetylation of p65, a key step for NF-κB transactivation [65] and downstream cytokine production.

This study focused on gene expression analysis; however, it is important to note that mRNA levels do not fully reflect the changes in the quantity and activity of clock proteins, primarily due to post-transcriptional regulation. Therefore, future research should aim to investigate the functions of the proteins encoded by clock genes. The topic of circadian clock mechanisms in fish warrants further study, as research on lower vertebrates may reveal many unknown interactions and mechanisms that are conserved through evolution, indicating their crucial role in the survival of higher organisms. Moreover, examining the chronobiology of fish and how it changes upon infections is essential for proper experiment planning and maintenance in aquaculture.

In conclusion, we observed that the expression of clock genes is present in the lymphoid organs, lymphoid-associated tissues, and peripheral blood leukocytes (PBLs) of common carp, and it changes throughout the day in a tissue-specific manner. Animals kept under different light regimes exhibited different clock gene expression patterns. Under constant darkness, the majority of the genes completely lost their expression patterns, while under constant lighting, the expression was affected, indicating which tissues may be more sensitive to light. Additionally, we found that both CyHV-3 and SVCV infections affect the expression of core clock genes in the organs of common carp. In most cases, the expression of clock genes was up-regulated in the early stages of infection and down-regulated later on. Furthermore, clock genes expression was shown to be negatively correlated with virus copy number, highlighting the interaction between infection and the circadian clock mechanism of the host.

Overall, this study demonstrates that the immune system and biological clock are interconnected and suggests that proper lighting conditions are crucial for the well-being and functionality of the circadian clock in fish. This consideration should always be taken into account both in experiments and especially in aquaculture.

CRediT authorship contribution statement

Mikolaj Mazur: Methodology, Formal analysis, Investigation, Visualization, Writing – review & editing. Lukasz Pijanowski: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Funding acquisition. Mikolaj Adamek: Investigation, Resources, Writing – review & editing. Marek Matras: Investigation, Krzysztof Rakus: Conceptualization, Methodology, Writing – review & editing. Magdalena Chadzinska: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Funding

This work was supported by the National Science Centre, Poland [Grant number 2018/31/D/NZ6/02321].

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2025.110525.

Data availability

Data will be made available on request.

References

[1] J. Aschoff, Desynchronization and resynchronization of human circadian rhythms, Aero. Med. 40 (1969) 844–849.
[2] V. Kumar, A. Sharma, Common features of circadian timekeeping in diverse organisms, Curr Opin Physiol 5 (2018) 58–67.
[3] J. Aschoff, S. Daan, K.I. Honma, Zeitgebers, entrainment, and masking: some unsettled questions, in: J. Aschoff, S. Daan, G.A. Groos (Eds.), Vertebrate Circadian Systems. Proceedings in Life Sciences, 1982.
[4] K.-A. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, M. Menaker, Entrainment of the circadian clock in the liver by feeding, Science 291 (2001) 490–493.
[5] C.L. Ehlers, Social zeitgebers and biological rhythms: a unified approach to understanding the etiology of depression, Arch. Gen. Psychiatry 45 (1988) 948.
[6] U. Albrecht, The mammalian circadian clock: a network of gene expression, Front. Biosci. 9 (2004) 48.
[7] P. Zhang, C. Yu, L. Sun, Japanese flounder (Paralichthys olivaceus) Bmal1 is involved in the regulation of inflammatory response and bacterial infection, Aquaculture 525 (2020) 735330.
[8] S. Zhang, M. Dai, X. Wang, S.-H. Jiang, L.-P. Hu, X.-L. Zhang, Z.-G. Zhang, Signalling entrains the peripheral circadian clock, Cell. Signal. 69 (2020) 109433.
[9] D. Whitmore, N.S. Foulkes, U. Strähle, P. Sassone-Corsi, Zebrafish clock rhythmic expression reveals independent peripheral circadian oscillators, Nat. Neurosci. 1 (1998) 701–707.
[10] M. Keller, J. Mazuch, U. Abraham, G.D. Eom, E.D. Herzog, H.-D. Volk, A. Kramer, B. Maier, A circadian clock in macrophages controls inflammatory immune responses, Proc. Natl. Acad. Sci. USA. 106 (2009) 21407–21412.
[11] C. Scheiermann, Y. Kunisaki, D. Lucas, A. Chow, J.-E. Jang, D. Zhang, D. Hashimoto, M. Merad, P.S. Frenette, Adrenergic nerves govern circadian leukocyte recruitment to tissues, Immunity 37 (2012) 290–301.
[12] K.D. Nguyen, S.J. Fentress, Y. Qiu, K. Yun, J.S. Cox, A. Chawla, Circadian gene Bmal1 regulates diurnal oscillations of Ly6Chi inflammatory monocytes, Science 341 (2013), https://doi.org/10.1126/science.1240636.
[13] N. Cermakian, D. Whitmore, N.S. Foulkes, P. Sassone-Corsi, Asynchronous oscillations of two zebrafish CLOCK partners reveal differential clock control and function, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4339–4344.
[14] H. Wang, Comparative analysis of teleost fish genomes reveals preservation of different ancient clock duplicates in different fishes, Mar. Genom. 1 (2008) 69–78.
[15] Y. Kobayashi, T. Ishikawa, J. Hirayama, H. Daiyasu, S. Kanai, H. Toh, I. Fukuda, T. Tsujimura, N. Terada, Y. Kamei, S. Yuba, S. Iwai, T. Todo, Molecular analysis of zebrafish photolyase/cryptochrome family: two types of cryptochromes present in zebrafish, Genes Cells 5 (2000) 725–738.
[16] H. Wang, Comparative analysis of period genes in teleost fish genomes, J. Mol. Evol. 67 (2008) 29–40.
[17] H. Wang, Comparative genomic analysis of teleost fish bmal genes, Genetica 136 (2009) 149–161.
[18] R.E. Sacksteder, J.M. Kimmey, Immunity, infection, and the zebrafish clock, Infect. Immun. 90 (2022) e00588, 21.
[19] H. Underwood, The Pineal and Melatonin: Regulators of Circadian Function in Lower Vertebrates, 1990.
[20] D. Whitmore, N.S. Foulkes, P. Sassone-Corsi, Light acts directly on organs and cells in culture to set the vertebrate circadian clock, Nature 404 (2000) 87–91.
[21] T.K. Tamai, L.C. Young, D. Whitmore, Light signaling to the zebrafish circadian clock by cryptochrome 1a, Proc. Natl. Acad. Sci. USA 104 (2007) 14712–14717.
[22] G. Vatine, D. Vallone, L. Appelbaum, P. Mracek, Z. Ben-Moshe, K. Lahiri, Y. Gothilf, N.S. Foulkes, Light directs zebrafish period2 expression via conserved D and E boxes, PLoS Biol. 7 (2009) e1000223.
[23] M. Mazur, M. Markowska, M. Chadzinska, L. Pijanowski, Changes of the clock gene expression in central and peripheral organs of common carp exposed to constant lighting conditions, Chronobiol. Int. 40 (2023) 145–161.
[24] M. Mazur, K. Rakus, M. Adamek, W. Surachetpong, M. Chadzinska, L. Pijanowski, Effects of light and circadian clock on the antiviral immune response in zebrafish, Fish Shellfish Immunol. 140 (2023) 108979.
[25] J.E. Gibbs, J. Blaikley, S. Beesley, L. Matthews, K.D. Simpson, S.H. Boyce, S. N. Farrow, K.J. Else, D. Singh, D.W. Ray, A.S.I. Loudon, The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines, Proc. Natl. Acad. Sci. USA 109 (2012) 582–587.
[26] A.C. Silver, A. Arjona, W.E. Walker, E. Fikrig, The circadian clock controls toll-like receptor 9-Mediated innate and adaptive immunity, Immunity 36 (2012) 251–261.
[27] K. Ella, R. Csépányi-Kömi, K. Káldi, Circadian regulation of human peripheral neutrophils, Brain Behav. Immun. 57 (2016) 209–221.
[28] W. He, S. Holtkamp, S.M. Hergenhan, K. Kraus, A. De Juan, J. Weber, P. Bradfield, J.M.P. Grenier, J. Pelletier, D. Druzd, C.-S. Chen, L.M. Ince, S. Bierschenk, R. Pick, M. Sperandio, M. Aurrand-Lions, C. Scheiermann, Circadian expression of migratory factors establishes lineage-specific signatures that guide the homing of leukocyte subsets to tissues, Immunity 49 (2018) 1175–1190.e7.
[29] J.M. Adrover, A. Aroca-Crevillén, G. Crainiciuc, F. Ostos, Y. Rojas-Vega, A. Rubio-Ponce, C. Cilloniz, E. Bonzón-Kulichenko, E. Calvo, D. Rico, M.A. Moro, C. Weber, I. Lizasoaín, A. Torres, J. Ruiz-Cabello, J. Vázquez, A. Hidalgo, Programmed 'disarming' of the neutrophil proteome reduces the magnitude of inflammation, Nat. Immunol. 21 (2020) 135–144.
[30] M. Amir, S. Campbell, T.M. Kamenecka, L.A. Solt, Pharmacological modulation and genetic deletion of REV-ERBα and REV-ERBβ regulates dendritic cell development, Biochem. Biophys. Res. Commun. 527 (2020) 1000–1007.
[31] T. Bollinger, A. Bollinger, L. Skrum, S. Dimitrov, T. Lange, W. Solbach, Sleep-dependent activity of T cells and regulatory T cells, Clin. Exp. Immunol. 155 (2009) 231–238.
[32] D. Druzd, O. Matveeva, L. Ince, U. Harrison, W. He, C. Schmal, H. Herzel, A. H. Tsang, N. Kawakami, A. Leliavski, O. Uhl, L. Yao, L.E. Sander, C.-S. Chen, K. Kraus, A. De Juan, S.M. Hergenhan, M. Ehlers, B. Koletzko, R. Haas, W. Solbach, H. Oster, C. Scheiermann, Lymphocyte circadian clocks control lymph node trafficking and adaptive immune responses, Immunity 46 (2017) 120–132.
[33] Q. Cao, X. Zhao, J. Bai, S. Gery, H. Sun, D.-C. Lin, Q. Chen, Z. Chen, L. Mack, H. Yang, R. Deng, X. Shi, L.-W. Chong, H. Cho, J. Xie, Q.-Z. Li, M. Müschen, A. R. Atkins, C. Liddle, R.T. Yu, S. Alkan, J.W. Said, Y. Zheng, M. Downes, R.M. Evans, H.P. Koeffler, Circadian clock cryptochrome proteins regulate autoimmunity, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 12548–12553.
[34] P. Bourin, I. Mansour, C. Doinel, R. Roue, P. Rouger, F. Levi, Circadian rhythms of circulating NK cells in healthy and human immunodeficiency virus-infected men, Chronobiol. Int. 10 (1993) 298–305.
[35] R.W. Logan, O. Wynne, D. Levitt, D. Price, D.K. Sarkar, Altered circadian expression of cytokines and cytolytic factors in splenic natural killer cells of Per1−/− mutant mice, J. Interferon Cytokine Res. 33 (2013) 108–114.
[36] J.E. Kaplan, R.D. Chrenek, J.G. Morash, C.M. Ruksznis, L.G. Hannum, Rhythmic patterns in phagocytosis and the production of reactive oxygen species by zebrafish leukocytes, Comp. Biochem. Physiol. Mol. Integr. Physiol. 151 (2008) 726–730.
[37] C.C. Lazado, P.V. Skov, P.B. Pedersen, Innate immune defenses exhibit circadian rhythmicity and differential temporal sensitivity to a bacterial endotoxin in nile tilapia (Oreochromis niloticus), Fish Shellfish Immunol. 55 (2016) 613–622.
[38] G. Taira, T. Onoue, J. Hikima, M. Sakai, T. Kono, Circadian clock components Bmal1 and Clock1 regulate tlr9 gene expression in the Japanese medaka (Oryzias latipes), Fish Shellfish Immunol. 105 (2020) 438–445.
[39] M. Adamek, M. Matras, A. Dawson, V. Piackova, D. Gela, M. Kocour, J. Adamek, R. Kaminski, K. Rakus, S.M. Bergmann, M. Stachnik, M. Reichert, D. Steinhagen, Type I interferon responses of common carp strains with different levels of resistance to koi herpesvirus disease during infection with CyHV-3 or SVCV, Fish Shellfish Immunol. 87 (2019) 809–819.
[40] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT–PCR, Nucleic Acids Res. 29 (2001) e45.
[41] M. Gómez-Boronat, N. Sáiz, M.J. Delgado, N. De Pedro, E. Isorna, Time-lag in feeding schedule acts as a stressor that alters circadian oscillators in goldfish, Front. Physiol. 9 (2018) 1749.
[42] M. Nakagawa, K. Okano, Y. Saratani, Y. Shoji, T. Okano, Midnight/midday-synchronized expression of cryptochrome genes in the eyes of three teleost species, zebrafish, goldfish, and medaka, Zoological Lett 8 (2022) 8.
[43] E. Velarde, R. Haque, P.M. Iuvone, C. Azpeleta, A.L. Alonso-Gómez, M.J. Delgado, Circadian clock genes of goldfish, *Carassius auratus* : cDNA cloning and rhythmic expression of *period* and *cryptochrome* transcripts in retina, liver, and gut, J. Biol. Rhythm. 24 (2009) 104–113.

[44] M. Iijima, S. Yamaguchi, G.T.J. Van Der Horst, X. Bonnefont, H. Okamura, S. Shibata, Altered food-anticipatory activity rhythm in Cryptochrome-deficient mice, Neurosci. Res. 52 (2005) 166–173.
[45] C. Lee, J.-P. Etchegaray, F.R.A. Cagampang, A.S.I. Loudon, S.M. Reppert, Posttranslational mechanisms regulate the mammalian circadian clock, Cell 107 (2001) 855–867.
[46] T. Ishikawa, J. Hirayama, Y. Kobayashi, T. Todo, Zebrafish CRY represses transcription mediated by CLOCK-BMAL heterodimer without inhibiting its binding to DNA, Genes Cells 7 (2002) 1073–1086.
[47] M. Iversen, T. Mulugeta, A.C. West, E.H. Jørgensen, S.A.M. Martin, S.R. Sandve, D. Hazlerigg, Photoperiod-dependent developmental reprogramming of the transcriptional response to seawater entry in Atlantic salmon (*Salmo salar*), G3 Genes|Genomes|Genetics 11 (2021) jkab072.
[48] Y. Du, S. Fan, Q. Meng, G. Wang, J. Tong, Circadian expression of clock and screening of clock-controlled genes in peripheral lymphocytes of rat, Biochem. Biophys. Res. Commun. 336 (2005) 1069–1073.
[49] C. Giannetto, F. Fazio, D. Alberghina, E. Giudice, G. Piccione, Clock genes expression in peripheral leukocytes and plasma melatonin daily rhythm in horses, J. Equine Vet. Sci. 84 (2020) 102856.
[50] A.J. Davison, T. Kurobe, D. Gatherer, C. Cunningham, I. Korf, H. Fukuda, R. P. Hedrick, T.B. Waltzek, Comparative genomics of carp herpesviruses, J. Virol. 87 (2013) 2908–2922.
[51] U. Ashraf, Y. Lu, L. Lin, J. Yuan, M. Wang, X. Liu, Spring viraemia of carp virus: recent advances, J. Gen. Virol. 97 (2016) 1037–1051.
[52] B. Costes, V.S. Raj, B. Michel, G. Fournier, M. Thirion, L. Gillet, J. Mast, F. Lieffrig, M. Bremont, A. Vanderplasschen, The major portal of entry of koi herpesvirus in *Cyprinus carpio* is the skin, J. Virol. 83 (2009) 2819–2830.
[53] G. Benegiamo, G. Mazzoccoli, F. Cappello, F. Rappa, N. Scibetta, J. Oben, A. Greco, R. Williams, A. Andriulli, M. Vinciguerra, V. Pazienza, Mutual antagonism between circadian protein period 2 and hepatitis C virus replication in hepatocytes, PLoS One 8 (2013) e60527.
[54] J. Jin, R. Li, C. Jiang, R. Zhang, X. Ge, F. Liang, X. Sheng, W. Dai, M. Chen, J. Wu, J. Xiao, W. Su, Transcriptome analysis reveals dynamic changes in coxsackievirus A16 infected HEK 293T cells, BMC Genom. 18 (2017) 933.
[55] K. Gagnidze, K.H. Hajdarovic, M. Moskalenko, I.N. Karatsoreos, B.S. McEwen, K. Bulloch, Nuclear receptor REV-ERBα mediates circadian sensitivity to mortality in murine vesicular stomatitis virus-induced encephalitis, Proc. Natl. Acad. Sci. USA 113 (2016) 5730–5735.
[56] T. Matsuzawa, Y. Nakamura, Y. Ogawa, K. Ishimaru, F. Goshima, S. Shimada, A. Nakao, T. Kawamura, Differential day-night outcome to HSV-2 cutaneous infection, J. Invest. Dermatol. 138 (2018) 233–236.
[57] X. Zhuang, S. Tsukuda, F. Wrensch, P.A.C. Wing, M. Schilling, J.M. Harris, H. Borrmann, S.B. Morgan, J.L. Cane, L. Mailly, N. Thakur, C. Conceicao, H. Sanghani, L. Heydmann, C. Bach, A. Ashton, S. Walsh, T.K. Tan, L. Schimanski, K.-Y.A. Huang, C. Schuster, K. Watashi, T.S.C. Hinks, A. Jagannath, S. R. Vausdevan, D. Bailey, T.F. Baumert, J.A. McKeating, The circadian clock component BMAL1 regulates SARS-CoV-2 entry and replication in lung epithelial cells, iScience 24 (2021) 103144.
[58] E.A. Mosser, C.N. Chiu, T.K. Tamai, T. Hirota, S. Li, M. Hui, A. Wang, C. Singh, A. Giovanni, S.A. Kay, D.A. Prober, Identification of pathways that regulate circadian rhythms using a larval zebrafish small molecule screen, Sci. Rep. 9 (2019) 12405.
[59] H.L.E. Midttun, M.A. Vindas, P.J. Whatmore, Ø. Øverli, I.B. Johansen, Effects of *Pseudoloma neurophilia* infection on the brain transcriptome in zebrafish (*Danio rerio*), J. Fish. Dis. 43 (2020) 863–875.
[60] A.R. Ellison, D. Wilcockson, J. Cable, Circadian dynamics of the teleost skin immune-microbiome interface, Microbiome 9 (2021) 222.
[61] R.S. Edgar, A. Stangherlin, A.D. Nagy, M.P. Nicoll, S. Efstathiou, J.S. O'Neill, A. B. Reddy, Cell autonomous regulation of herpes and influenza virus infection by the circadian clock, Proc. Natl. Acad. Sci. USA 113 (2016) 10085–10090.
[62] T. Majumdar, J. Dhar, S. Patel, R. Kondratov, S. Barik, Circadian transcription factor BMAL1 regulates innate immunity against select RNA viruses, Innate Immun. 23 (2017) 147–154.
[63] A. Ehlers, W. Xie, E. Agapov, S. Brown, D. Steinberg, R. Tidwell, G. Sajol, R. Schutz, R. Weaver, H. Yu, M. Castro, L.B. Bacharier, X. Wang, M.J. Holtzman, J.A. Haspel, BMAL1 links the circadian clock to viral airway pathology and asthma phenotypes, Mucosal Immunol. 11 (2018) 97–111.
[64] A.M. Curtis, C.T. Fagundes, G. Yang, E.M. Palsson-McDermott, P. Wochal, A. F. McGettrick, N.H. Foley, J.O. Early, L. Chen, H. Zhang, C. Xue, S.S. Geiger, K. Hokamp, M.P. Reilly, A.N. Coogan, E. Vigorito, G.A. FitzGerald, L.A.J. O'Neill, Circadian control of innate immunity in macrophages by miR-155 targeting *Bmal1*, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 7231–7236.
[65] M.L. Spengler, K.K. Kuropatwinski, M. Comas, A.V. Gasparian, N. Fedtsova, A. S. Gleiberman, I.I. Gitlin, N.M. Artemicheva, K.A. Deluca, A.V. Gudkov, M. P. Antoch, Core circadian protein CLOCK is a positive regulator of NF-κB–mediated transcription, Proc. Natl. Acad. Sci. USA 109 (2012).
[66] S. Babicki, D. Arndt, A. Marcu, Y. Liang, J.R. Grant, A. Maciejewski, D.S. Wishart, Heatmapper: web-enabled heat mapping for all, Nucleic Acids Res. 44 (2016) W147–W153.