Introduction

Fish are universally acknowledged as a healthy food and are the major source in our diet of the essential and highly beneficial omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) [1]. With stagnating or diminishing wild capture fisheries, aquaculture now produces around half the global supply of fish and seafood for human consumption [2]. European aquaculture is dominated by carnivorous species whose natural diets are dominated by protein and lipid with the latter being the prime energy source [3]. Therefore, fish lipid nutrition and metabolism is crucially important issues in aquaculture and, two of the most important issues are the level and source of dietary lipid [4]. Traditionally, dietary lipid was supplied by fish oil, but this is a finite commodity and global supplies are at their sustainable limit and cannot support continued development [5]. As a result, vegetable oils devoid of both omega-3 LC-PUFA and cholesterol are replacing dietary fish oil with potential consequences for both fish health and nutritional quality of the product [6]. Developing a better understanding of the molecular mechanisms controlling lipid metabolism in farmed fish such as Atlantic salmon (Salmo salar) will greatly improve the efficiency and sustainability of aquaculture [7].
to influence the positive arm of the molecular clock through repression of the BMAL1 transcription factor. REV-ERB 1α is not essential for the cycling of the molecular clock; however it is fundamental in the accuracy and fine-tuning of the clock and has been implicated in adiposity and fatty acid metabolism [10].

The regulation of genes involved in cholesterol and lipid homeostasis by REV-ERB 1α is mediated through sterol regulatory element binding protein (SREBP) pathways and SREBP target genes [10] that include genes of lipid and fatty acid metabolism (SREBP1) or cholesterol biosynthesis (SREBP2) [16]. Srebp1 transcription can also be activated through the LXRE response element by liver X receptor (LXR), a nuclear receptor that regulates the metabolism of several important lipids, including cholesterol and bile acids [17]. Other transcription factors that are direct targets of the daily clock in mammals are the peroxisome proliferator-activated receptor-α (PPARα) and PPARγ, which are known to regulate lipid metabolism and energy homeostasis by coordinated actions in a variety of tissues [18].

The cDNAs of transcription factors key to lipid, fatty acid and cholesterol metabolism including Srebps, Pparα and Lxr have been cloned and studied in salmon [19,20,21]. Similarly, cDNAs for various clock genes including Clock, Per1, Per2 and Bmal1 have been isolated in salmonids [22,23,24]. However, whereas the circadian regulation of lipid and cholesterol metabolism and the genes and enzymes involved has been shown in rodents [10], nothing was known of this regulation in teleost fish. The primary aim of the present study was to investigate the relationships between the daily expression of key components of the daily clock and genes of lipid metabolism including the transcription factors and their target genes in salmon liver. As REV-ERB 1α had been shown in rodents to be a critical factor in daily regulation of SREBP pathways [10], a further aim was to clone the Rev-erb 1α cDNA and determine its pattern of expression in a 24 h cycle in salmon.

Materials and Methods

Experimental animals and sampling procedures

Stock Atlantic salmon parr (100 fish; mean 24.9 ± 5.4 g, 14.2 ± 0.8 cm) were maintained in a single 1 m³ (1000 L) tank at the Niall Bromage Freshwater Research Facilities (Institute of Aquaculture, Stirling, UK). The fish were acclimated to a long day photoperiod cycle (LD 16 h light: 08 h dark) in early March when water temperature was on average 4.6 ± 0.7 °C. Feed, EWOS micro parr diet (EWOS Ltd., Bathgate, UK), was offered continuously in excess, during the day and night via a clockwork belt feeder. After 1 month, liver tissue samples were collected from 6 individuals every 4 h over a 24 h period. Briefly, experimental animals were sacrificed under lethal anaesthesia and decapitation. Feeding activity in all animals sampled was confirmed by video recording.

RNA extraction and cDNA synthesis

Approximately 100 mg of liver tissue was homogenised in 1 ml of TRIzol and RNA extracted according to manufacturer’s instructions (Invitrogen, Life Technologies Ltd., Paisley, UK). The resulting RNA pellets were resuspended in MilliQ water to a final RNA concentration of approximately 100 ng/μl. Total RNA concentration was determined by ND-1000 Nanodrop spectrophotometer (Labtech Int., East Sussex, UK). In order to eliminate DNA contamination 5 μg of total RNA was treated with DNase I enzyme following DNA-free kit guidelines (Applied Biosystems, Warrington, UK). cDNA was synthesized using 1 μg of DNase-treated RNA and random primers in 20 μl reactions and the High capacity reverse transcription kit without RNase inhibitor according to the manufacturer’s protocol (Applied Biosystems). Final reactions were diluted with DNA/RNA-free water to a final volume of 200 μl. All cDNA samples were stored at −20°C until use in qPCR.

Identification and cloning of Atlantic salmon Rev-erb 1α

Salmo salar Rev-erb 1α was cloned as follows: two Atlantic salmon expressed sequence tag clones (Genbank ID: DY724083 and DY731913) were identified by BLAST analysis of published vertebrate Rev-erb 1α sequences. 5‘ and 3‘ ends from the constructed contig were amplified using Rapid Amplification of cDNA Ends (RACE)-PCR with the RACE cDNAs generated from 1 μg of salmon whole brain total RNA using the SMART RACE kit as described in the user manual (Clontech, Mountain View, CA). The 5′ and 3‘ RACE amplicons were generated by two rounds of PCR using Rev-erb 1α 1 5’R1 and Rev-erb 1α 2 5’R2 primers or Rev-erb 1α 2 3’F1 and Rev-erb 1α 2 3’F2, respectively (Table S1). The final full-length sequence was confirmed by two rounds of PCR using nested primers designed to amplify end-to-end full-length cDNAs (REV-ERB 1α_full_F1: REV-ERB 1α_full_R1 and REV-ERB 1α_full_F2: REV-ERB 1α_full_R2). All PCRs were run at annealing temperatures as listed in Table S1 with an extension time of 1 min/Kb of predicted PCR product, with 3 min applied for unpredictable RACE PCR products. All primers were designed using Primer Select Ver. 6.1 program (DNASTAR, www.dnastar.com). Sequencing was performed using a Beckman 8800 autosequencer and Lasergene SEQman software (DNASTAR) used to edit and assemble DNA sequences.

Quantitative real-time PCR (qPCR)

With the exception of Rev-erb 1α, all qPCR assays were established and verified previously [21,22,25]. In order to determine diel patterns of gene expression, qPCR was carried out on clock genes Bmal1, Clock, Per1, Per2 and Rev-erb 1α; lipid-associated transcription factor genes, Srebp1, Srebp2, Lxr, Pparα and Pparγ; and key genes involved in major lipid pathways including fatty acid synthesis (Fas, D6fad, D5fad, Elov5 and Elov12) and catabolism (Cpt1 and Aco), cholesterol metabolism (Hmgcr, Mv, Hsd17b, Ip1, Acal1 and Cyp11a2) and lipoprotein metabolism (Apoa1, Apob, AposIId, Ldli, El, Lplb, Lpb and Lplc) (Table S2). qPCR primer sequences and annealing temperatures are described in Table S3. All samples were run in duplicate and assays were performed as follows, 95°C for 15 min and 45 cycles of 95°C for 15 s, anneal for 15 s and 72°C for 30 s. This was followed by a temperature ramp from 70–90°C for melt-curve analysis to verify that no primer–dimer artefacts were present and only one product was generated from each qPCR assay. Quantification was achieved by a parallel set of reactions containing standards consisting of serial dilution of spectrophotometrically-determined, linearised plasmid containing partial cDNA sequences.

qPCR normalisation and statistical analysis

GeNorm analysis was carried out on three potential housekeeping genes over the long day liver diel profile to determine the
most stable and appropriate reference gene for this tissue. Of the three genes studied (β-actin, EF-a and GAPDH) analysis highlighted elongation factor a (EF-a) as the most appropriate housekeeping gene with the greatest stability (M value = 0.9). Analysis of Variance (ANOVA) was used to determine significant effects of time and Tukey’s test was used to determine the significance of differences between sample time points and mean of different sample sets (InStat 3.1, Graphpad software inc.). Data were then fitted to a cosine wave in order to determine the presence of a significant daily rhythm. Raw data was analysed using Acro circadian analysis programs (University of South Carolina, USA; http://www.circadian.org/softwar.html). Acro analysis determines both the significance, acrophase (peak in expression) mean and amplitude of raw data using the equation:

\[ Y = A + B \cos(C \times X - D) \]

where Y is level of gene expression as a percentage of the mean, A is the baseline, C is the frequency multiplier (set to fixed period of 24 h), and D is the acrophase (peak time of the cosine approximation) of the data set [22]. A significant daily rhythm was deemed present when \( p \) value was less than 0.05 for all statistical analysis. All results are presented as % of mean expression whereby each normalized copy number value was converted to a % of the average total copy number of the gene of interest. Data were then presented as mean \( \pm \) SEM in relation to zeitgeber time (ZT) whereby ZT 00:00 occurred at lights on and lights off was at ZT 16:00.

Results

Salmon REV-ERB 1α sequence and phylogenetics

A 2987 bp full-length cDNA sequence (Accession number: 1714461) was obtained by several runs of 5′ and 3′ RACE PCR. This contained an open reading frame (ORF) of 1824 bp encoding a putative protein of 608 amino acids and 3′ and 5′ untranslated regions (UTR) of 349 bp and an 814 bp, respectively. Atlantic salmon putative REV-ERB 1α possessed a ligand binding domain, and the predicted DNA binding domain composed of two C4-type zinc fingers, each one containing a group of four coordinating cysteine residues, as well as one crystal form similar to human 1HLZ-A [26] (Fig. S1). Alignment and phylogenetic analysis of the deduced amino acid sequence of salmon Rev-erb 1α in relation to other previously characterised vertebrate Rev-erb 1α and Rev-erb 1β sequences showed clustering in two different clades containing mammalian REV-ERB 1α and β (Fig. S2). Homologous proteins exist in other fish species such as tilapia (Oreochromis niloticus) and fugu (Takifugu rubripes), with the Atlantic salmon protein displaying 57% and 71% identities with human and zebrafish (Danio rerio) Rev-erb 1α sequences, respectively.

Clock gene expression in liver

Of the five clock genes investigated all were expressed in the liver, but only Bmal 1 displayed a significant daily pattern of expression when results were fitted to a cosine wave using Acro analysis (Fig. 1; Table 1) [27]. The acrophase of Bmal1 occurred at 3 h prior to lights off at ZT 13:00 ± 3.9.

Transcription factor gene expression

Of the studied metabolism-related genes, those belonging to the transcription factors presented the most consistent rhythmicity. Thus, four of the five studied transcription factor genes presented 24 h rhythmic expression patterns in the liver of LD Atlantic salmon (Fig. 2; Table 1). Lxr, Srebpl, Ppara and Pparγ exhibited significant rhythmic variations in mRNA expression, whereas Srebpl2 did not show such a tendency (Fig. 2). However, for Lxr unlike Srebpl, Ppara and Pparγ while expression was rhythmic there were no significant temporal differences in expression levels.
The acrophase of expression cycles was comparable for Lxr, Srebp1 and Ppar 
but 4 h advanced for Pparc (Table 1).

Lipid and cholesterol homeostasis gene expression

The daily expression profiles of the cholesterol metabolism genes showed that only Ipi and Hmgcr displayed a significant daily rhythm, showing peak expression around ZT 09:00, seven hours before the dark phase (Fig. 3; Table 1). Whilst Dhcr7 did not display a significant daily rhythm, significant differences in expression between time points was observed, with maximal transcript abundance at ZT 22:00, at the end of the dark phase (Fig. 3). None of the genes involved in fatty acid synthesis or catabolism displayed a significant daily profile of expression, or any significant differences among the time points (Fig. 4; Table 1). Of the lipoprotein metabolism genes, ApoCII and EI followed a rhythmic expression, reaching their acrophase at ZT 10:00 and 22:00 respectively, although there were no significant temporal differences in expression levels (Fig. 5).

Discussion

While in mammals the interaction of the clock gene mechanism and liver transcriptome had been established, the importance of daily regulation on diverse metabolic processes in fish remained to be determined. The results of the present study demonstrated, for the first time, daily regulation of specific genes of lipid metabolism and homeostasis in liver of Atlantic salmon, and provided an insight into the molecular control mechanisms involved.

The molecular clock mechanism is based on an autoregulatory feedback loop that takes approximately 24 h to complete and synchronises a multitude of molecular, physiological and behavioural processes to the daily 24 h cycle [8]. As in other tissue types, the hepatic oscillator is believed to be centred on two transcriptional-translational activators, BMAL1 and Clock, and two classes of repressors, Period and Cryptochrome [15]. In the present study the expression of four core clock genes, Bmal1, Clock, Per 1 and Per 2 was characterised, with Bmal1 the only one to display a

| Table 1. P value of 24 h profiles of gene expression Acro and ANOVA analysis and acrophase where significant rhythm is present. |
|---------------------------------------------------------------|
| **Gene**          | **Acro (p value)** | **Acrophase ZT** | **ANOVA (p value)** |
| Bmal1             | <0.05             | 13:00 ± 3.09    | <0.05               |
| Clock             | n.s.              | -               | <0.05               |
| Per 1             | n.s.              | -               | n.s.                |
| Per 2             | n.s.              | -               | <0.05               |
| Rev-erb 1a        | n.s.              | -               | n.s.                |
| Lxr               | <0.05             | 13:00 ± 2.73    | n.s.                |
| Srebp1            | <0.05             | 13:00 ± 2.41    | <0.05               |
| Srebp 2           | n.s.              | -               | n.s.                |
| Pparα             | <0.05             | 13:00 ± 2.26    | <0.05               |
| Pparγ             | <0.05             | 9:00 ± 2.33     | <0.05               |
| Hmgcr             | <0.05             | 9:00 ± 3.12     | <0.05               |
| Mev               | n.s.              | -               | n.s.                |
| Ipi               | <0.05             | 9:00 ± 2.57     | n.s.                |
| Dhcr7             | n.s.              | -               | <0.05               |
| Abca1             | n.s.              | -               | n.s.                |
| Cyp71a            | n.s.              | -               | n.s.                |
| DSFad             | n.s.              | -               | n.s.                |
| D6Fad             | n.s.              | -               | n.s.                |
| Elovil2           | n.s.              | -               | n.s.                |
| Elovil5a          | n.s.              | -               | n.s.                |
| Fas               | n.s.              | -               | n.s.                |
| Aco               | n.s.              | -               | n.s.                |
| Cpt1              | n.s.              | -               | n.s.                |
| ApoA1             | n.s.              | -               | n.s.                |
| ApoB              | n.s.              | -               | n.s.                |
| ApoCII            | <0.05             | 9:00 ± 2.97     | n.s.                |
| Ldh               | n.s.              | -               | n.s.                |
| El                | <0.05             | 21:00 ± 3.23    | n.s.                |
| Lplb              | n.s.              | -               | n.s.                |
| Lplc              | n.s.              | -               | n.s.                |
| n.s. denotes no statistical differences between the different sampling points. Acrophases (circadian peak times) were calculated by non-linear regression fit of a cosine function. Data are expressed as acrophase ± 95% confidence intervals. doi:10.1371/journal.pone.0106739.t001 |
significant daily profile of expression. Such expression was previously observed in zebrafish and gilthead sea bream (Sparus aurata) liver [28,29] and the salmonid brain [22,30]. In addition to its role in the control of daily rhythm, BMAL 1 has been suggested to contribute to lipid metabolism control and adipogenesis in mammals [31]. It has also been shown to regulate...
rhythmic gene expression throughout the liver transcriptome [32,33]. However the daily profile of Bmal1 expression identified in this study, particularly in the absence of additional rhythmic expression of other clock genes, may indicate an additional function of Bmal1 in the liver of the Atlantic salmon. Interestingly Bmal1 itself can be regulated by elements of lipid metabolism pathways. Specifically PPARγ has been shown to control Bmal1 expression in the liver via direct binding to a PPRE located in the Bmal1 promoter [34,35]. With regard to the present investigation, mRNA expression of Bmal1 and Pparγ in the liver appeared to be in phase with their acrophases occurring at 13:00±3.09 and 13:00±2.73, respectively, indicating that expression and regulation of these genes may be related as in mammals. In addition, Bmal1 is a direct target of vascular Pparγ [36] although its role in the regulation of the clock gene Bmal1 is unknown in the liver.

In contrast to previous studies on teleost liver [28,29,37,38], Clock, Per1 and Per2 did not display oscillatory patterns in a 24 h cycle, however, the results for Clock and Per2 are consistent with those previously obtained in the brain of Atlantic salmon [26]. While in the teleost brain light dark cycle is considered to be the primary zeitgeber [39], hepatic clocks appear to demonstrate a greater variety in entrainment pathways [32]. Both light as well as food availability can act as a potential entraining signal [29,40]. Equally in mammals it has been demonstrated that the phase of the daily clock of the liver can be altered by feeding time, decoupling it from the unaltered rhythm of the suprachiasmatic nucleus (SCN), which is the central daily pace maker in mammals [41]. Due to the liver being fundamental to the metabolism and overall health and welfare of an organism it is hypothesised that in both mammals and teleosts the hepatic clock can be decoupled from central clock mechanisms and non-photic entrainment allows adaptation to immediate environmental changes such as food availability [29,32].

With regard to the temporal regulation and expression of the liver lipid metabolism it was necessary to determine if Rev-erb 1α, a key connection between the molecular clock and liver lipid metabolism in mammals, played a similar role in Atlantic salmon. Alignment and phylogenetic analysis showed that the sequence identified displayed considerable similarity to other vertebrate Rev-erb 1α sequences, and presented homologous predicted structural components with human REV-ERB 1α protein [26]. In contrast to mammals, analyses of Rev-erb 1α expression over the 24 h period revealed no temporal differences in levels of mRNA expression in the salmon liver. This finding does not
discount regulation of protein as opposed to transcript level or the potential presence of additional copies or homologs of Rev-erb 1x. Salmonids have undergone two further genome duplication events in comparison to mammals [42] yet the relevance of this to the clock system is unknown.

One of the most interesting findings of the present study was the strong rhythmicity displayed by the transcription factors. Of the 31 genes examined in the present study, 9 followed a rhythmic pattern in Atlantic salmon liver, with transcription factors representing the most consistent group (four out of five genes). Although precisely how the circadian clock acts to control metabolic rhythms is still unclear, for instance, Rev-erb 1x was shown to regulate the expression of Srebps and its target genes [10]. However, rhythmic expression of Srebpl1 was observed in the salmon liver despite the lack of rhythmic expression of Rev-erb 1x, which may infer protein level or non-Rev-erb 1x regulation of the pathway. Although Srebpl1 target genes did not similarly display significant rhythmic expression, the peak expression of Srebpl1 at ZT 14:00 was coincidental with higher expression of several genes including D6 Fad, Elovl5a, Fas, Cpt1 and Aco, all known to be target genes of Srebpl1 in mammals [43].

The PPARs family members are known to regulate lipid metabolism and energy homeostasis in several teleost species [20,44,45]. However, in fish it was not as clear whether Ppar expression showed rhythmic variation as described for mammals [9] and, similarly, there was limited information regarding temporal fluctuations of lipid metabolism genes over the daily LD cycle. The present study showed that both Pparz and γ expression followed a rhythmic pattern, with Pparz reaching a peak at ZT 14:00 concurrently with Srebps, whereas Pparγ peaked at ZT 9:00 which contrasts to the results of Huang et al. [37], but is in agreement with Paredes et al. [46]. In the present study the difference in acrophase could be correlated with the known functions of PPARs, with z expression being high under low feeding conditions, just prior to the scotophase, whereas γ expression was highest when lipid levels would be high in the middle of the light phase. It is important to note that salmonids are visual feeders, thus it is expected a decrease in the feed intake at the scotophase [47]. Similarly, the rhythmic expression observed in El and ApoCHI, two genes involved in lipoprotein metabolism, appeared to be linked to lipid availability. Thus, an increase in the expression of El, a phospholipid and triacylglycerol hydrolysing enzyme, was observed during the dark phase, a period when feeding is low and release of lipid from lipoproteins is likely required. In contrast, ApoCHI, which is important in the formation of very-low density lipoproteins, is higher at the middle of the light phase when there is greatest feeding and thus high plasma lipid levels promoting liver lipoprotein production. The physical drivers

Figure 5. Twenty-four hour expression of lipoprotein metabolism genes in the liver of salmon parr acclimated to LD photoperiod. Results are displayed in relation to Zeitgeber time (ZT), where ZT 0 is the onset of light. Gene expression data is displayed as the percentage of the mean ± SEM and includes the spread of the data. The presence of a cosine wave denotes a significant circadian rhythm by acro analysis. The grey bar at the bottom of the graph represents the dark period. The presence of different letters represents statistically significant differences between samples by ANOVA and Tukey’s test (P < 0.05).

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of rhythmic expression in both the clock genes as well as lipid metabolism remain to be determined, however it is likely that either photoperiod, feed availability or an interaction of the two will control the expression patterns observed. Interestingly in sea bream, while Vera et al. [29] reported feeding patterns to be the driver of liver clock gene rhythms, Paredes et al. [46] concluded that photoperiod itself was the strongest regulator of liver lipid metabolism cycles.

Another transcription factor that displayed a significant daily rhythm was Lxr, which reached its zenith at dusk when both Srebp1 and 2 were at their nadir. Mammalian Lxr is activated by binding oxysterols (cholesterol metabolites) arising from increased intracellular cholesterol [48,49]. This suggests that the increase in Lxr transcript could be related to the daily rhythm of cholesterol biosynthesis in the salmon liver, displaying higher de novo production during the dark phase when there is a fasting state, as reported for humans [50].

In addition, when intracellular levels of cholesterol are low, SREBPs are cleaved and released to act as transcription factors. In mammalian systems SREBPs accumulate in the nucleus and trigger the synthesis of HMG-CoA reductase (Hmgcr), the rate-limiting enzyme in cholesterol biosynthesis [10]. In salmon liver, Hmgcr acrophase was reached at ZT 10:00, whereas Srebp1 acrophase occurred three hours later, inconsistent with Hmgcr being a Srebp target gene in this species. In agreement with the present data, recent studies, both in vivo and in vitro, have shown that this pathway is likely to be regulated differently in salmon compared to mammals [7,21]. In contrast, however, negative regulation appeared to exist between \( Lxr \) and \( Lxr \), as similarly described for mammals [36], when one gene reaches its zenith the other is at its lowest expression level.

In mammals, the mechanisms involved in the conversion of cholesterol to bile acid appear to be under a degree of daily regulation via REV-ERB 1 \( \alpha \) [10]. However, in Atlantic salmon liver this was not the case, suggesting that regulation may be at the protein level as opposed to transcriptional level or a differential pathway as previously described in mammals may be in place. Isopentenyl diphosphate isomerase (Ipp), an enzyme in the mevalonate pathway from mevalonate pyrophosphate to farnesyl pyrophosphate, displayed a rhythmic expression, reaching its peak at around ZT 9:00. This enzyme did not follow the tendency observed by Srebps, Lxr or Cyp71a, but it must be noted that each one of the synthetic steps is under rigid negative feedback regulation by some intermediate substrates and not only by the final product, cholesterol. This could also explain why the other cholesterol metabolism genes \( Mv \), \( Dhcr7 \) and \( Abca1 \) show different expression profiles, depending on which step is being promoted at that moment.

Overall the current study provided clear evidence for the conservation of rhythmic daily regulation of liver lipid metabolism in teleosts as has been reported in mammals [14]. However it is the practical significance of this study that warrants further research as understanding the mechanisms involved in the regulation of absorption, transport and metabolism of lipids and fatty acids is of increasing importance in aquaculture [21]. The current work is the first evidence that Atlantic salmon lipid metabolism is under the influence of environmental parameters that are routinely manipulated in culture for other production reasons e.g. photoperiod management of sexual maturation [51]. As such, it is suggested that the possibility of environmental manipulation to optimise lipid metabolism in farmed salmon should be further explored.

In conclusion, the current study has provided the first evidence for the daily expression of genes involved in cholesterol and lipid homeostasis in the liver of Atlantic salmon under an LD cycle. Transcription factors appear to present a strong rhythmic expression, which could indicate their role as synchronisers. However, the expression of some target genes did not display a significant daily expression, denoting that this activation pathway may differ among mammals and teleosts or that other entraining factors may regulate their expression in liver. These findings provide the basis towards understanding the role of the circadian clock in the regulation of lipid metabolism in teleost fish and provide a novel approach to improve fish aquaculture by optimising feeding protocols and/or environmental conditions to match fish lipid metabolism rhythms.

**Supporting Information**

**Figure S1** Alignment of the deduced protein sequence for Atlantic salmon REV-ERB 1\( \alpha \) along with tilapia, zebrafish, as well as human. The conserved amino acids are shaded in grey. Predicted DNA binding domain (top) and ligand binding domain (bottom) identified using a CDD search [52] are boxed. The structurally coordinating cysteine residues belonging to the two C4 Zinc fingers are identified with an asterisk.

**Figure S2** Phylogenetic analysis of the deduced amino acid sequence for REV-ERB 1\( \alpha \) in relation to other vertebrate REV-ERB 1\( \alpha \) and REV-ERB 1\( \beta \) sequences. The evolutionary history was inferred using the neighbour-joining method [53]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [54]. The evolutionary distances were computed using the maximum composite likelihood method [55] and are presented as the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA5 [55]. GenBank Accession Numbers: zebrafish REV-ERB 1\( \alpha \) (NP_991292.1), tilapia REV-ERB 1\( \alpha \) (XP_003442479.1), zebra mbuna REV-ERB 1\( \alpha \) (XP_004355667.1), sheep REV-ERB 1\( \alpha \) (NP_001124501.1), human REV-ERB 1\( \alpha \) (NP_068370.1), mouse REV-ERB 1\( \alpha \) (NP_663409.2), tilapia REV-ERB 1\( \beta \) (XP_005459783.1), fugu REV-ERB 1\( \beta \) (XP_003909152.1), zebrafish REV-ERB 1\( \beta \) (NP_001092087.1), mouse REV-ERB 1\( \beta \) (NP_035714.3) and human REV-ERB 1\( \beta \) (NP_001138897.1).

**Table S1** Abbreviation, full name and function of all genes investigated.

**Table S2** Primer pairs and sequences for Rev-erb\( \alpha \) identification including primer name, purpose, sequence and annealing temperature.

**Table S3** Primers used for qRT-PCR.

**Author Contributions**

Conceived and designed the experiments: AD HM DRT. Performed the experiments: EM. Analyzed the data: MM EM MBB AD. Contributed to the writing of the manuscript: MBB DRT EM HM MM AD.
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