Tissue Distribution of Peroxisome Proliferator - Activated Receptors in Salmon Fish (*Salmo salar L.*)

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Abstract

   Peroxisome Proliferator-activated Receptors (PPARs) are ligand-inducible transcription factors, that can be activated by both dietary fatty acids and their metabolic derivates in the body, as by eicosanoids, hypolipidemic agents or antidiabetic drugs. To date three PPAR isotypes have been identified in mammals, birds, and amphibians, termed PPARα, PPARγ, and PPARβ/δ. Each isotype is a product of a separate gene and each one has a distinct tissue distribution . The importance of this receptors is nowadays a focus on different studies, due to medical and pharmacological interest, most studies on PPARs have concentrated on mammalian genes and proteins but also some research has also been done on fish. This recent interests on fish is due to the fact that aquaculture production is increasing and it's being a challenge feeding fish using fish oil, trying to replace it with vegetable oil. Changing the dietary pattern influences the fatty acid composition in fish tissues with the decrease of the n-3 highly unsaturated fatty acids, as main consequence rendering fish less beneficial for human health. One way to find new approaches for using vegetable oil or other ways to feed farmed fish, is to study changes on gene expression, or on receptors that affect lipid's genome and one of those are PPARs.

   The main objective of this study is to learn about PPAR, focusing on the tissue distribution of his different isotypes, and associate it with their functions on the organism. While studying the PCR methodology in order to know the gene expression of each PPAR.

Keywords: PPAR, Atlantic salmon, fatty acid, PCR, qPCR, RT-PCR

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Abbreviations

PPAR Peroxisome Proliferator-activated Receptors
CD36 Cluster of differentiation 36
NHR Nuclear hormone receptor
FA Fatty acid
PPRE PPAR response element
CPT1 Carnitine palmitoyltransferase 1
SR-B1 Scavenger receptor class B member 1
TZD Thiazolidinediones
TG Triglycerides
LDL Low-density lipoprotein
HDL High-density lipoprotein
LPL Lipoprotein lipase
PUFA Polyunsaturated fatty acids
ALA α-Linolenic acid
LA Linoleic acid
ARA Arachidonic acid
EPA Eicosapentaenoic acid
DHA Docosahexaenoic acid
RT-PCR Reverse Transcription PCR
qPCR Quantitative PCR
PCR Polymerase chain reaction
NTC No-template controls (NTCs
miRNA MicroRNA
NUOR NADH-ubiquinone oxidoreductase
ETIF Eukaryotic translation initiation factor 3

1   Introduction

1.1. PPAR definition

Peroxisome Proliferator-activated Receptors (PPARs) are ligand-inducible tran-scription factors belonging to the nuclear hormone receptor (NHR) superfamily. To date three PPAR isotypes have been identified in mammals, birds, and amphi-bians, termed PPARα, PPARγ, and PPARβ/δ. Each isotype is a product of a sepa-rate gene and each one has a distinct tissue distribution and, like typical sibling, are often functionally in disagreement with each other [5].

PPARs have been identified as targets for regulation by fatty acids or their meta-bolites. Fatty acids (FAs) bind to and regulate the activity of PPARs. All PPARs bind 20-carbon polyunsaturated fatty acids, *e.g.* 20:4,n6 and 20:5,n3 (ω6 and ω3) [6]. They can be activated by both dietary fatty acids and their metabolic derivates in the body, and thus serve as lipid sensors which when activated can markedly redirect metabolism. They are also activated by various natural and synthetic li-gands like eicosanoids, hypolipidemic agents, and antidiabetic drugs [3]

In terms of genomics for these receptors, transcriptional activation of target genes by PPARs requires the presence of peroxisome proliferator, or PPAR response elements (PPREs) in the promoter of target genes. The DNA consensus sequence for the PPRE is AGGTCAXAGGTCA, with X being a random nucleotide [9]. These PPREs are direct repeat elements of the DR1 type. PPARs bind as hetero-dimers with the retinoid-X-receptor, conformational changes of the receptors li-gand binding domain result in the release of corepressor proteins, recruitment of coactivator proteins, and subsequent assembly of protein complex that enhances transcription of the target gens [5].
A number of PPAR target genes have been characterized to date. Most of these genes are known to have roles in lipid and glucose metabolism, whereas PPAR ligands are themselves, in many cases, the substrates and/or products of the en-zymes whose genes PPARs have emerged as critical regulators of lipid and glu-cose homeostasis in mammals [5].

Due to obvious medical and pharmacological interest, most studies on PPARs have concentrated on mammalian genes and proteins. But some research has also been done on fish. Although differences in the genomic organization of the fish PPAR genes compared with their mammalian counterparts are evident, sequence alignments and phylogenetic comparisons show the fish genes to be homologs of mammalian PPARα, PPARβ, and PPARγ [5].

All different subtypes of PPARs have been identified in Atlantic Salmon (Salmo salar L.). Four genes coding for four different subtypes of PPARβ have been identified, these subtypes were grouped into two families based on differences in exons and exon-flanking regions. Each subtype had a characteristic expression pattern varying between tissues. Furthermore, two forms of PPARγ been described in Atlantic salmon liver [9]. This kind of research has not been done just in Salmon, grass carp (Ctenopharyngodon idella), brown trout (Salmo trutta), plaice (Pleuronectes platessa) and gilthead sea bream (Sparus aurata) are other examples of cross-hairs.

1.2. PPAR tissue distribution

The three PPAR family members have distinct patterns of tissue distribution and consistent with their expression profiles, the PPARs each carry out unique functions in the regulation of energy metabolism
In mammals, PPARα is expressed in metabolically active tissues, *e.g.* the liver, and induces a range of genes involved in lipid transport, oxidation and thermogenesis. PPARγ is found in white adipose tissues, where they are involved in lipid synthesis. PPARβ is abundantly expressed throughout the body markedly on brain, adipose tissue, and skin but at lows levels in liver, and has less defined functions. [12].

A study about PPARs in grass carp (*Ctenopharyngodon idella*) found that PPARα predominates in the liver, as mammal PPARα does, PPARβ is widely expressed, particularly abundant in heart, liver and muscle and PPARγ is abundant in the liver, and to a lesser extent in brain, muscle and visceral adipose tissue [6] . Thus here we can find a coincidence between PPARα and PPARβ in humans and this fish. Another study about the brown trout (*Salmo trutta* f. *fario*), concluded that PPARα predominates in white muscle, heart and liver. PPARβ is more expressed in testis, heart, liver, white muscle and trunk kidney, so throughout the whole fish, as humans and the other fish species do, and PPARy is found in trunk kidney and liver [1]. There is some research done on the plaice (*Pleuronectes platessa*) and the gilthead sea bream (*Sparus aurata*) affirming that in sea bream, PPARα is mostly expressed in liver and heart, and PPARγ in intestine and adipose tissue. PPARβ is expressed in all sea bream tissues, so as the others cases is the most widely expressed. In plaice, the PPAR expression profile is generally similar to sea bream. The major differences are the higher level of PPARβ over PPARα in liver and the low level of PPARα in red muscle [5].

In these studies it is of interest to note that PPARγ is widely expressed in different tissues at a level at least similar of PPARβ. This is in contrast to both mammals and amphibians where this isotype exhibits a restricted expression pattern, being present mainly in adipose tissue, and only at low level in most other tissues. But, like its mammalian homolog, fish PPARα is mostly expressed in tissues with high β-oxidation capacity, namely liver or heart. As PPARα does PPARβ has a similar distribution pattern in both, fish and mammals.

1.3. The significance of PPAR in physiology and human diseases

As said before, PPARs play a major role in whole body lipid and glucose metabolism, as well as in inflammatory and immune responses. Based on in vitro binding and cell culture studies PPARs have emerged as prospective monitors of intracellular non-esterified FA levels, and in liver this receptors would respond accordingly by altering metabolism to prevent lipid and cholesterol overload. [6]

PPARα has been shown to activate the gene coding for the β-oxidation enzyme, carnitine palmitoyltransferase 1 (CPT1) by binding to a PPREs in the promoter region of the gene, thus playing an important role in regulating β-oxidation in rodents, fish and humans. Others target genes on β-oxidation series for PPARα are genes coding for enzymes like Acyl-CoA oxidase, and hydroxyacyl dehydrogenase, or fatty acid binding proteins and transmembrane fatty acid trasnporters such as CD36 and FAT. Both PPARα and PPARγ have shown to induce the transcription of the transmembrane fatty acid transporter CD36 and SR-B1. SR-B1 is a cell surface, high density lipoprotein receptor and a member of CD36 receptor family. It's expressed in all tissues engaged in cholesterol metabolism, and so this seems to be the reason for what PUFAs increase hepatic cholesterol uptake [9].

The PPAR activators correct the levels of plasma cholesterol and TGs, as a consequence of glucose, and exert anti-inflammatory actions. As a consequence they appear good drug targets for the treatment of dyslipidemia, type 2 diabetes, and for the reduction of the progression of coronary atherosclerosis and so the decrease of the incidence of coronary heart disease. [2], [3]

Nowadays, there is some pharmacological compounds that use them as targets. Fibrates are lipid lowering drugs that act through PPARα activation, and thiazolidinediones (TZDs), a class of insulin sensitizers, are synthetic ligands for PPARγ. They have different effects because of the activation of different PPARs, due to the fact that they have different tissue distribution.
Dyslipidemia associated with metabolic syndrome is characterized by elevated plasma levels of small dense low-density lipoprotein (LDL) and TGs and by reduced high-density lipoprotein (HDL) cholesterol levels. Fibrates, which are relatively weak PPARα ligands, stimulate intravascular lipoprotein lipase (LPL) activity and reduced expression of apoCIII, a natural LPL inhibitor.

They also inhibit TG synthesis and very-low-density lipoprotein production by favoring FA uptake and retention, enhancing FA catabolism and reducing FA synthesis in hepatocytes. As hypotriglyceridemic drugs, fibrates also induce the expression of apoAV, an important determinant of plasma TG levels. Fibrates improve LDL clearance by changing the plasma LDL distribution profile, from small dense to large LDL particles that display higher affinity for the LDL receptor. Moreover, fibrates stimulate the production of apoAI and apoAII in human liver, thus leading to increased HDL production.
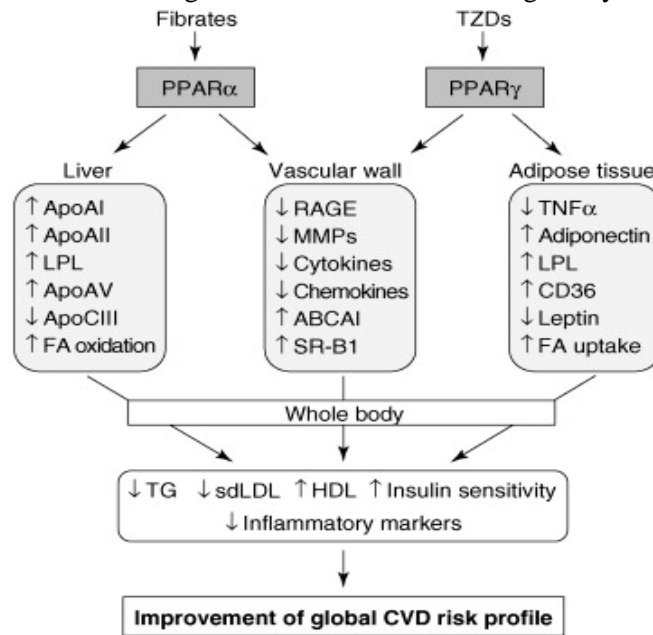
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Figure 1. Functions of PPARα and PPARγ depending on their agonists.

TZDs are PPARγ ligands that affect the levels of circulating free FAs and cholesterol (by increasing the level of HDL cholesterol). Activation of PPARγ induces the expression of genes controlling adipocyte FA metabolism, including those that encode LPL and FA transport proteins, conducting to lypolisis of plasma TGs, uptake of FAs and storage of TGs in adipocytes. In addition, TZDs improve insulin sensitivity by reducing peripheral insulin resistance and so they low blood glucose levels in patients with type 2 diabetes. As a consequence, PPARγ activation reduce the release of free FAs and insulin-resistance-mediating adipocytokines, such as tumor necrosis factor (TNFα), leptin and resistin, and increase production of adiponectin, which has anti-atherosclerotic and anti-diabetic properties.

There are new therapeutic approaches that are trying to find selective PPAR modulators, PPARγ antagonists, and combined PPARα and PPARγ agonists [2].

1.4. The importance of PPAR in salmon.

It has been shown that dietary fat influence the gene expression by controlling the activity or abundance of central transcription factors, and one nuclear receptor that influences this transcription are PPARs. As said before PPARs can be activated by FA [9].

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There are two families of FA needed to be present in adequate and balanced quantities within the human body for man to stay healthy. These two groups of FA are the omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA). α-Linolenic acid (ALA) is the key essential n-3 FA, and linoleic acid (LA) is the corresponding essential n-6 FA. Both of cannot be synthesized in higher vertebrates including humans. The essential FA can be found in many different sources of food, especially in nuts, various plants, seeds and oils. In the body, LA can be metabolized to its longer chain derivate, arachidonic acid (ARA), and ALA can be methabolized to eicosapentaenoic acid (EPA) and further on to docosahexaenoic acid (DHA). However, in most vertebrates, the ALA conversion to EPA or DHA is very limited. Fish and shellfish being naturally high in EPA and DHA are the most important source for the highly unsaturated n-3 fatty acids. Furthermore, fish is naturally low in n-6 FA and consequently generate a low n-6/n-3 ratio, which is known to be beneficial for human health [9], [12].

Omega-6 series

Linoleic acid
18:2n-6
↓ Δ-6 desaturase
18:3-n6
↓ Elongase
20:3n-6
↓ Δ-5 desaturase
Arachidonic acid
20:4n-6
↓ Elongase
22:4n-6
↓ Elongase
24:4n-6
↓ Δ-6 desaturase
24:5n-6
↓
Docosapentaenoic acid
22:5n-6

Omega-3 series

α-Linolenic acid
18:3n-3
↓
18:4n-3
↓
20:4n-3
↓
Eicosapentaenoic acid
20:5n-3
↓
22:5n-3
↓
24:5n-3
↓
24:6n-3
↘ Peroxisomal β-oxidation
Docosahexaenoic acid
22:6n-3

Figure 2. Elongation and desaturation of n-6 and n-3 FA. Adapted from (Voss et al.,1991) and modified after (Trattner, 2009)
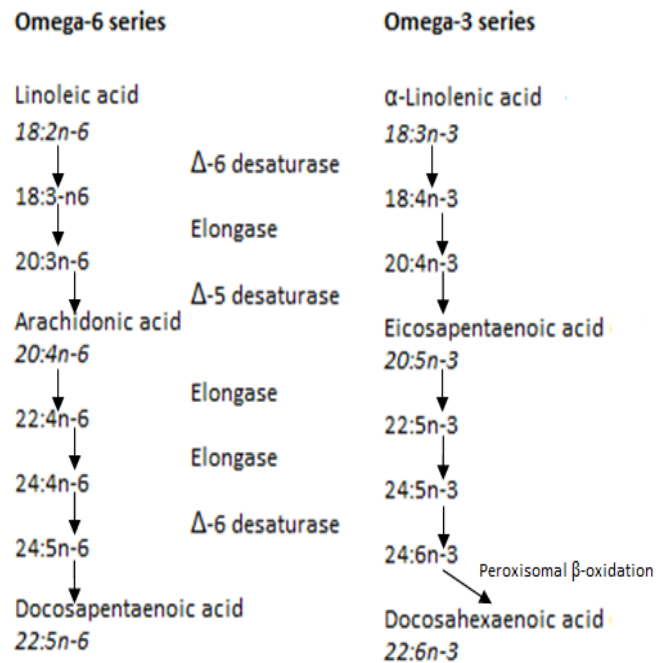
Despite that, meeting the dietary demands of a growing human population for balanced PUFA intake is actually a challenge. One way to meet this demand is through aquaculture of fish, such as salmon, tuna, sardines, trout etc. that all contain high levels of n-3 long chain PUFAs and are low in n-6 FAs. Another way is to achieve a better understanding of the regulation of the PUFA metabolism by monitoring the underlying internal molecular mechanisms [9].
It's known that global production of fish from aquaculture has grown substantially with almost 10% per year over the past decades. Roughly 50% of fish used for human consumption are now farmed, and this proportion increases more every year [9]. This growth on aquaculture was made possible by the development of formulated fish feed including aminoacids, FAs, minerals and vitamins fulfilling all essential requirements. The diets have been relaying on fish meal and fish oil rich in n-3 PUFAs from natural fisheries to generate a diet high in both lipids and proteins [12].

The development of aquaculture has been heavily depending on the availability of fish oil supply. Fish oil is basically made by pelagic fish, naturally high in both EPA and DHA. However the landings from global fisheries of pelagic fish cannot keep up with this increasing demand from the industrial aquaculture [9]. Therefore, it has been an important cause for a lot of researches to find new lipid sources for fish feed. This investigations focus on finding a replacement of the fish oil with vegetable alternates, but doing it a decrease in long chain n-3 FA is noticed. Growth and fish health have been shown to not be negatively affect by replacement of up to 50% of fish oil in the diet with vegetable oils, but more knowledge of the metabolic effects of these compounds is needed. And a way of understanding this metabolism is to learn more about the effects on PPARs of different bioactive compounds that can be added in this oils, as tocopherols, carotenoids, sterols, certain fatty acids, sesamin, lipoic acid and other lipophilic compounds [9],[12].

1.4. PCR methodology

In order to quantify the gene expression of PPAR is necessary to understand the Polymerase chain reaction (PCR) method. PCR is a molecular biological technique used to amplify a specific region of a DNA strand, the DNA target, across several orders of magnitude, generating thousands to millions of copies of this particular DNA sequence. The versatility of PCR has brought a large number of variants, in this study we used two of them, the RT-PCR (Reverse Transcription PCR) and the qPCR (Quantitative PCR).

qPCR or also called Real-time PCR has the ability to monitor the complete DNA amplification process of a target DNA molecule throughout the PCR reaction. It quantitatively measures amounts of cDNA, it's used to determine whether a DNA sequence is present in a sample and the number of its copies, in other words, to quantify the gene expression of a molecule in the sample [14].
After a correct setup, run and technical quality control of a qPCR the C_T value must be determined [8].The C_T is defined as the number of cycles needed for the fluorescence signal to reach a specific threshold level of detection and is inversely correlated with the amount of template nucleic acid present in the reaction.
Another quality control is using reference genes, to compare their amplification with the sample genes. The accurate quantification of a true reference gene allows the normalisation of differences in the amount of amplifiable cDNA in individual samples generated by different amounts of starting material, the quality of the starting material; and differences in RNA preparation and cDNA synthesis, since the reference gene is exposed to the same preparation steps as the gene of interest [7]. The use of no-template controls (NTCs) in qPCR runs is also required. NTCs are controls where water instead of DNA is included in the reaction mixture; this checks for purity of the PCR components. Moreover, they are necessary to check the proper function of the primers used on the PCR, to control the bindings to each other [8].

2   Materials and Methods

3.1. Fish

Atlantic salmon of approximately 10g (Älvkarleby Research Station, Älvkarleby, Sweden) was kept in tanks with water from the river "Dalaälven" at 10°C and fed a commercial diet (Alleraqua Performa pellet size 2 and 9% fat content, Aller Aqua A/S, Christiansfeld, Denmark) prior to sampling [13].

3.2. mRNA and miRNA extraction

The interest of this study is to analyze different samples of RNA from different tissues of Atlantic Salmon, therefore an extraction of miRNA was done from white muscle, intestine, gill, kidney, brain, liver, stomack, heart, spleen, and red muscle from a salmon fish. Three different samples from three different individuals were taken from this tissues. A summary of the samples are shown in *Table 1*. The tissue samples used for miRNA analysis were collected and transferred to RNA later (Life Technologies, Carlsbad, CA, US) and stored at – 80°C until further analysis [13].

These miRNA samples were isolated with **mirVana™ PARIS™ Kit**. The tissue samples were disrupted in Cell Disruption Buffer. For RNA isolation, the lysate was mixed with 2X Denaturing Solution and subjected to Acid-Phenol:Chloroform extraction which provides a robust front-end RNA purification that also removes most DNA. Then, the RNA was purified adding Etanol to the samples, and they were passed through a Filter Cartridge containing glass-fiber which immobilizes the RNA. The filter was then washed a few times, and finally the RNA was eluted with a low ionic-strength solution.

3.3. RT-PCR

The gene expression on these tissue samples was investigated by quantitative Real-Time PCR. The first step on this procedure was analyze the exact concentration of RNA that the samples contained in order to know the volume to use in the RT-Reaction, this have been done using an spectrophotometer and the results are shown in *Table 1*.

After diluting the less concentrated samples (20 to 28), samples not present in triplicates were excluded from the study (8,17,19, 29), the total RNA was quantified and reverse transcription First strand cDNA was synthesized using the **High Capacity RNA-to-cDNA™ Kit**.

So we mixed our samples with the RT Buffer and the RT enzyme mix that contains the kit, in 20 µL aliquots and ran them in the adequate thermal cycler conditions using an special thermal cycler.

	Tissues	RNA concentrations (µg/µl)
1.	White muscle 1	0.038
2.	Intestine 1	0.023
3.	Gill 1	0.015
4.	Kidney 1	0.018
5.	Brain 1	0.044
6.	Liver 1	0.015
7.	Stomach 1	0.031
8.	Heart 1	Discarded
9.	Spleen 1	0.015
10.	White muscle 2	0.017
11.	Intestine 2	0.019
12.	Gill 2	0.014
13.	Kidney 2	0.033
14.	Brain 2	0.036

15.	Liver 2	0.046
16.	Stomach 2	0.034
17.	Heart 2	Discarded
18.	Spleen 2	0.329
19.	Red muscle 2	Discarded
20.	White muscle 3	0.274
21.	Intestine 3	1.455
22.	Gill 3	0.854
23.	Kidney 3	0.992
24.	Brain 3	0.307
25.	Liver 3	2.154
26.	Stomach 3	2.538
27.	Not extracted	
28.	Spleen 3	0.162
29.	Red muscle 3	Discarded

Table 1. *Samples and RNA concentrations to run on RT-PCR*

3.4. PCR (cDNA synthesis)

At this point we had the samples of RNA converted to cDNA. The PCR reactions were run on plates on an Applied Biosystems real-time quantitative PCR instrument, in 20 µL reaction volumes.
In order to analyze the gene expression of PPARs in every tissue we used the **Fast SYBR® Green Master Mix** and performed a quantitative real-time PCR in our samples.
Therefore, we used 5 different primers to obtain 5 different genes. The primers were designed based on available Atlantic salmon sequences from the online version of GenBank® (NCBI) using Primer Express® software or copied from literature references and custom-made at Invitrogen Part of Life Technologies (Carlsbad, CA, USA) [11]. The two reference genes used were NADH-ubiquinone oxidoreductase (NUOR) and Eukaryotic translation initiation factor 3 (ETiF) [9].

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The other genes that were looked for the study were the genes coding for PPARα, PPARβ and PPARγ. We mixed the primers with our samples, and the Fast SYBR® Green Master Mix that contains uracil-DNA glycosylase for preventing the reamplification of carryover PCR products.

3.4. qPCR

In order to know the dilutions to be used for qPCR analysis the first plate was ran with different dilutions, but the results showed better values with the non diluted ones. The PCR products without dilutions were used directly in the analysis, without doing the dilutions, and we ran the first two plates with the first 6 samples for each gene in triplicate with three non-template control, using 2 µL of each sample. A melt curve was performed after each run in order to control for primer-primer bindings and in samples with 2µl cDNA the CT-value was too high. In order to optimize the results, we changed our method, and used 6 µL of each sample, but we didn't have enough sample to run them in triplicate, so we decided to run them alone, with non repeated cDNA samples, even if this means that the results could be less statistically significant.

3.6. Data evaluation

In order to evaluate the data from gene expression we viewed the amplification plots, adjusted the baseline and threshold values to determine the threshold cycles (C_T) for the amplification curves. An statistical analysis with DataAssist ™ version 3.0. was made to compare the relative quantitation of gene expression in the different tissues. This data analysis tool for sample comparison uses the comparative C_T ($\Delta\Delta C_T$). So, the C_T of PPARs gene expression were compared and this way, decided where they are more or less expressed.

3   Results

The relative expression of PPARs was compared with the expression showed in liver. So, the results obtained using the liver as reference group are shown in different graphics below.
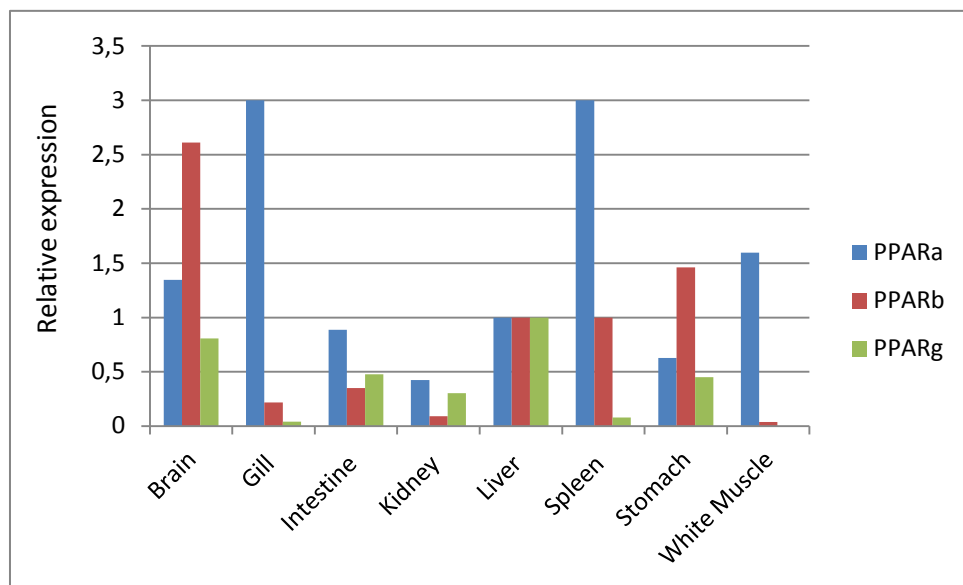

Figure 3. Relative gene expression of the three types of PPAR in different tissues, compared to the liver. In order to understand better the values, some of them were discarded, as PPARα in gill, PPARα in spleen, and PPARγ in white muscle

PPARα is the most expressed type, in fact the value of the relative expression on gill compared to the liver was 21151.08, far exceeding all other values, being those close to 1. Something similar happened with the spleen value for PPARα, that was 33.7423. On white muscle and brain also the values for this PPAR are quite high, at least the expression is higher than the one in the liver.

PPARβ shows a remarkable higher expression in the brain than in the liver, and it's also a higher value to consider the one in the stomach, having the spleen the same value, the other tissues show lower ones.

PPARγ showed a similar problem as PPARα, being this higher expressed in white muscle, the exact value of the relative expression was 6.7571. The results show too that the most gene expression seems to be in the liver, as the other values are totally lower.
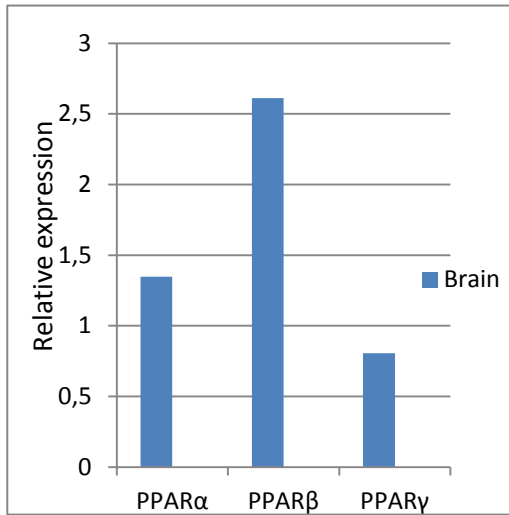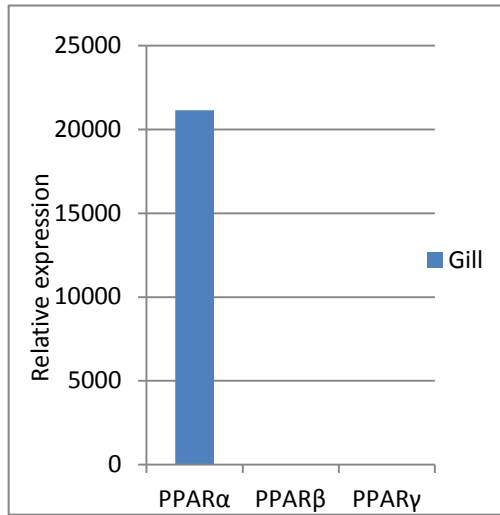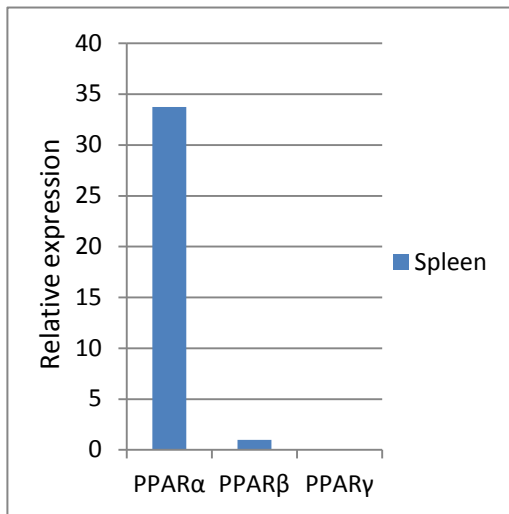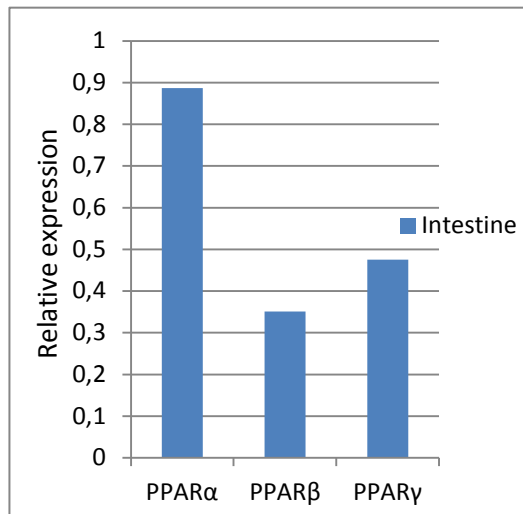

Figure 4

Figure 5

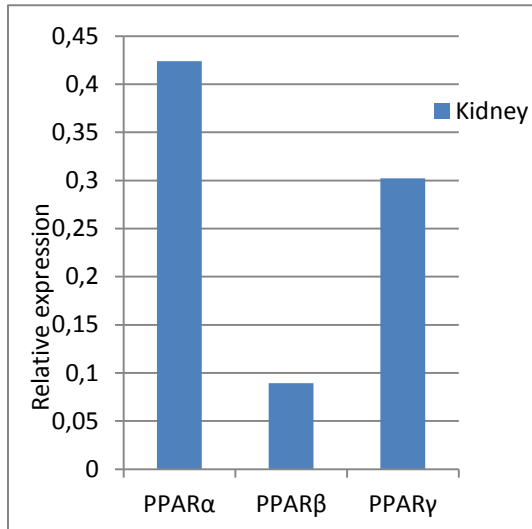*Figure 6*

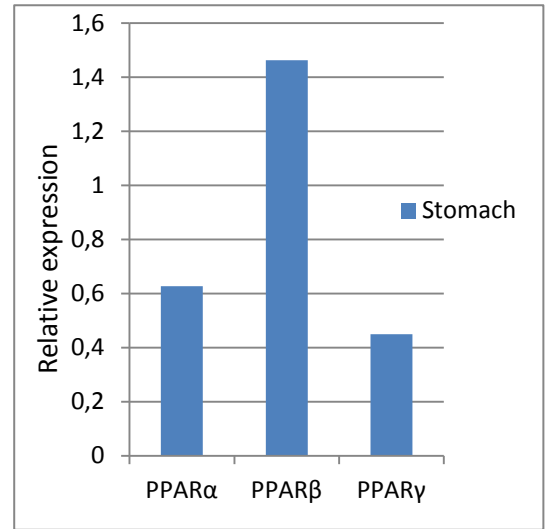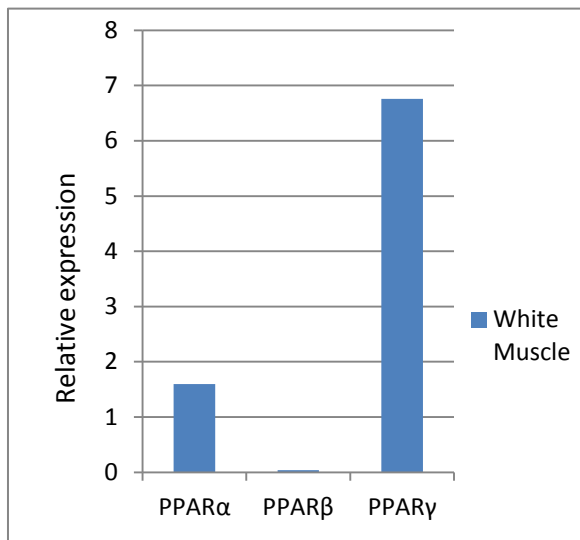*Figure 7*

Figure 8

Figure 9

Figure 10

Figures 4,5,6,7,8,9,10. Relative expressions of the three types of PPAR in each analyzed tissue, compared to the liver.

As we can see in the previous graphics, each tissue has a different distribution of each PPAR. In gill, and spleen the PPARα is most widely expressed with difference from the other tissues. In intestine PPARα is also the most expressed, but PPARγ and PPARβ have also a remarkable value of expression. The kidney is the other organ where we can see that PPARα has the most expression, but in this case PPARγ is close to his value, and PPARβ has a really low value in comparison. In brain and stomach we can see that PPARβ is the main character, and also PPARγ the less expressed. White muscle is the only one where PPARγ is widely expressed, and hasn't got any PPARβ expression, or at least not a remarkable one.

4   Discussion

The main objective of this study was to investigate the gene expression of the three different types of PPAR (α,β,γ) in some tissue samples of the Atlantic Salmon, as to learn about the methodology to do it, focusing on PCR method.
The results were quite difficult to evaluate, due to the fact that just one replicate of each sample has been analyzed with the quantitative real-time PCR, so in this research some points are remarkable to consider, but not all are totally agree with the literature.
Some explanation about the difficulty to obtain results in this project work could be the weaknesses of PCR such as the low quantities of DNA that are required, thus the need of accurate manipulation of them. Also, the concentration of DNA samples may be optimized, so they can be finished, then having the possibility of not being able to get more amount of sample to continue the development of more plates. This is what exactly happened in this experiment, we couldn't use more than one replicate because the samples were run out. Also when using a PCR if the requirement is amplify a specific gene, some knowledge of the gene's DNA sequence is needed, in order to design some primers, therefore the need of having good primers is important to notice, they cannot bind to each other and they have to work properly to obtain the gene expression.
The relative expression of PPARs was compared with the expression showed in liver, as is one tissue that for sure presents PPAR activity, being one tissue with more metabolically activity, as high β-oxidation capacity [8]. About the results obtained we could evaluate that PPARα is supposed to be found in tissues that catabolize large amounts of fatty acids, at least in metabolically actives tissues [12]. However, the most expression was found in the gill, and the other tissues where is widely expressed are spleen, kidney and intestine. So, maybe in this point the literature is not totally agree with the results. It's important to say also that the most expressed isotype was this PPARα.
PPARβ is supposed to be widely expressed on the whole animal, and some literature reflects that is markedly found on brain, adipose tissue and skin in mammals, and also in some fishes [5], [12].Thus our results on this isotype are the most similar ones, because we could find PPARβ in most of the tissues, except the white muscle, being the brain the most representative one.

The widespread distribution of PPARβ could indicate that it plays several roles in cell physiology, including basic lipid metabolism, epidermal cell proliferation and differentation, adipocyte function and placentogenesis [5].

PPARγ has been the most tricky sample to analyze, being always the results of this one a little bit difficult to trust. However, the other fishes literature explain the fact that it's widely expressed in the whole fish, being this a difference with mammals, where we can find it being present mainly in adipose tissue, and only at low level in most other tissues. Being the major functions of PPARγ to induce adipogenesis and affect the storage of fatty acids [5]. Therefore, in our fish we didn't analyze adipose tissue, so it could be an explanation of why the expression of PPARγ was difficult to find in the other tissues.

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