



UNIVERSITY OF STIRLING



Universidad Complutense De Madrid



CRIPESA

Cria de Pescado SA

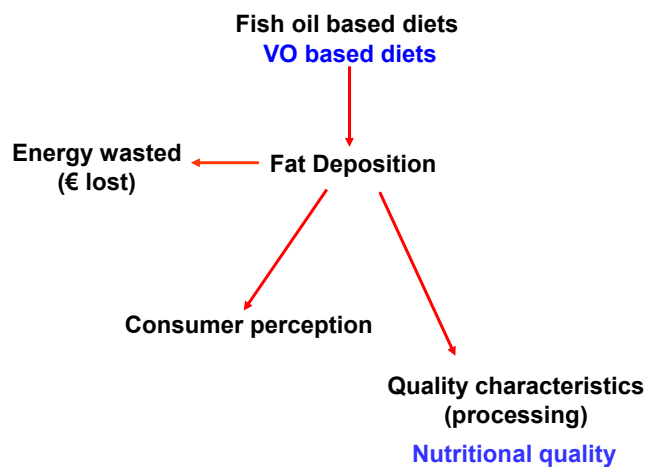
Cloning and functional analysis of fish Peroxisome Proliferator-Activated Receptors: The transcriptional control of lipid metabolism in farmed fish species (fPPARs)

EU Fifth Framework Q5RS-2000-30360

PARTICIPANTS

- 1. NAGREF-Fisheries Research Institute (Greece).
- 2. University of Stirling-Institute of Aquaculture (UK).
- 3. Universidad Complutense de Madrid-Faculty of Veterinary Medicine (Spain).
- 4. Nutreco Aquaculture Research Centre (ARC, Norway).
- 5. Cria de Pescado (Spain).

Fatty acid metabolism-associated problems in aquaculture



Need a better understanding of underlying physiology

Peroxisome proliferator-activated receptors (PPARs)

Ligand-induced Transcription factors

- Activated by PUFA and their eicosanoid derivatives (fatty acid sensors)
- Functions in lipid and carbohydrate metabolism, inflammation, cell cycle and differentiation
- Discovered in 1990, to date >3000 publications

Three isotypes in mammals, birds, amphibians

α	β/δ	γ
Peroxisomal and mitochondrial fatty acid β-oxidation. Liver, muscle.	Widespread regulator of fat burning. Ubiquitous.	Fat accumulation/ Adipogenesis. Adipose tissue, spleen.

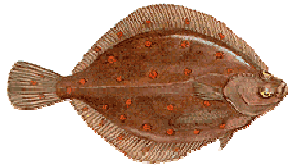
Questions:

- Do fish have PPARs?
- Functional properties of PPARs in fish (expression, DNA and ligand binding, transactivation), i.e. the role of PPARs in fatty acid metabolism in fish?

Aim:

- Manipulate fish fatty acid metabolism *via* PPARs.

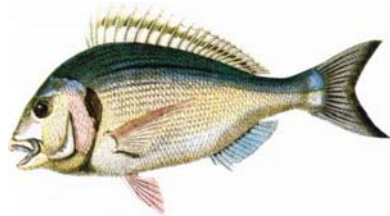
Looked for PPARs in:



Plaice (*Pleuronectes platessa*)



Atlantic salmon (*Salmo salar*)



Sea bream (*Sparus aurata*)

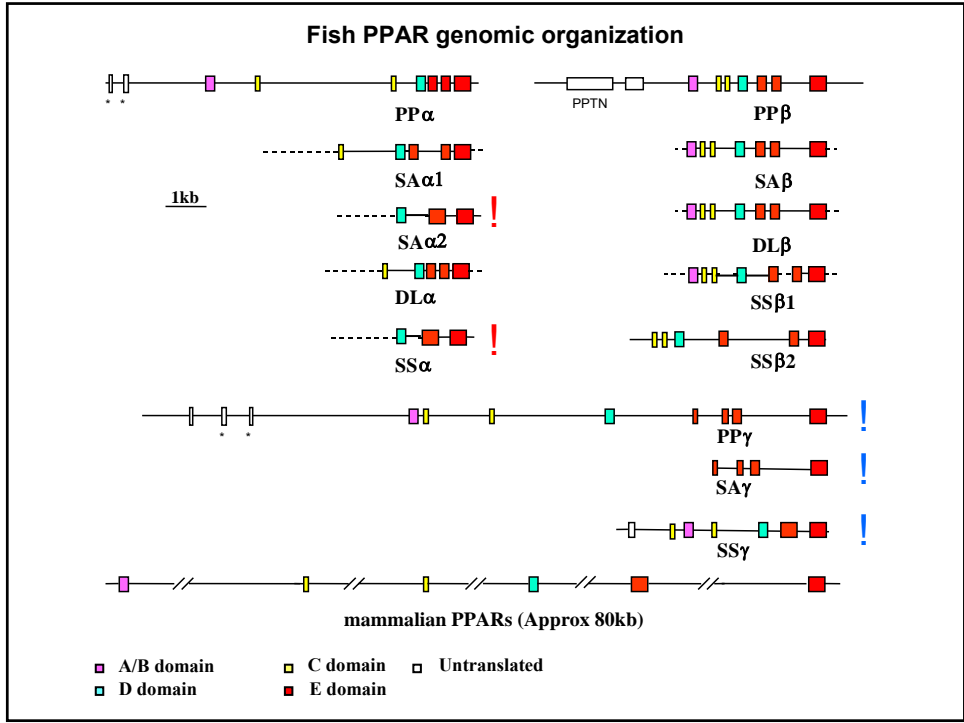


Sea bass (*Dicentrarchus labrax*)

Found:

- Genes and corresponding cDNAs encoding 3 distinct PPAR isotypes in plaice and sea bass.
- In sea bream, 2 PPAR α isoforms, each encoded by a distinct gene, were identified in addition to the PPAR β and γ isotypes.
- In Atlantic salmon, 2 PPAR β isoforms, each encoded by a distinct gene, were identified in addition to the PPAR α and γ isotypes.

=> More complex level of PPAR isotype variety in fish than in mammals



Alignment of the Ligand Binding Domain of PPARs

PPAR α			PPAR β				
PFVHIDMETLQAKRTIYAKLQANG	I	QNSAEVRIE	Human	PFVHIDMETLQAKRTIYAKLQANG	I	QNSAEVRIE	Human
PFVHIDMETLQAKRTIYAKLQANG	Q	QNSAEVRIE	Sea bream1	PFVHIDMETLQAKRTIYAKLQANG	Q	QNSAEVRIE	Sea bream
PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Sea bream2	PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Salmon 2
PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Salmon	PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Salmon 1
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Human	IPGQVDFLNDQVTLKKYGVHEAIFAMLSLNKDG	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Human
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Sea bream1	IPGQVDFLNDQVTLKKYGVHEAIFAMLSLNKDG	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Sea bream
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Sea bream2	IPGQVDFLNDQVTLKKYGVHEAIFAMLSLNKDG	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Salmon 2
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Salmon	IPGQVDFLNDQVTLKKYGVHEAIFAMLSLNKDG	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Salmon 1
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Human	FSEIMKFEFAVFNALDELDDSLALFAAILCGDRPGLANVAVI	S	ELDDSDLALEVAALICCG	Human
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Sea bream1	FSEIMKFEFAVFNALDELDDSLALFAAILCGDRPGLANVAVI	S	ELDDSDLALEVAALICCG	Sea bream
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Sea bream2	FSEIMKFEFAVFNALDELDDSLALFAAILCGDRPGLANVAVI	S	ELDDSDLALEVAALICCG	Salmon 2
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Salmon	FSEIMKFEFAVFNALDELDDSLALFAAILCGDRPGLANVAVI	S	ELDDSDLALEVAALICCG	Salmon 1
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Human	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Human
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Sea bream1	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Sea bream
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Sea bream2	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Salmon 2
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Salmon	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Salmon 1
QIKKTR	S	DTSLHPLLQEIYRDMY	Human	KDMY	S	DTSLHPLLQEIYRDMY	Human
QIKKTR	S	DTSLHPLLQEIYRDMY	Sea bream1	KDMY	S	DTSLHPLLQEIYRDMY	Sea bream
QIKKTR	S	DTSLHPLLQEIYRDMY	Sea bream2	KDMY	S	DTSLHPLLQEIYRDMY	Salmon 2
QIKKTR	S	DTSLHPLLQEIYRDMY	Salmon	KDMY	S	DTSLHPLLQEIYRDMY	Salmon 1
PPAR γ							
PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Human	PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Human
PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Sea bream	PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Sea bream
PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Salmon	PFVHIDMETLQAKRTIYAKLQANG	S	SKDREAEVRIE	Salmon
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Human	HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Human
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Sea bream	HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Sea bream
HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Salmon	HCCQCTSVETVETLFEFAKVFQGF	S	SLDNDQVTLKKYGVYEAIFALFASLNKDG	Salmon
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Human	LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Human
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Sea bream	LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Sea bream
LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Salmon	LVAVCSGFITREFLKSIRPFPSMMEKFKQFAIFNF	S	ELDDSDLALEVAALICCG	Salmon
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Human	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Human
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Sea bream	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Sea bream
RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Salmon	RPGLVNYVTEPQKQVAVVLEHLLANHPDPTLFLF	S	LQKADLRQVTEHAQW	Salmon
QIKKTR	S	DTSLHPLLQEIYRDMY	Human	KDMY	S	DTSLHPLLQEIYRDMY	Human
QIKKTR	S	DTSLHPLLQEIYRDMY	Sea bream	KDMY	S	DTSLHPLLQEIYRDMY	Sea bream
QIKKTR	S	DTSLHPLLQEIYRDMY	Salmon	KDMY	S	DTSLHPLLQEIYRDMY	Salmon

Conclusions from the molecular characterization of the fish PPARs

Likely that PPAR α 1 and β fulfill the same functions in marine fish as in mammals, i.e. important regulators of fatty acid catabolism in fish.

Questions remaining:

- What does PPAR γ do?
- PPAR α 2?

Nutritional stimuli and gene expression

- Qualitative aspects of diet and gene expression
 - 6 experimental diets tested in sea bream and Atlantic salmon (FO replacement, CLA supplementation)
 - PPAR, CPT I, ELO, and DES gene expression
- Effects of fasting/feeding on gene expression in sea bream.
 - PPAR, CPT I, α -amylase gene expression

The Partners

- Dr. M. J. Leaver, Dr. D. R. Tocher (Stirling)
- Dr. J. M. Bautista, Dr. A. Diez (Madrid)
- Dr. A. Obach (ARC)
- Mr. G. Bores (Cripesa)
- E. Boukouvala, Dr. E. Antonopoulou, L. Favre (Kavala)