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de **BIOLOGIA**

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Jornada Anual de la Secció d'Aqüicultura de la Societat Catalana de Biologia

Avenços en Recerca en Aqüicultura

Barcelona, 12 de juny de 2015
Sala Prat de la Riba, Institut d'Estudis Catalans

PROGRAMA

9.20-9.30	Rebuda/Welcome
9.30-10.15	*Invited speaker*: **Manuel Manchado**, IFAPA, Càdiz *"Generation and application of genomic resources in Senegalese sole aquaculture"*
10.15-10.35	**Francois Chauvigné**, IRTA-CSIC-University of Bergen, Norway *"Reproductive endocrinology of the Senegalese sole (Solea senegalensis): recent advances and applications to aquaculture"*
10.35-10.55	**Dafni Anastasiadi**, Institut de Ciències del Mar-CSIC, Barcelona *"Environmental influences on the epigenome and transcriptome of the European sea bass"*
10.55-11.15	**Enric Gisbert**, IRTA, Sant Carles de la Ràpita, Tarragona *"The benefits of the Mediterranean diet in fish: the effects of concentrated olive oil in growth performance and gut condition in gilthead sea bream (Sparus aurata)"*
11.15-11.45	Coffee Break
11.45-12.30	*Invited speaker*: **Leonor Cancela**, University of Algarve, Faro, Portugal *"Blue Biotech: applications to aquaculture"*
12.30-12.55	**Laia Ribas**, Institut de Ciències del Mar-CSIC, Barcelona *"The usefulness of zebrafish (Danio rerio) as a model for aquaculture research"*
12.55-13.15	**Roderick Nigel Finn**, University of Bergen, Norway *"The molecular evolution and function of aquaporins in the parasitic salmon louse in relation to its host the Atlantic salmon"*
13.15-13.35	**Neil Duncan**, IRTA, Sant Carles de la Ràpita, Tarragona *"A review of the reproductive behaviour of Senegalese sole (Solea senegalensis)"*
13.35-15.15	Lunch/Poster session
15.15-16.00	*Invited speaker*: **Yann Guiguen**, INRA, Rennes, France *"Diversity and evolution of genetic sex determination in fish "*
16.00-16.20	**Noelia Carrasco**, IRTA, Sant Carles de la Ràpita, Tarragona *"Field strategies to reduce the Ostreid Herpesvirus (OsHv-1μvar) impact on Pacific oysters C. gigas cultured in the Mediterranean"*
16.20-16.40	**Monica Boj**, IRTA-CSIC, Barcelona *"Deciphering the role of aquaporins during sperm motility in the marine teleost gilthead seabream"*
16.40-17.00	**Josep V. Planas**, Universitat de Barcelona, Barcelona *"Cytokine mediation of the maturational and pro-ovulatory effects of luteinizing hormone in trout"*
17.00-17.05	Cloenda/Closing

ENVIRONMENTAL INFLUENCES ON THE EPIGENOME AND TRANSCRIPTOME OF THE THE EUROPEAN SEA BASS

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Temperature is an environmental factor known to influence both DNA methylation and gene expression in many types of animals, whereas farming conditions can alter the morphology and the gene expression in fish. Here, we explored the effects of temperature and farming on the genome-wide DNA methylation and the transcriptome of adult European sea bass (*Dicentrarchus labrax*). For this purpose, we used Reduced Representation Bisulfite Sequencing (RRBS) and and RNA sequencing to measure DNA methylation and gene expression levels, respectively. Temperature effects were evaluated using 3-year-old fish that were raised either at low (17°C) or high (21°C) temperature during the thermosensitive period, while farming effects were evaluated comparing fish raised at low temperature with wild fish. Cytosines in the CpG context were affected in all comparisons, with more differentially methylated cytosines (DMC) found in the muscle of farmed fish and fewer but still more than ~12000 in the testis of high temperature fish. Focusing on genes that contained DMCs in their known regulatory regions, especially the promoter and the first exon, we performed enrichment tests of gene ontology (GO) terms to gather functional information In addition, we found genes that not only contained DMCs in their regulatory elements but also were differentially expressed. Together, these data show that environmental factors acting during the early development have long lasting effects on both the epigenome and the transcriptome of the sea bass, since these effects are detected later in life. It remains to be determined their actual functional consequences. *Supported by MINECO grant AGL2013-41047-R “Epifarm” to FP.*

DECIPHERING THE ROLE OF AQUAPORINS DURING SPERM MOTILITY IN THE MARINE TELEOST GILTHEAD SEABREAM

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In the marine teleost gilthead seabream (*Sparus aurata*) different aquaporin water channels, such as Aqp1aa, -1ab, -7 and -8b, are expressed during the hyperosmotic induction of spermatozoon motility in seawater (SW). However, the physiological roles of these channels are unknown. Single and double immunofluorescence microscopy studies indicate that Aqp1aa and -7 are respectively localized in the entire flagellum or the head, while Aqp1ab and -8b are both in the head and the anterior tail of ejaculated spermatozoa. Upon SW activation, Aqp1ab and -8b are rapidly phosphorylated and translocated to the head plasma membrane and the mitochondrion, respectively, whereas Aqp1aa and -7 remain unchanged. Immunological inhibition of Aqp1aa function reduced the rise of intracellular Ca^{2+} that normally occurs upon activation, and strongly inhibited sperm motility. Impaired Aqp1aa function also prevented the intracellular trafficking of Aqp8b to the mitochondrion, where it acts as a peroxiporin allowing H_2O_2 efflux and ATP production during flagellar motility. However, restoring the Ca^{2+} levels in spermatozoa with immunosuppressed Aqp1aa fully rescued mitochondrial Aqp8b accumulation and sperm motility. In contrast, exposure of sperm to Aqp1ab and -7 antibodies did not affect motility during the initial phase of activation, but latently compromised the trajectory and the pattern of movement. These data reveal the coordinated action of spatially segregated aquaporins during sperm motility activation in seabream, where flagellar-localized Aqp1aa plays a dual Ca^{2+}-dependent role controlling the initiation of motility and the activation of mitochondrial Aqp8b-mediated detoxification mechanisms, while Aqp1ab and -7 in the head and anterior tail direct the motion pattern. These findings provide new insights into the molecular mechanisms regulating sperm motility in marine teleosts and the causes of male infertility under culture conditions.

FIELD STRATEGIES TO REDUCE THE OSTREID HERPESVIRUS (OSHV-1μVAR) IMPACT ON PACIFIC OYSTERS *C. GIGAS* CULTURED IN THE MEDITERRANEAN

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Crassostrea gigas represent an important specie for molluscs European aquaculture and its currently the main oyster cultured in Europe. However, recurrent mortalities of oyster spat and juveniles in spring season represent the major limitation in its production. Such mortality episodes have been attributed to a particular virulent variant of the Ostreid Herpesvirus (OsHv-1), the OsHv-1 μvar. The Ebro Delta bays are the second molluscs production area in Spain. Currently, most of the spat is imported from French natural captation and hatcheries. French natural captation spat is known to be infected by OsHv-1 μvar. Thus, in the Ebro Delta *C. gigas* cultures are being affected recurrently by the impact of OsHv-1 μvar causing high economical loses for local producers and representing, thus, a relevant socio-economical in the region. However, an appropriated management of the *C. gigas* cultures can reduce the mortalities caused by such pathogen. Thus, this study was focused in studding the viability of free virus local production of spat and field strategies to reduce the OsHv-1 μvar impact. Free OsHv-1 μvar spat using Mediterranean broodstock was produced in an experimental local hatchery in order to produce free virus spat adapted to local Mediterranean environment. The local produced spat contributed to not increase the virus loads in the bays and allowed spat availability for an appropriated planification of the field practices in order to reduce the mortalities produced by OsHv-1 μvar. Furthermore, it has been demonstrated that the immersion calendar, spat immersion size and culture system are key issues to increase *C. gigas* survival during the OsHv-1 μvar critical episodes.

REPRODUCTIVE ENDOCRINOLOGY OF THE SENEGALESE SOLE (*SOLEA SENEGALENSIS*): RECENT ADVANCES AND APPLICATIONS TO AQUACULTURE

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The aquaculture of Senegalese sole is hampered by the absence of methods to control reproduction, and in particular by the low amount and quality of the sperm produced by cultured males. In Senegalese sole, spermatogenesis is semi-cystic and its endocrine control is largely unknown. In recent years, we have made significant progress in the understanding of sole spermatogenesis by focusing on the mechanism of action of the major reproductive hormones, the gonadotropins follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh). The isolation of the transcripts encoding the sole Fsh and Lh subunits, as well as the Fsh and Lh receptors (Fshra and Lhcgrba), allowed us to produce recombinant hormones and specific antibodies to investigate the role of Fsh and Lh during spermatogenesis. Cell-based functional assays and immunolocalization studies showed that the sole Fshra is promiscuos (i.e. activated by both Fsh and Lh), and expressed in Sertoli cells enclosing the germ cells as well as in the androgen-producing Leydig cells. By contrast, the sole Lhcgrba is Lh-specific and only present in Leydig cells and spermatids free in the tubular lumen. Both Fsh and Lh control similar steroidogenic pathways in the testis, although Fsh also regulates genes encoding Sertoli cell growth factors, and Lh specifically activates spermatid-specific genes encoding spermatozoon proteins through direct interaction with the Lhcgrba in these cells. Finally, we have developed specific enzyme-linked immunosorbent assays (ELISAs) for Fsh and Lh that are being employed to determine the plasma levels of both gonadotropins during the reproductive cycle. All these molecular tools will allow the design of novel methods based on recombinant hormones for the control of sperm production in Senegalese sole, and the establishment of biomarkers to monitor reproductive success in cultured stocks.

A REVIEW OF THE REPRODUCTIVE BEHAVIOUR OF SENEGALESE SOLE (*SOLEA SENEGALENSIS*)

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This paper reviews studies that have described the reproductive courtship in wild caught Senegalese sole (*Solea senegalensis*), the reproductive behavioural dysfunction in captivity bred G1 (1st generation) sole and that more recently indications of behavioural solutions to the G1 reproductive dysfunction. Sole breeders (n=16-29) were held in tanks (7.5-14m^3) with >300% water exchange, under natural conditions and fed natural prey and broodstock diet. Microsatellites paternity analysis was used to identify successfully spawning breeders. Wild breeders were observed to complete a complicated courtship behaviour that included a following behaviour and paired synchronised swimming to the surface to spawn. These following and synchronised spawning behaviours were not observed in G1 breeders and all eggs collected were not fertilised. Hormone induction (GnRHa, hCG and PGF2alpha) of G1 breeders did not induce the spawning behaviours, following and synchronised swimming, but did increase the production of unfertilised eggs. Mixed origin groups were studied and the group of G1 males and wild females did not produce any eggs, whilst the group of wild males with G1 females produced 2 fertilised spawns. Lastly groups containing successfully spawning wild breeders with G1 breeders (males and females) were studied. In the first year G1 males were observed to participate in following swimming behaviours, but did not participate in gamete release. In the second year the G1 breeders were observed and assigned (microsatellite) to participate in a few isolated spawns. This may indicate that the presence of spawning wild fish increases the participation of G1 males in courtship and spawning.

The studies were funded by INIA-FEDER projects RTA2011-00050 and RTA2005-00113 and MAPAMA - JACUMAR Sole Projects II and III

DIVERSITY AND EVOLUTION OF GENETIC SEX DETERMINATION IN FISH

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Fish show a great variety of sex determination mechanisms, which in the case of genetic sex determination is linked to a similarly high variability of sex chromosome differentiation. But this diversity does not follow any obvious phylogenetic pattern. To obtain a better understanding of the biological meaning of the diversity of sex determination and the mechanisms driving sex chromosome evolution we are attempting to decipher the molecular basis of the primary sex determination mechanisms and the structure and genetic organization of sex chromosomes across a broad diversity of rayfin fish. On the one hand we are analyzing a

broad collection of species that represent major branches of the fish tree of life and on the other hand we focus on closely related species within branches of the phylogenetic tree (Salmoniformes, Esociformes, Danios, Poeciliids). We use high throughput marker mapping in 40 species as well as transcriptomics and genome sequencing to delineate sex-specific chromosomal regions and to identify candidate sex determining genes. These strategies already led to the identification of sex-specific markers, allowing delineating the extent of recombination suppression, and candidate sex determining genes in some species. Examples taken from Salmoniformes and its sister taxonomical clade i.e, the Esociformes will be described.

GENERATION AND APPLICATION OF GENOMIC RESOURCES IN SENEGALESE SOLE AQUACULTURE

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The soles are important economically important flatfish species in Europe. The increasing demand of new high-quality marine products has determined a rapid development of there aquaculture in Sourthern Europe. Neverthess, producers have to face particular biological challenges in reproduction, larval quality and diseases management that require novel and sophisticated tools to accurately and rapidly advance in their aquaculture and convert it in a profitable activity.

The genomic research is a powerful approach to provide high-throughput information about the cellular responses that mediates key production parameters facilitating the improvement and optimization of production procedures. Moreover, genomic information is required to implement successfully breeding program that assist in the selection of high-performance animals.

The arrival of NGS technologies have favoured the development of a high-bulk of genomic resources in non-model species including the Senegalese sole, the main species produced in aquaculture. In this way, abundant information on transcriptome has been generated and hosted in a sole-specific database (named *SoleaDB*). In total, more than 1,800 millions reads from different larval and adult tissues were *de novo* assembled resulting in 701,767 tentative transcripts. Orthology analysis using zebrafish as reference identified at least 45,063 putative different transcripts, 18,738 of which were reconstructed with a complete ORF. Moreover, cross-species comparison with the closely-related species *S. solea* and other teleosts identified a set of 14,451 putative transcripts for sole- or lineage-specific genes. As a result, a reference transcriptome including 59,514 transcripts was defined and used to print a sole oligonucleotide microarray containing 43,303 probes. Moreover, a search of molecular markers identified a total of 266,434 SSRs and 337,315 SNPs in the transcriptome to be used as genetic tools.

In addition to the microarray, a platform for RNA-seq analysis using orthology annotation with zebrafish for functional analysis has been established. Moreover, some mid-density openarray chips based on qPCR have been used to validate expression results. To check the usefulness of tools developed, they have been applied to evaluate the responses that govern the adaptation to salinity of larval developmental stages. Senegalese sole develops its osmoregulatory capacity at mouth opening. Nevertheless, when larvae are exposed to low salinity before 3 days post hatching, they develop lethal morphological anomalies. Application of RNA-seq, microarrays and openarray confirmed that larvae at early stages were able to mobilize important energy reserves to cope the ionic challenge and maintain the cellular homeostasis. But, later, a collapse of osmoregulatory mechanisms induces edemas and destabilizes cell membranes provoking cellular autolysis by uncontrolled releasing of pancreatic enzymes and subsequent activation of inflammatory and antioxidative defence mechanisms.

In addition, genome analysis has been commenced. A total of ~3.000×10^6 raw reads (including single, paired-end and mate-pair reads from both 454/Roche and Illumina platforms) were processed Sequences were cleaned using SeqTrimNext, assembled with Ray, reconciled with Gam-NGS, scaffolded with SOAPdenovo2 and SSPACE, and finally gaps closed with Gap closing tool of SOAPdenovo2. Several in-house scripts were developed for contig and scaffold validation and mapping. The 132,712 contigs obtained provide 34,176 scaffolds with a N50 of 85,602 nt. The whole draft genome was ~600 Mb in size and the longest scaffold was 638 kb in length. Mapping of scaffolds onto *Cynoglossus semilaevis* draft genome located 95% of scaffolds (569 Mb in total) onto 21 chromosomes. *In silico* comparison of genetic map markers and scaffold positioning confirmed a linkage correspondence higher than 90%. Moreover, the amplification of 111 predicted SSR markers distributed through the chromosomes confirmed the accuracy of the assembly obtained. These results confirmed the high similarity of both flatfish genomes and represent new powerful tools for genomic analysis in *S. senegalensis*.

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Keywords: Sole, transcriptome, genome, draft, database, microarray

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THE MOLECULAR EVOLUTION AND FUNCTION OF AQUAPORINS IN THE PARASITIC SALMON LOUSE IN RELATION TO ITS HOST THE ATLANTIC SALMON

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The saltwater salmon louse (*Lepeophtheirus salmonis*) is an ectoparasitic crustacean copepod that causes severe infections of salmonids across the Atlantic and Pacific regions. Increasing levels of resistance to established drugs requires alternative approaches to solving the salmon louse epidemic. In contrast to its salmonid host, which is an osmoregulator, the louse´s cell volume regulation breaks down in fresh and brackish waters. These features suggest that aquaporins, which facilitate the transmembrane transport of water and other small uncharged solutes could represent potential therapeutic targets. However almost nothing is known concerning the functional diversity of aquaporins in crustaceans. To redress this lack of knowledge, we screened the genomes of the salmon louse and the Atlantic salmon and phylogenetically characterised their aquaporin superfamily repertoires in relation to those present in 131 arthropod and 121 vertebrate genomes. Subsequently we isolated and cloned seven full-length aquaporin transcripts from the louse and determined the functional transport properties of the encoded channels using a heterologous *Xenopus laevis* oocyte expression system. Our data reveal that the aquaporin repertoires in crustaceans and other arthropods can be classified into 3 major grades of aquaporins (1) classical type aquaporins including Big brain and Prip-like channels (2) aquaglyceroporins and (3) unorthodox aquaporins, while vertebrates have an additional Aqp8-type grade of channels. Functional characterization of the louse channels, reveal that the permeation properties of the arthropod grades of aquaporin are largely conserved to the vertebrate counterparts. Transcript abundance analyses and subcellular localisation studies indicate that selective aquaporins may be suitable as therapeutic targets.

CYTOKINE MEDIATION OF THE MATURATIONAL AND PRO-OVULATORY EFFECTS OF LUTEINIZING HORMONE IN TROUT

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In all vertebrates, luteinizing hormone (LH) stimulates oocyte maturation (OM) and ovulation in the female ovary through the stimulation of the local production of steroids and eicosanoids. In addition, there is evidence in mammals for the ability of proinflammatory cytokines such as TNFα and interleukin-6 (IL6) to exert LH-like effects in the ovary. Our group aims at elucidating the possible involvement of intraovarian cytokines in key reproductive events in teleost fish by investigating the mediatory role of TNFα and IL6 in the stimulatory effects of LH on OM and ovulation in salmonid fish. We show that in vitro treatment of immature preovulatory trout follicles with salmon LH (sLH) increases mRNA expression and protein secretion of TNFα2. Furthermore, trout recombinant TNFα2 (trTNFα2) stimulates OM through its stimulation of the production of the maturation-inducing progestin and inhibition of TNFα secretion completely obliterates sLH-stimulated OM, evidencing that TNFα mediates the maturational effects of` sLH in the salmonid ovary. In mature preovulatory trout follicles, trTNFα2 also mimics the stimulatory effects of LH on follicle contraction, apoptosis, proteolysis and ovulation. Interestingly, all LH- and trTNFα2-stimulated pro-ovulatory activities are in turn mediated by PGF2α. On another hand, LH and trTNFα2 stimulate IL6 mRNA expression levels in preovulatory follicles. In contrast to trTNFα2, recombinant trout IL6 did not affect OM nor proteolytic activity but stimulated follicle contraction. In summary, we provide evidence for the important, albeit different, role of TNFα and IL6 as intraovarian mediators of the action of LH in the teleost ovary.

THE USEFULNESS OF ZEBRAFISH (*DANIO RERIO*) AS A MODEL FOR AQUACULTURE RESEARCH

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In the last fifteen years, zebrafish (*Danio rerio*) has become a popular fish model in many finfish aquaculture research fields. Recently, this popularity has been fueled by the fact that in many cultured species genomic resources are becoming available, a situation that allows fruitful comparative structural and functional genomics across species. Here, we assess the characteristics that zebrafish offers as a suitable model for finfish aquaculture research describing not only the advantages but also its limitations. We describe the biological features (phylogenetic relationships, development, growth, reproduction, etc.) in wild and domesticated zebrafish strains. We first comment the work already carried out in zebrafish that is related to different aspects of aquaculture research such as stress, pathology, toxicology nutrition and growth and later explore the different resources implemented so far to facilitate research with zebrafish and also highlight the remaining shortcomings. We illustrate the usefulness of using zebrafish as a model for reproduction-related research by presenting data performed in our laboratory, describing, among others, the environmental effects on sex differentiation process in zebrafish. Finally, we conclude that the implementation of zebrafish as a model organism in aquaculture research will continue in the following years and zebrafish will likely play an important tool for many finfish aquaculture research areas.

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1. DESAPARICIÓN DE OYSTER HERPES VIRUS TIPO 1 (OSHV-1) DEL DELTA DEL EBRO, ESPAÑA

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De hace más de una década que se detectan mortalidades en las ostras del Pacífico en Cataluña. La vigilancia por parte del IRTA del virus OsHV-1 en *C. gigas* aumentó en 2005 debido a las mortalidades recurrentes. En 2008 y 2009 las zonas de cultivo de la ostra del Pacífico (*C. gigas*) a lo largo de toda la costa francesa experimentaron gran aumento de la mortalidad. OsHV-1 se detectó por primera vez en Cataluña en el año 2005 con una prevalencia muy baja. La vigilancia de la presencia de OsHV-1 y/o OsHV-1 µvar ha sido continua durante los últimos ocho años en el marco de varios proyectos del grupo. Presentamos aquí un breve resumen de algunos de los análisis genéticos de muestras de ostras recolectada durante este período de tiempo.

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2. EFFECT OF DIETARY FATTY ACID COMPOSITION ON FOOD INTAKE IN *SOLEA SENEGALENSIS* LARVAE IN RELATION TO THE EXPRESSION OF KEY REGULATORY GASTROINTESTINAL AND NEUROPEPTIDES

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Food intake is one of the key factors governing growth. However, larval nutritional studies have so far mostly focused on essential fatty acid requirements and there are practically no studies on the effects of diet composition on food intake in fish larvae. Furthermore, results from juvenile or adult animals cannot be extrapolated given that fish larvae have much higher requirements for energy and structural components for rapid growth and organogenesis/metamorphosis, as well as a structurally and functionally less developed digestive tract, which is likely tied to differences in metabolism. However, these types of studies are especially difficult to conduct in fish larvae for a wide number of biological and technical reasons. In this study we employed a method of labeling *Artemia* using fluorescent microspheres to quantify ingestion and investigate food selectivity in response to the fatty acid (FA) composition of the diet in Senegalese sole larvae and post-larvae. We tested four treatments of live prey enriched with lipid emulsions differing in the oil source that was used - cod liver oil (CLO), linseed oil (LSO), soybean oil (SBO) and olive oil (OO) – from first feeding (enriched rotifers from 2 to 8 days post hatching -dph; enriched *Artemia* metanauplii from 6dph onwards). The results showed that larvae fed the CLO diet had a significantly higher dry weight at 22dph and 30dph, followed by LSO (not significantly different from the SBO and OO treatments). The ingestion trials performed at 19dph and 35dph showed a significantly higher intake (number of *Artemia* per mg of wet weight of larvae) of the CLO diet. However, when presented with a choice of the four diets, post-larvae at 29dph and 30dph did not show a preference for any of the diets. We were therefore able to demonstrate that the FA composition of enriched *Artemia* can affect food intake during the larval (pelagic) and post-larval (benthonic) stages and that the most ingested diet (CLO) was also responsible for higher growth. The expression of several anorexigenic (POMCa, POMCb, CRH, CCK-L, CART1, CART5, PYYa, PYYb) and orexigenic (NPY, AgRP2) gastrointestinal and neuropeptides was assessed just before feeding and at 30min, 1h 30min and 3h after feeding, in whole larvae (at 16dph) and separate head and body compartments (at 34dph). Most significant changes in gene expression with relation to time were observed at t0 and t1 and the CLO diet induced most significant differences in levels of gene expression, especially in relation to OO (up or down regulated depending on gene).

3. PRELIMINARY TESTS GROWING SALICORNIA (SALICORNIA EUROPEA) & SEABASS (*DICENTRACHUS LABRAX*) WITH MARINE AQUAPONIC TECHNOLOGY IN THE EBRO DELTA

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Aquaponics is a combination of both hydroponics and aquaculture. Marine aquaponics system applies this technology with marine aquatic organisms and plants that can be grown in sea water systems. Aquaponics systems are a symbiotic system where fish effluent provides nourishment to plants, while plants, in return, filters toxic fish waste from the fish tank water. Salicornia is a halophytes plant, which can be irrigated with salt water. It is a plant with a high content of n-3 fatty acids and a high market price, usually used in salads.

A preliminary tests was conducted for growing Salicornia (Salicornia europea) & Seabass (Dicentrachus labrax) with marine aquaponic technology . With this technology it is possible to reduce the water consumption and the environmental impact with a double benefit .During the trial we test the growing of fishes and plants compared with blank test. We also did a comparative between the composition of wild and cultivated salicornia.

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4. PRELIMINARY TESTS GROWING TENCH (TINCA TINCA) WITH BIOFLOC TECHNOLOGY

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Biofloc Technology (BFT) is based on bioflocs. The bioflocs are conglomerates of microbes, algae, protozoa and others, together with detritus, dead organic particles. The water exchanged along the growing process is zero or minimal. Organic residues accumulating in the pond under such conditions degrade, and ammoniums nitrified or assimilated, by an intensive microbial community. This series of processes replace the conventional external biofilter or a high and expensive water exchange, by maintaining a high C/N ratio and inducing the uptake of ammonium by the microbial community (Avnimelech et al., 1994; McIntosh, 2000). A by-product of this is the growth of the microbial community and the production of microbial protein harvesting of the bio-flocs by fish serves as an addition of high value feed, recycling of the non-utilized fraction of the feed and was shown to double the utilization of protein and feed by fish or shrimp (Avnimelech et al.,1989; McIntosh, 2000; Velasco et al., 1998). A preliminary trial was conducted in IRTA to test the viability of on-growing of Tenca (*Tinca tinca*) with BFT system.

5. NEW ADVANCES IN MEAGRE (*Argyrosomus regius*) CULTURE. RESULTS OF THE EU DIVERSIFY PROJECT IN 2014 AND 2015

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Introduction

The meagre, A*rgyrosomus regius,* is a sciaenid distributed in the Mediterranean and Black Sea and along the Atlantic coasts of Europe and Africa (Poli *et al.*, 2003). The fish can grow up to 2 m and reach 50 kg in the wild (FAO, 2005-2010). Aquaculture of meagre began in the late 1990s in France with an annual production of 30 t in 1997. In 2009, production reached 2200 t in Egypt, 1348 t in Spain, 418 t in France, 102 t in Italy and 44 t in Portugal (FAO, 2011). Ongrowing techniques are similar to those used for European seabass, *Dicentrarchus labrax*and gilthead seabream, *Sparus aurata*), principally large circular (25 m diameter) sea surface cages. Meagre is a promising aquaculture species for its high growth rate (around 1 kg per year) and good feed conversion ratio. Meagre flesh quality is very well considered by the consumer due to its low muscle fat content (Poli *et al.*, 2003; Monfort 2010; Grigorakis *et al.*, 2011). The meagre is also interesting for recreational purposes (aquaria), considering its high adaptation to captivity and the wide range of temperature and salinity tolerated. Until recently, all juvenile production of meagre was from a few hatcheries (FAO, 2005-2010). This situation has at times restricted the availability of juveniles to the industry and, therefore, potentially restricted the growth of aquaculture of meagre. DIVERSIFY project identified several bottlenecks for aquaculture production of meagre and in this presentation the most important contributions of DIVERSIFY project to the culture of meagre during the years 2014 and 2015 are summarized. Advances obtained in reproduction, genetics, larval culture, nutrition, ongrowing and health of this species will enable the industry to solve the bottlenecks in meagre culture. Collected results for each of these areas of investigation will be presented during the congress.

Results and Conclusions

1.- Reproduction. Genetic screening of the available captive meagre broodstocks in Europe using 2 microsatellite multiplexes has been completed in FCPCT. The broodstocks examined seem to pertain into 3 clusters and had enough genetic variation to start a breeding program with selected families. Moreover, transcriptome sequencing through RNA sequencing (RNA-Seq) in Illumina HiSEq platform was performed and lead to the identification of thousands of SNP and polymorphic microsatellite markers in the species. Paired crossing has been carried out in IRTA using hormone induction and natural spawning. Differences in egg production and quality not related to maturity status or repeated spawnings or inductions were observed. On the other hand experiments carried out at HCMR to determine the number of successful spawnings after weekly hormonal inductions resulted in 17 consecutive spawns with high quality eggs and high hatching rates and larval survival. Thus, paired crossing in meagre is possible and can be used in selective breeding programs

2.- *Larval culture*. Weaning was identified as one of the main bottleneck for the species. To solve the problem, 2 trials were performed in 2014 and 2015 to establish the best weaning practice. In 2014 high cannibalism was observed related to early weaning (starting at 12 days post hatch). In 2015 a new trial in collaboration with HCMR was carried out.

3.- *Nutrition*. Requirements of meagre for n-3 LCFA and its interrelation with vitamins E and C were studied by FCPCT in 2014. The results obtained suggest that meagre has a high requirement for HUFA to promote growth and vit E and C to prevent fatty acid oxidation. Weaning diets for meagre larvae should include these components to avoid stress related to handling. The requirements for essential fatty acids during ongrowing were also studied by FCPCT and Skretting using six different levels of DHA, EPA and ARA.

4.- *Ongrowing*. Two experiments of size variability in juveniles were carried out in IRTA in 2014 and 2015. Juveniles from 6 families were mixed and stocked in tanks with the same initial density and feeding. After several months the size distribution in the tanks was compared, and the fish graded (large, medium, small) and monitored. Growth potential was similar among the graded groups. The effect of cage depth was studied at HCMR in cages of 180 and 290 m^3. Vertical distribution was monitored using an echo integrator showing that fish are usually located in the lower half of the cage during the daylight period while they show a homogeneous distribution in the whole cage volume during the dark period of the day.

5.- Health. The study of the immune system in meagre and its ontogeny is being carried out in collaboration between IRTA and University of Aberdeen. Several tissues (spleen, head kidney, gills, intestine) were collected during the larval development and ongrowing of meagre. Genes for the characterization of the immune system of meagre are being identified and after all gene expression assays have been fully validated, the expression of the chosen target genes will be assessed for different developmental stages in each tissue. Granulomatosis has been identified as a major pathological condition for meagre and is considered to be related to nutritional factors. In the first trial we have examined the effect of various levels of vitamin D addition on the development of the disease in juvenile fish. The initial assessment of the results of a 3-month feeding trial indicated that vitamin D did not affect the development of granulomas at any of the doses tested. Further, various attempts have been made to isolate bacteria from affected meagre and to investigate if the disease is related to *Nocardia* sp. However up to date the few bacterial strains isolated are not pathogenic and no one has been identified as *Nocardia* sp. An acceptance trial, feeding juveniles with mediacated feeds containing essential oils with vermicide characteristics was conducted, samples were taken in the end for histology and immunology. Results will be presented during the congress.

Acknowledgements

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6. THE BENEFITS OF THE MEDITERRANEAN DIET IN FISH: THE EFFECTS OF CONCENTRATED OLIVE OIL IN GROWTH PERFORMANCE AND GUT CONDITION IN GILTHEAD SEA BREAM (*SPARUS AURATA*)

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The benefits of the Mediterranean diet and olive oil are well known in human health. In this study, concentrated olive oil rich in polyphenols and triterpenic compounds (MAC-60, Natac Biotech SL) was tested at five different levels (0; 0.78; 1.63; 3.15; and 5.90 g MAC-60/Kg feed) using a basal diet (53% protein, 18% lipids; fish oil in feeds was substituted by MAC-60). Diets were tested by quadruplicate for 90 days at 22ºC in gilthead sea bream (initial weight = 5.4 g). At the end of the study, fish fed diets containing 1.63 and 3.15 g MAC-60/Kg feed showed the best results in terms of growth performance ($P<0.05$), which improved on average 5% over those fed the control diet (60.7 vs 57.7 g). No differences in the proximate composition of the fillet were found, although fish fed diets containing concentrated olive oil tended to have higher levels of proteins and lipids. The histological analysis of samples revealed that fish fed 1.63 g MAC-60/Kg feed had a higher number of goblet cells in the intestinal mucosa when compared to the control ($P<0.05$), whereas no differences in villi height were observed among groups. The analysis of 90 gene markers of the intestinal mucosa by quantitative PCR revealed that 33% of the analysed genes were differentially expressed. In particular, MAC-60 increased the expression of genes associated with pathogen recognition, proinflammatory processes and interleukins, and reduced the oxidative stress in enterocytes' mitochondria. These results indicated that the inclusion of concentrated olive oil in on-growing diets for fish may be beneficial in terms of growth performance, as well as regarding the health and condition of the intestinal mucosa, a key organ in digestion and one of the the first barriers to entry of pathogens into the body.

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7. FISH MEAL SUBSTITUTION IN DIETS FOR FLATHEAD GREY MULLET (*MUGIL CEPHALUS*) FRY

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The flathead grey mullet is among the species considered as having an interesting aquaculture potential due to its lowest trophic level and tolerance to a relatively wide range of culture conditions. The formulation of a grow-out feed with a low inclusion of fish meal (FM) is a must for the sustainable development of the aquaculture of this species. Thus, the effects of different levels of FM substitution (0, 50 and 75%) in diets (36% protein, 16% fat; corn gluten, wheat gluten and soy protein concentrate were used as alternative protein sources) for flathead grey mullet fry was evaluated in terms of growth performance, survival, proximate composition and digestive enzyme activities. For this purpose, early weaned flathead grey mullet fry (0.20 g) were reared in quadruplicate in 100 L tanks (100 fish/tank) at 18.1 ± 0.3ºC in freshwater (1-1.5 ‰) in a RAS system for 40 days. At the end of the study, there were not differences in weight (0.68-0.71 g), standard length (3.1-3.2 mm) nor survival (96-98%) of fish fed diets with different levels of FM substitution, neither in their proximate composition or the activity of intestinal (alkaline phosphatase, maltase, aminopeptidase-N) and pancreatic (lipase, α-amylase, total alkaline proteases and trypsin) digestive enzymes. These results indicate that feeding grey mullet fry with diets with a 75% of FM substitution does not compromise their growth performance and digestive capacities.

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8. REGULATION OF LIPID METABOLISM IN GILTHEAD SEA BREAM LIVER AND ADIPOSE TISSUE BY A LONG-TERM RECOMBINANT BOVINE GROWTH HORMONE TREATMENT

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Growth hormone (GH) is the main factor regulating growth, but direct lipolytic effects under catabolic situations have also been described. We have studied *in vivo* the effects of long-term sustained-release of recombinant bovine GH (rBGH) in gilthead sea bream. Twelve weeks post-injection, blood, liver and adipose tissue samples were collected. Results from plasma analyses showed a significant decrease in triglyceride levels, whereas no differences were observed in glycerol and non-esterified fatty acids, suggesting a moderate increase in the lipolytic capacity of rBGH-treated fish. qPCR analyses showed up-regulation of hormone sensitive lipase (HSL) in response to rBGH in the liver, supporting the lipolytic effect of GH. A significant increase was observed also in liver X receptor (LXR) and glucokinase gene expression in rBGH-treated fish compared to controls, suggesting an involvement of GH in glucose or cholesterol homeostasis too, whereas the other genes analysed did not show any differences between treatments. Regarding adipose tissue, lipoprotein lipase was upregulated in rBGH-treated fish, while HSL levels decreased, indicating a metabolic adjustment of lipogenic genes. No differences were found in fatty acid synthase, peroxisome-proliferator activated receptor-γ or LXR expression. Additional qPCR analyses are being performed to further elucidate the effects of this treatment on the GH/insulin-like growth factors axis. These findings demonstrate the important role of GH in lipid mobilization and energy balance in fish, and help us to better understand how GH acts regulating gilthead sea bream metabolism. Thanks to Elanco Animal Health for kindly providing the rBGH. Supported by MINECO (AGL2011-24961; AGL2012-39768); 2014SGR-01371.

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9. IN SILICO SINGLE NUCLEOTIDE POLYMORPHISM (SNP) SEARCH IN GENES ASSOCIATED WITH IMMUNE RESPONSE AGAINST INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) IN ATLANTIC SALMON (*SALMO SALAR*)

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Currently, studies for the understanding of fish-IPNV interaction are based on functional genomics using microarrays to find differentially expressed genes (DEG), or cDNA libraries to annotate candidate genes associated with a given phenotype. Since the identification and selection of DEG do not explain the individual phenotypic variability in the response against IPNV, it is necessary to identify molecular markers being the most common the single nucleotide polymorphisms (SNPs). The present work was focused on the search, identification and selection of SNPs in genes of immunological interest in Atlantic salmon challenged with IPNV. All available sequences for immune-related genes were taken from Unigene database (NCBI) and used as target to search SNPs from the same transcription locus using bioinformatic tools. The effect of synonymous and non-synonymous substitutions was analyzed based on the predicted amino acid sequence. Several synonymous SNPs were found in CCL19, CD18, HSP70, and MHC class I, while three non-synonymous SNPs were found with high-predicted risk factor on the gene encoding the glutathione S-transferase omega 1 (GSTO-1). By homology modeling, we confirmed these SNPs in GSTO-1 affect the 3D protein structure, particularly the beta1 sheet at the N-terminal domain (affecting the beta/alpha structure), the glutathione binding site (G-site) at the active site, and the helix alpha8 at the C-terminal domain (H-site). The presence of these SNPs opens the possibility that immune-relevant genes would be associated with individual phenotypic variations in the response against IPNV in salmonids.

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10. EFFECTS OF LONG-TERM RECOMBINANT BOVINE GROWTH HORMONE TREATMENT ON GH/IGFS AXIS AND OSTEOGENIC-RELATED GENES EXPRESSION IN GILTHEAD SEA BREAM BONE TISSUE

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Fish growth is regulated by environmental, nutritional and hormonal factors, among which, growth hormone (GH) plays a major role. In mammals, both GH and the insulin-like growth factor-I (IGF-I) stimulate in bone, osteoblasts recruitment, proliferation and activity; however, the specific role of GH/IGFs in fish bone

homeostasis has been less explored. In the present study we investigated *in vivo* the involvement of GH in bone tissue regulation in gilthead sea bream (*Sparus aurata*). We assessed the effects of long-term (12-weeks) recombinant bovine GH (rBGH) administration on osteogenic and GH/IGFs axis genes expression. Results showed a significant down-regulation of IGF-I receptors (IGF-IRa and IGF-IRb) mRNA levels on rBGH-treated fish, whereas both IGFs, GH receptors (GHR-I/II) and IGF binding proteins (IGFBP-4/5) did not show any differences. These findings suggest that GH treatment may have indirectly leaded to down-regulation of IGF-IRs as a negative feedback response due to elevated IGF-I plasma levels. Regarding bone-related genes, rBGH-treated fish showed up-regulation of collagen1A1 and non-collagenic proteins such as osteocalcin, osteopontin and alkaline phosphatase, indicating increased activation of bone extracellular matrix synthesis and mineralization as well as bone resorption. Nevertheless, other osteogenic-related genes like fibronectin1a, osteonectin and matrix gla-protein remained unaffected after treatment. These results lead us to conclude that GH plays an essential role regulating bone homeostasis in gilthead sea bream, helping to better understand the endocrine regulation of fish skeletal growth, which may contribute to improve aquaculture production. Thanks to Elanco Animal Health for kindly providing the rBGH. Supported by MINECO (AGL2010-17324; AGL2012-39768); 2014SGR-01371.

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11. EFFECT OF DIETARY FISH OIL SUBSTITUTION BY VEGETABLE OILS ON GROWTH PERFORMANCE AND PLASMA PARAMETERS IN GILTHEAD SEA BREAM

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Aquaculture is one of the fastest growing sectors nowadays; nevertheless, the production of fish meal (FM) and oil (FO), the main sources of nutrients for aquafeeds, may not be enough to satisfy the increasing demand. Vegetable products are interesting source alternatives; however, their use may cause imbalances being inadequate to obtain optimum product growth and quality. The aim of this experimental trial was to study the effect of partial dietary FO substitution by palm (PO), rapeseed (RO) or soybean (SO) oils, each one with different fatty acids profile, on somatic and plasma parameters in gilthead sea bream. Significant differences among groups were not found for body weight and length, condition factor, liver and adipose tissue weights, hepatosomatic and mesenteric fat indexes and gut length, although we observed certain tendencies where the PO diet appeared to have caused a bit higher fat deposition compared to the other groups. Plasma values for glycerol, glucose and non-esterified fatty acids (NEFA) showed significant differences among diets, indicating different adaptations regarding lipid and glucose metabolism depending on the dietary lipid source; however, no differences were found in the amount of triglycerides. In this sense, the PO diet presented the highest glucose and glycerol plasma levels and the lowest circulating NEFA, contrary to the SO diet, whereas the RO diet was intermediate for those parameters. Currently we are performing analysis of lipid metabolism-related genes expression to better understand the effects of these diets in gilthead sea bream growth and metabolism in order to optimize diet formulation.

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12. PROTEIN-NANOPARTICLES AS IMMUNOSTIMULANT-DELIVERY PLATFORM FOR FISH IMMUNIZATION

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The massive increase in farmed animal production and its subsequent related diseases have put greater emphasis on the development of new prophylactic tools in the field of animal health. In this context, fish diseases have been identified as an important limiting factor for aquaculture production being bacteria the most common cause of infectious disease. Thus, the development of efficient and cost-effective prophylactic tools is becoming a critical need in this field. Bacterial inclusion bodies (IBs) are protein deposits naturally occurring during recombinant protein production in microbial hosts. These aggregates are sub-micron, mechanically stable and biocompatible protein particles that have been shown able to retain a certain grade of the forming protein biological activity. IBs have extensively shown their potential in biotechnological approaches such as their straightforward use as biocatalysts. In addition, some applications of IBs in the biomedical context have recently risen, from their use as topographical modifiers for tissue engineering to the development of protein nanopills for protein-replacement cell therapies[1].
The present work aims to propose bacterial IBs as a *protein delivery platform* for the immunization and further protection against a model bacterial infection in zebrafish (*Danio rerio*)[2]. Specifically, the IBs used in this study are formed by the reporter green fluorescent protein (GFP) fused to the VP1 protein of the capsid of the foot-and-mouth disease virus produced in *E. coli*. *In vitro* assays using the ZFL cell line and macrophages from rainbow trout showed a clear uptake of the protein particles and non-toxic effects were observed due to the addition of the IBs to the cell cultures. Moreover, *in vivo* survival tests after immunization with IBs and posterior infection with *Pseudomonas aeruginosa* revealed that IB protects the fish in a dose dependent manner. Comparative analysis using particles produced in LPS free *E. coli* strains and different recombinant proteins showed that the protective effects observed were not related with the LPS content in IBs.

In summary bacterial IBs show promising properties to become an efficient immunization system for farmed fish based on the slow protein release and high particle stability.

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13. GENE EXPRESSION ANALYSIS IN GONADS OF ZEBRAFISH (*DANIO RERIO*) SUBJECTED TO MASCULINIZATION TREATMENTS

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Fish exhibit all types of reproduction systems known in vertebrates in which sex determination and sex differentiation establish the final gender. Several environmental factors such as density, temperature or toxicity have been described to affect these two processes in fish, altering the final sex ratio. It is known that fish subjected to stress release higher cortisol plasma levels. Cortisol can, in turn, masculinize fish. In previous experiments using zebrafish (*Danio rerio*) in our laboratory, we have observed masculinization in fish subjected to either high density or cortisol-treated diets. Here, we aimed to understand the molecular mechanisms by which fish are masculinized by density and cortisol. For that purpose, we assessed gene expression in juvenile ovaries and testes by RT-PCR of four canonical reproduction-related genes, five genes involved in the glucocorticoid pathway and one epigenetic gene in fish subjected to different densities and cortisol treatments, during 6–90 and 10–45 days post fertilization, respectively. Results showed a significant down-regulation of glucocorticoid receptor (*nr3c1*) in ovaries and testes and also down-regulation of DNA-methyltransferase 1 (*dnmt1*) and gonadal aromatase (*cyp19a1a*) in ovaries of fish subjected to high density. On the other hand, no differences in any of the studied genes were found in testes of fish fed with cortisol-treated diets. In conclusion, this study shows that high density induced masculinization, involving changes in the expression of key sex differentiation genes, notably the downregulation of aromatase.

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14. CORTISOL MODULATES THE IL-1B GENE EXPRESSION

in primary head kidney cell culture: A comparative response between rainbow trout (*Oncorhynchus mykiss*) ad gilthead seabream (*Sparus aurata*).

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Stress is a situation in which an animal is subjected to a challenge that may result in a danger for its integrity. Fish are regularly exposed to severe environmental and pathophysiological stressors which activate a signal transduction cascade and gene expression that mediate the physiological response and may cause many different alterations in the immune system affecting performance, health and welfare. There is little information on the interaction between stress-related hormones and mediators of the immune system in fish and their effects on health performance. In this ambit, the effect of antagonists to reverse the hormone effect and its consequence at gene expression level in cells exposed to pathogens has not been elucidated. Thus, we designed an in vitro experiment to evaluate the correlation between immune and endocrine responses in the head kidney, a main organ regarding immuno-endocrine interactions, focusing in the effects of cortisol and ACTH on IL-1ß expression in trout and seabream. Primary head kidney cell cultures (pHKCC) were preincubated with antagonists for cortisol (RU486, spironolactone) and ACTH (RU486) and then stimulated with their respective hormones and formalin-killed *Vibrio anguillarum* (FKVA). The results in sea bream show a down-regulation in IL-1ß expression mediated by cortisol, both in non-treated and FKVA cells. This effect was reversed when pHKCC were preincubated with the antagonists. The same effect is observed in trout, although the response against FKVA is much higher. A higher response in trout than in seabream was observed when cells were stimulated with ACTH, although without significant differences. The results suggest a direct correlation between cortisol in the IL-1ß gene expression modulation, providing new evidence of the neuroendocrine and immune system interaction in teleosts.

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