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ASOCIACIÓN IBÉRICA DE ENDOCRINOLOGÍA COMPARADA

BOOK OF ABSTRACTS

16 - 17 September 2021

XIII Congress of AIEC | Online

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16 - 17 September 2021
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WELCOME

The XIII AIEC Meeting reunites online

The Organizing Committee of the XIII Congress of the Iberian Association of Comparative Endocrinology - AIEC (online), the Center for Marine Sciences and the University of Algarve, together with the AIEC Board of Directors, welcome all participants to the 13th AIEC.

This will be the first time that the congress is held online, we hope it will be an exciting congress to meet and discuss our latest results. We will look forward for the next presential meeting, to be held at Bilbao in 2023.

The organizing committee

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PROGRAMME

Thursday, 16th of September 2021

	14:30		**Welcome ceremony**
	14:45		**General organization**
	15:00 - 15:30	**OL**	**Opening Lecture (OL) - Prof. Oliana Carnevali** Environmental contaminants of anthropogenic origin: a threat to wildlife and human health *Chairs: Isabel Navarro, Jose Luis Soengas*
Session 1: Neuroendocrinology and rhythms *Chairs: Mª Jesús Delgado, Jesús Míguez*	15:45	**O1**	**Inmaculada Rodríguez** "Daily and seasonal rhythms of lipid metabolism and fatty acids profile in the European sea bass"
	16:00	**O2**	**Elisa Samori** "Daily rhythms in the stress response of the European sea bass (*Dicentrarchus labrax*): effects of seasonality and feeding time"
	16:15	**O3**	**Alejandra Godino** "The role of melanocortin system in the regulation of circadian rhythms in fish"
	16:30	**O4**	**Diego Madera** "Temperature and fasting: two synchronizers of nocturnin expression in goldfish?"
	16:45	**O5**	**Zhi Li** "Allatostatin-C-like (AST-C-like) as a putative regulatory factor of the mantle in the Mediterranean mussel (*Mytilus galloprovincialis*)?
	17:00	**O6**	**Nuria Saiz Aparicio** "Looking for peripheral photoreception in goldfish (*Carassius auratus*)"
	17:15		**Coffee break**
Session 2: Immune system and osmoregulation *Chairs: Thomas Knigge, Juan Miguel Mancera*	17:30	**O7**	**Maoxiao Peng** "Complement *c345* subunit *"a"* in protostomes is a chemotaxin of bivalve haemocytes and a functional homologue of deuterostome *c5a"*
	17:45	**O8**	**Yawei Shen** "Expression and functional analysis of hepcidin from mandarin fish (*Siniperca chuatsi*)"
	18:00	**O9**	**Esther Leal** "Role of melanocortin system in the zebrafish skin physiology"
	18:15	**O10**	**Andre Barany** "Two different corticosteroids, cortisol and dexamethasone, modulate the osmoregulatory response after hypersaline transfer in the gilthead seabream (*Sparus aurata*)"
Posters sessions	18:30 - 19:45	**P1 - P30**	**P1 - P30** displayed in different ZOOM breakout rooms (details in the posters list)

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Session 3: *Reproduction* *Chairs: Francesc Piferrer, Alicia Felip*	09:00	**O11**	**Núria Sánchez Baizan** "Gonadal transcriptome dynamics during sex differentiation across vertebrates"
	09:15	**O12**	**Alessia Mascoli "**Sex-specific subfunctionalization of gsdf duplicates in early gametogenesis of sea bass"
	09:30	**O13**	**Gonzalo de Alba "**Combined effects of rearing temperature regime and thermal treatment on the sex differentiation process of nile tilapia"
	09:45	**O14**	**Paul George Holhorea "**Male sex reversal is nutritionally and genetically regulated in sea bream: insights in welfare and reproductive resilience"
	10:00		**5 minutes break**
	10:05	**O15**	**Cinta Zapater "**New role of *amh* as a steroidogenesis promoting hormone in European sea bass (*Dicentrarchus labrax*)"
	10:20	**O16**	**Laura Sempere "**Changes in body weight and plasma levels of reproductive hormones in prepubertal sea bass females with advanced gonadal growth"
	10:35	**O17**	**Anthony Nzioka "**Molecular pathways involved in ovarian follicular atresia in European hake (*Merluccius merluccius*)"
	10:50	**O18**	**Diana Santos** "Expression of estrogenic biomarkers in the liver of brown trout males in distinct reproductive phases"
	11:05		**Coffee break**
Session 4: *Growth and development* *Chairs: Joaquim Gutiérrez, Deborah Power*	11:15	**O19**	**Sandra Navarro** "Decreased activity of the melanocortin system in zebrafish enhances growth during early adulthood but has no impact in puberty"
	11:30	**O20**	**Laura Guerrero-Peña** "Brains in metamorphosis. Spatiotemporal transcriptome dynamics in hatchery-reared flatfishes"
	11:45	**O21**	**Silvia Beato** "Reprogramming of epigenetic marks during early development across vertebrates"
	12:00	**O22**	**Lisen Li** "IGF2BPs as novel RNA-binding proteins that modulate early fish development"
	12:15		**5 minutes break**
	12:20	**O23**	**Babak Najafpour** "Ontogeny of insulin-like growth factor binding proteins (IGFBP) during gilthead sea bream, *Sparus aurata*, development"
	12:35	**O24**	**Fernando Naya-Català** "Transcriptome profile of GH/IGF system shows an early tissue-specific differentiation and hypoxia responsive genes in sea bream"
	12:50	**O25**	**Sheida Azizi** "First results on gene transfection in fish muscle cells"
	13:05 - 13:45		**Lunch**

Session 5: *Food intake and metabolism* *Chairs: Jaume Pérez-Sánchez, Pedro Guerreiro*	14:00	**O26**	**Mauro Chivite** "Dopamine, a controversial player in the feeding regulation in teleost fish"
	14:15	**O27**	**Sara Comesaña "**LAT1 and SNAT2 carriers are involved in leucine sensing in brain of Atlantic salmon (*Salmo salar*)"
	14:30	**O28**	**Jessica Calo "**First evidence for the presence of fatty acid sensing mechanisms in the rainbow trout gastrointestinal tract"
	14:45	**O29**	**Sara Balbuena-Pecino "**Benefits of dietary hydroxytyrosol on musculoskeletal growth and hepatic metabolism in gilthead sea bream (*Sparus aurata*)"
	15:00	**O30**	**Catarina Basto-Silva "**Dietary protein/carbohydrate ratio and feeding frequency affect gilthead seabream (*Sparus aurata*) appetite regulation mechanism"
	15:15	**O31**	**Aitana Alonso-Gómez "**IGFBP1 and IGFBP2 in goldfish: distribution, rhythmicity and influence of environmental zeitgebers"
	15:30		**5 minutes break**
	15:35 - 16:05	**CL**	**Closing Lecture (CL) - Prof. Rui Oliveira** Title here: *Chair: Adelino Canário*
	16:20		**General Assembly**
	16:50		**Closing ceremony**

OPENING LECTURE (OL)

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ENVIRONMENTAL CONTAMINANTS OF ANTHROPOGENIC ORIGIN: A THREAT TO WILDLIFE AND HUMAN HEALTH

Oliana Carnevali

Email: o.carnevali@staff.univpm.it

Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

Abstract

The lifestyle of the last fifty years has altered our planet, many chemical compounds that have become part of our daily life can have negative effects on the health of organisms and concern for environmental contaminants with hormonal disrupting activity is increasing. Exposure to endocrine disrupting chemicals (EDCs) has emerged as a major cause of health damage in wild populations and humans. EDCs can cause alteration of reproductive processes (fertility, sperm count and quality reduction, abortion rate rise...) and metabolic disorders (lipid metabolism alterations, obesity, diabetes, etc) as well as impair intellectual capacity (attention deficit, hyperactivity disorder...). Given the high consumption of plastics, concern about plasticizer and microplastic effects is increasing. Clear evidence on the negative effects of phthalates and BPA on metabolism and reproduction of marine and freshwater organisms are available. Significant reductions of fecundity have been found with these pollutants, and in addition, environmental levels of DiNP can induce follicular atresia after chronic exposure, affecting reproductive performance of female zebrafish. Plasticizers can also alter the Endocannabinoid System and consequently, gonadal function in both male and female fish.
Stable epigenetic effects of chronic exposure to environmental concentration of BPA in zebrafish, evidencing functional and transgenerational alterations have been also proven.
Regarding microplastics, generated by the mechanical disruption of plastic waste, they can move from the environment to living organisms. Recently, by means of Raman microspectroscopy, microplastics were identified either on the fetal and maternal side of human placentas and in the chorioamniotic membranes.
Additional studies to establish whether these microplastics, deriving from polypropylene, thermoplastic polymers, or from artificial coatings, cosmetics and personal care products, can be harmful for pregnancy. An overview of the EDC hazards on wildlife and human health will be presented.

Acknowledgments

Thanks to my collaborators for their commitment to provide guidance to improve the environmental sustainability.

CLOSING LECTURE (CL)

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EVOLUTIONARY CONSERVED ROLE OF OXYTOCIN IN THE REGULATION OF SOCIAL BEHAVIOR

Rui F. Oliveira

Email: ruiol@ispa.pt

ISPA – Instituto Universitário, Lisboa, Portugal & Instituto Gulbenkian de Ciência, Oeiras, Portugal

Abstract

Here, using mutant and transgenic lines of zebrafish we show a key role of oxytocin signaling in the regulation of social behavior. First, we show that early developmental perturbation of oxytocin neurons affects the neurodevelopment of specific dopaminergic neurons and blunts neuronal and behavioral responses to social stimulus in adulthood. These findings indicate that oxytocin neurons have a developmental role, namely to shape the anatomical and functional maturation of neural systems that are necessary for social behavior. Secondly, we show that oxytocin is necessary and sufficient for social contagion of fear to occur in zebrafish. Using molecular reporters of brain activity, we also identify subpallial brain regions whose activity responds to the behavioral task. Moreover, we disentangle the contribution of motor imitation and emotion recognition for social contagion of fear, showing the involvement of the latter. Given the established role of oxytocin in the development of social behavior and in social contagion of emotions in mammals, our results show that the involvement of oxytocin in social behavior is evolutionary conserved in deep time (i.e. since the divergence of teleost fish and tetrapods in vertebrate evolution) despite the fact that it modulates different neural substrates in fish and mammals.

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ORAL COMMUNICATIONS

DAILY AND SEASONAL RHYTHMS OF LIPID METABOLISM AND FATTY ACIDS PROFILE IN THE EUROPEAN SEA BASS

Inmaculada Rodríguez [1], E. Samori [1], D. González-Silvera [2], J.A. López-Jiménez [1], F.J. Sánchez-Vázquez [1], J.F. López-Olmeda [1]

Email: inmaculada.rodriguez4@um.es

[1] *Department of Physiology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain*

[2] *Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, FK9 4LA Stirling, Scotland, UK*

Abstract

Fish have daily rhythms synchronised mainly to the light/dark cycle. However, food may be a stronger synchroniser than light for some peripheral organs. In fish, the daily rhythms of mRNA expression of enzymes involved in lipid metabolism have been studied in several species. However, the daily rhythms of the fatty acid profile have not yet been studied. The aim of this experiment was to investigate the existence of daily rhythms in the lipid profile liver and plasma, as well as the rhythms of expression of enzymes involved in lipid metabolism in the European sea bass. In addition, we tested the relative strength of two synchronisers, to the light/dark cycle and feeding cycles, and whether these rhythms present seasonal differences. To this end, two samplings were carried out, the first one in December and the second one in June. For each sampling animals were divided into two groups: one fed in the middle of the light phase (ML) and the other one fed in the middle of the dark phase (MD). Throughout the experiment, fish were kept under natural photoperiod and temperature conditions. Samples were collected at different points over a 24-hour period (ZT0.5, ZT4, ZT7.5, ZT12, ZT16, ZT20 and ZT24.5 h), with ZT0 h belonging to light onset. The lipids profile was analysed by gas-liquid chromatography. In addition, the mRNA expression of several genes from lipid metabolism processes (lipogenesis and lipolysis) was analysed by qPCR. Several fatty acids displayed rhythms in ML and MD group, the acrophases were located around the middle of the dark phase and at the beginning of the dark phase for ML and MD, respectively. Lipolysis-related genes showed nocturnal acrophases, while lipogenesis-related genes showed diurnal acrophases.

Acknowledgments

This research was funded by project "CHRONOLIPOFISH" (RTI2018-100678-A-I00), granted by the Spanish Ministry of Science to JFLO.

DAILY RHYTHMS IN THE STRESS RESPONSE OF THE EUROPEAN SEA BASS (*Dicentrarchus labrax*): EFFECTS OF SEASONALITY AND FEEDING TIME

Elisa Samorì [1], J.A. Paullada-Salmerón [2], I. Rodríguez [1], L. Sánchez-Alacid [1], J.A. Muñoz-Cueto [2], F.J. Sánchez-Vázquez [1], J.F. López-Olmeda [1]

Email: elisa.samori@um.es

[1] *Department of Animal Physiology, Faculty of Biology, University of Murcia, Murcia, Spain*
[2] *Department of Biology, Faculty of Environmental and Marine Sciences, University of Cádiz, Cádiz, Spain*

Abstract

In the natural environment, fish are subjected to environmental variables that act as exogenous synchronizing factors (zeitgebers) for biological rhythms. As other physiological processes in fish, the hypothalamus-pituitary-interrenal (HPI) axis, or stress axis, displays rhythmicity in their components, with a species-specific pattern. However, less is known about the mitochondrial function and antioxidant enzymes that respond to stress. The main objective of this study was to evaluate the presence of daily rhythms in the stress response of the European sea bass, and the influence of two different environmental synchronizers: photoperiod (season of the year) and feeding time. For this reason, fish were kept under natural photoperiod and fed either at mid-light (ML) or at mid-dark (MD). Samples were collected in December (10:14 LD) and June (15:9 LD). At each season, samples were collected in 7 points, every 4 h, during a 24h cycle. At each sampling point, two groups were collected: control (unstressed) groups, which were sampled immediately at every point; and stressed groups, which were sampled 1h after 1 minute of air exposure. Hypothalamus, pituitary , liver and plasma samples were collected. The analysis revealed rhythmicity in the control group for plasma cortisol and the mRNA expression of catalase (cat), uncoupling protein 1 (ucp1), glutathione reductase (gsr), cytochrome c oxidase (cox IV) and glutathione peroxidase (gshpx) in liver, with most acrophases located in the dark phase. In the stressed group, rhythms in liver were found in peroxiredoxin 3 (prdx3), superoxide dismutase 1 (sod1), gsr, coxIV, gshpx, with acrophase at the beginning of the dark phase. These data underly that the moment in which stress occurs can trigger a different amplitude in the response and potentially impact the welfare status differently.

Acknowledgments

This research was funded by project "CHRONOLIPOFISH" (RTI2018-100678-A-I00), granted by the Spanish Ministry of Science to JFLO.

THE ROLE OF MELANOCORTIN SYSTEM IN THE REGULATION OF CIRCADIAN RHYTHMS IN FISH

Alejandra Godino-Gimeno, E. Leal, R. Guillot, J. Rotllant, J.M. Cerdá-Reverter

Email: a.godino@csic.es

[1] *Aquaculture Institute Torre de la Sal. (CSIC)*

Abstract

The melanocortin complex system is composed of agonists, antagonists, several receptors, and accessory proteins. They are expressed in different tissues and participate in several physiological functions. The endogenous antagonist, agouti-signaling protein (ASIP), binds MC1R and MC4R decreasing their constitutive activities to regulate skin pigmentation and energy expenditure, respectively. Overexpression of ASIP1 in zebrafish (Danio rerio) disrupts the dorsoventral pigmentation pattern and enhances growth yet no obesity. Melanocortin system is also involved in the regulation of fish behavior. ASIP1 transgenic fish show higher levels of anxiety-related behaviour, are sight, display reduced exploratory behaviour, and are less aggressive than wild-type fish yet no less active. For that reason, we were interested in evaluating circadian rhythms (LCRs). During a month, we measure the locomotor activity of both wild type and ASIP1 transgenic fish using a custom system composed of photosensitive cells connected to Automata software. Data were analyzed with "El Temps" software that also allows creating chronograms and periodograms. Chronograms showed heightened locomotor activity in ASIP1 fish during the dark phase, and periodograms revealed the absence of circadian periodicity in locomotor activity in these fish. The brain and liver of 10 animals were dissected every 4 hours for a period of 24 hours on order to evaluate the daily expression patterns of clock related genes including: cry1a, per1a, clock1a, bmal1 and nr1d1. All three per1a, clock1a and nr1d1 showed enhanced expression levels during the dark phase of the photoperiod in ASIP1 fish. On the contrary, bmal1 expression ASIP1 fish raised coinciding with the lights turned on and during the light phase. These findings provide evidence for the role of the melanocortin system in the regulation of circadian rhythms.

Acknowledgments

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TEMPERATURE AND FASTING: TWO SYNCHRONIZERS OF NOCTURNIN EXPRESSION IN GOLDFISH?

Diego Madera, A. Alonso-Gómez, A.I. Valenciano, M.J. Delgado, A.L. Alonso-Gómez

Email: dmadera@UCM.es

[1] *Department of Genetics, Physiology and Microbiology. Faculty of Biology, Complutense University, Madrid, Spain*

Abstract

Nocturnin (NOC) is a phosphatase discovered in Xenopus laevis retina that displays a daily rhythmic expression. Recent studies suggest its implication in the transformation of NADP(H) to NAD(H), being a key molecule for the regulation of metabolism. A relationship among nocturnin, the circadian system and environmental temperature has been recently suggested in mice, but the physiological role of this enzyme in poikilotherms is yet unknown. Our aim was to investigate the effect of acclimation to different temperatures and food availability on the relative abundance of the three noc paralogs (noc-a1, noc-a2 and noc-b1) expressed in goldfish (*Carassius auratus*). For this purpose, fish under 12L:12D photoperiod were acclimated at 3 temperatures (17, 22 and 27ºC) and fed 4 times daily at ZT1, ZT4, ZT7, ZT10 or kept unfed. After 30 days, hypothalamus, liver and adipose tissue were sampled and noc paralogs expression was analyzed by RT-qPCR. In liver, the three paralogs had temperature-dependent expression levels. While noc-a2 and noc-b1 are induced by low temperature, noc-a1 is induced by both cool and warm. Fasting during the last 7 days of acclimation does not modify noc-a1 or noc-b1 expression but represses noc-a2 expression at all temperatures. The effects of temperature and fasting on noc-a1 expression in adipose tissue matched the ones observed in liver, except for its non-induction when fish are fasted at 27ºC. Besides, in adipose tissue, neither temperature nor fasting modified noc-a2 or noc-b1 expression. No significant effects of temperature or fasting were found on any paralog expression in hypothalamus. These results suggest a possible functional specialization of noc paralogs in the different tissues and aim to investigate its possible role as a nexus between the environment and energy metabolism in ectotherms.

Acknowledgments

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ALLATOSTATIN-C-LIKE (AST-C-LIKE) AS A PUTATIVE REGULATORY FACTOR OF THE MANTLE IN THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*)

Zhi Li, J.C.R. Cardoso, M.X. Peng, J.P.S. Inácio, D.M. Power

Email: a73168@ualg.pt

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

Abstract

Allatostatin-C (AST-C) belongs to a group of small neuropeptides described in insects. The peptides are characterised by a conserved C-terminal PISCF motif and activate a specific receptor (AST-CR) that is a member of the rhodopsin G-protein coupled receptor (GPCRs) superfamily. AST-CR arose from the same ancestral gene that gave rise to Somatostatin receptors (SSR) in vertebrates. AST-C has multiple and varied functions across the insects and common actions include the inhibition of muscle contraction, suppression of feeding and reduced fecundity. Homologues of the insect AST-C system have been identified in the molluscs, the second most diverse group of animals, but their structure and function remain poorly characterised. In this study, we aimed to characterize the AST-C system in molluscs and assign potential functions by conducting meta-analysis of available transcriptomes and through experimentation. In mollusc genomes and transcriptomes a single AST-C peptide precursor and multiple AST-CRs were identified that had a widespread tissue distribution and were particularly abundant in the shell producing mantle. In the Mediterranean mussel, *Mytilus galloprovincialis*, an economically important aquaculture species, mantle transcriptome data revealed that AST-CR expression was significantly modified by a lipopolysaccharide challenge. Exposure of M. galloprovincialis to the marine pathogenic bacteria Vibrio harveyi by introduction into the bathing seawater significantly up-regulated ($p < 0.05$) the AST-C precursor but down-regulated ($p < 0.05$) its receptors. In summary, molluscs like insects have an AST-C system that has a widespread tissue distribution. It is most abundant in the mantle where one of its functions appears to be related to the immune response.

Acknowledgments

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LOOKING FOR PERIPHERAL PHOTORECEPTION IN GOLDFISH (*Carassius auratus*)

Nuria Saiz, S. Bustamante-Martínez, A.L. Alonso-Gómez, M.J. Delgado, E. Isorna

Email: nursaiz@ucm.es

[1] *Fish Neuroendocrinology Research Group, Department of Genetics, Physiology and Microbiology, Complutense University of Madrid*

Abstract

Reports in the last decades have revealed that photosensitivity is not exclusive to visual and neural tissues. In zebrafish, all studied cell types display direct clock-gene induction by light in vitro, and a vast variety of non-visual opsins have been identified. This work aimed to determine whether such a broadly distributed light sensitivity is a specific trait of zebrafish or if it is present in other teleost fish, focusing in the goldfish liver. In this direction, we searched goldfish opsin 7 (strongly expressed in zebrafish liver) paralogs and we measured clock gene expression (by RT-PCR) after light exposure (with different wavelengths, white, red, green, and blue) in vivo, and in cultured liver and eyecups. In silico analysis revealed 8 opn7 paralogs from 4 gene families (opn7a-d), consequence of both whole-genome and tandem duplications. Subsequent cDNA amplification of two of them showed that opn7a1b was expressed in all studied tissues (including brain, retina, liver, and other peripheral tissues), while opn7b was only found in retina, suggesting different functions among paralogs. The abundance of the directly light-induced clock gene per2a was increased by 6-h white-light exposure in the retina in vitro. This gene was unaffected in the liver by any of the employed lights, however short-wavelength lights (green and blue), induced hepatic per1a and cry1a expression in vivo, while in vitro light exposure of liver explants hinted a weak induction of cry1a and per1a by white and red light. The differences observed among in vivo and in vitro approaches suggest that, while the liver circadian clock can respond to light, upstream signals play a stronger role than direct light sensitivity. Further research is needed to assess the implications of local photosynchronization and the role of non-visual opsins in teleosts.

Acknowledgments

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COMPLEMENT C345 SUBUNIT "A" IN PROTOSTOMES IS A CHEMOTAXIN OF BIVALVE HAEMOCYTES AND A FUNCTIONAL HOMOLOGUE OF DEUTEROSTOME C5A

Maoxiao Peng[1,2], Z. Li[1,2], J.C.R. Cardoso[1], D. Niu[2], X. Liu[2], Z. Dong[3], J. Li[2], D. M. Power[1]

Email: a66019@ualg.pt

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[2] *Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, 201306, China*

[3] *Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang 222005, China*

Abstract

The complement system is a complex proteolytic cascade that first emerged in protostomes where an ancestral C3-like gene exists. Previous studies have confirmed that the complement C3/C4/C5 family is derived from the TEP family found in protostomes (starting from cnidarians) and vertebrates. Activation of complement in vertebrates leads to proteolytic cleavage near the N-terminus of the α chain of the zymogens C3, C4 and C5 and liberates the C3a, C4a and C5a anaphylatoxins and C3b, C4b and C5b that are involved in the reaction cascade that forms MAC. Vertebrate C3a, C4a, and C5a have differing potencies and mediate diverse activities including chemotaxis. The present study investigated complement genes in the Chinese razor clam (Sinonovacula constricta) with a view to establishing how many C345i genes exist and if the proteins they encode are zymogens that generate an "a" subunit that functions as a chemotaxin. Three C345i gene isoforms were identified in Chinese razor clam and arose through lineage and species-specific duplications. To determine subunit, function a recombinant protein was generated for each of the "a" subunits of the three C345i proteins in razor clam. In Transwell® and μ-Slide chemotaxis assays the recombinant proteins caused chemotaxis of razor clam hemocytes and vertebrate macrophage cell lines (J774A.1) but with differing potency. The three recombinant protein "a" subunits also promoted with differing potency phagocytosis of heat killed bacteria (S. aureus and V. anguillarum) by razor clam hemocytes. It was observed that small changes in the sequence of the thioester bond cleavage sites (α-β cleavage site and α-γ cleavage site), and the a-subunit protein (ANATO domain) caused the functional differentiation of the razor clam C345i proteins. In summary, evolution of C345 across the deuterostomes and protostomes occurred through parallel evolution and the "a" subunit of the three C345i proteins in razor clam are chemotaxins.

Acknowledgments

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EXPRESSION AND FUNCTIONAL ANALYSIS OF HEPCIDIN FROM MANDARIN FISH (*Siniperca chuatsi*)

Yawei Shen [1], H. Li [1], Z. Zhao [1], J. Zhao [2], X. Chen [3], M. Cao [4], M. Wu [5]

Email: yshen@ualg.pt

[1] *National Demonstration Center for Experimental Fisheries Science Education (Shanghai Ocean University), Shanghai 201306, China*

[2] *Shanghai Engineering Research Center of Aquaculture, Shanghai 201306, China*

[3] *Shanghai Collaborative Innovation for Aquatic Animal Genetics and Breeding, Shanghai 201306, China*

[4] *Guangdong Provincial Fishery Germplasm Conservation Center, Guangzhou 511400, China*

[5] *Fisheries Research Institute, Anhui Academy of Agricultural Sciences, Hefei, 230000, China*

Abstract

Hepcidin is a liver-derived peptide hormone that is related to iron balance and immunity in human. However, its function in fish has not been well elucidated. In this study, we analyzed the expression and function of the *Siniperca chuatsi hepcidin* (Sc-hep) gene. Sc-hep was specifically expressed in the liver and one of the most highly expressed genes in the liver. After infectious spleen and kidney necrosis virus (ISKNV) infection and lipopolysaccharide (LPS) and polyinosinic-polycytidylic acid (Poly I:C) stimulation, the expression of Sc-hep increased by approximately 110, 6500, and 225 folds in liver, respectively. After ferrous sulfate (FS) injection, the expression of Sc-hep in the liver increased by approximately 520-fold. Interestingly, miR-19c-5p could inhibit Sc-hep expression, and five CpG dinucleotides distributed in the promoter region showed no differential methylation level compared with the stomach, both with high methylation rates. After FS or LPS injection, the expression of three iron balance-related genes (FPN1, TFR1, and FTN) and five immune-related cytokines (IL-1β, IL8, TNF-α, TLR22, and SOCS3) significantly changed. These results indicated that Sc-hep participated in the regulation of iron balance and played an important role in the immune system. Sc-hep increased by approximately 52-fold when mandarin fish were domesticated with artificial diets. Sc-hep might be used as a real-time biomarker of mandarin fish liver because Sc-hep expression markedly varied under different physiological conditions.

Acknowledgments

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ROLE OF MELANOCORTIN SYSTEM IN THE ZEBRAFISH SKIN PHYSIOLOGY

E. Leal [1], J.B. Ortiz-Delgado [2], J. Rotllant [3], J.M. Cerda-Reverter [1]

Email: esther@iats.csic.es

[1] *Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal, IATS-CSIC, 12595 Castellon, Spain*

[2] *Instituto de Ciencias Marinas de Andalucía-ICMAN, CSIC Campus Universitario Río San Pedro, 11510, Puerto Real, Cádiz, Spain*

[3] *Department of Biotechnology and Aquaculture. Instituto de Investigaciones Marinas, IIM-CSIC, 36208, Vigo, Spain*

Abstract

The melanocortin system is one of the most complex hormonal systems in vertebrates. The main melanocortin peptides are the melanocyte-stimulating hormones (MSHs) and adrenocorticotropic hormone (ACTH), all of them encoded by a common precursor named proopiomelanocortin (POMC), which is expressed mainly in the pituitary. Melanocortin signaling is mediated by a family of G protein-coupled receptors. In tetrapod species, five different subtypes (MC1R-MC5R) has been characterized yet number diverges in fish species. Melanocortin signaling is mediated also by endogenous antagonists which compete with melanocortin agonist by binding to MCRs. Agouti-signaling protein (ASIP) is mainly expressed in the skin where compete by binding to MC1R whereas agouti-related protein (AGRP) is expressed in the hypothalamus to regulate energy balance by binding central MC3R and MC4R. The skin plays a crucial role in the immune function, working as a physical impediment against infestation yet also as a chemical barrier through its ability to synthesizes and secretes mucus. In addition, the skin can be considered also as part of the adaptive immune response characterized by a much more specific response to pathogens. The melanocortin system has been described to regulate inflammatory process which constitutes a key point in the defense response against the damage factors and it is an important component of the innate immune system. Our recent studies using a zebrafish strain overexpressing ASIP1 have demonstrated the participation of the melanocortin system in the regulation of skin physiology. This transgenic model thus constitutes a key opportunity to evaluate the role of the melanocortins in the skin immune response. Our results show that transgenic fish exposed to lypopolysaccharide (LPS) are capable to increase significantly the mRNA levels of pro-inflammatory skin cytokines, suggesting better immune response than wild type fish against bacterial infections.

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TWO DIFFERENT CORTICOSTEROIDS, CORTISOL AND DEXAMETHASONE, MODULATE THE OSMOREGULATORY RESPONSE AFTER HYPERSALINE TRANSFER IN THE GILTHEAD SEABREAM (*Sparus aurata*)

Andre Barany [1], V. Valderrama [1], A. Broz-Ruiz [1], G. Martinez-Rodriguez [2], J. Fuentes [3], J.M. Mancera [1]

Email: andre.barany@uca.es

[1] *Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), Universidad de Cádiz, 11510 Puerto Real, Cádiz, España*

[2] *Instituto de Ciencias Marinas de Andalucía, Consejo Superior de Investigaciones Científicas (ICMAN-CSIC), E-11510 Puerto Real Cádiz, España*

[3] *Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

Abstract

Corticosteroids control inter alia, metabolism, immunology, osmoregulation, and stress responses. In the present work, we examined the different physiological and molecular responses evoked in the gilthead seabream acclimated to seawater (SW, 38 ppt) after chronic oral administration for 30 days of cortisol (F; 400 mg/kg fish feed) and the synthetic glucocorticoid dexamethasone (DEX; 300 mg/kg fish feed), prior- and post-hypersaline seawater (HSW, 60 ppt) direct transfer for 3 days. After the feeding trial, plasma cortisol levels showed the following pattern: CT > F > DEX. HSW transfer significantly enhanced plasma cortisol levels in the CT group, whereas F and DEX groups showed about half of these values. Na+/K+-ATPase (NKA) activity increased dramatically in the DEX group prior to HSW transfer. Surprisingly gill NKA activity levels in this tissue were recovered to the initial lowest levels for all the groups. Plasma and intestinal fluid osmolalities enhanced significantly after HSW transfer in the DEX group. Intestinal short circuit-current (Isc) showed a more anion absorptive capacity in the posterior intestine (PI) than the anterior. Moreover, F treatment significantly increased this anion absorptive capacity in the PI after HSW transference. Surprisingly HSW significantly decreased the anion absorptive capacity in all intestinal regions, specifically in the PI, indicating the appearance and/or switch to secretory mechanisms in the intestine to counteract hypersalinity. Furthermore, molecular hypophyseal markers such as the pomcb and the gh were significantly altered/modulated by both pharmacological treatments and HSW challenge.

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GONADAL TRANSCRIPTOME DYNAMICS DURING SEX DIFFERENTIATION ACROSS VERTEBRATES

Núria Sanchez-Baizan, I. Jarne, F. Piferrer

Email: nsbaizan@icm.csic.es

[1] *Institut de Ciències del Mar (ICM), Consejo Superior de Investigaciones Científicas (CSIC), Passeig Marítim, Barcelona, Spain*

Abstract

Gonadal sex differentiation is the process by which the bi-potential gonads transform into testis or ovaries after sex determination. A large number of genes and pathways involved in this process have been discovered and are fairly conserved across vertebrates. However, the temporal and relative expression of these genes may be different in the major five vertebrate groups, although a comparative study has never been carried out. To investigate these possible differences, we used both published and unpublished studies concerning sex differentiation in different vertebrate species including the European sea bass, platyfish, red-eared slider turtle, frog, chicken, and mouse. First, we determined the differentially expressed genes, within each sex, between the beginning and towards the end of sex differentiation. Male- and female-enriched genes were defined based on their higher expression in males and females, respectively, towards the end of sex differentiation. Next, we compared the transcriptome dynamics and specific gene expression profiles of a set of key genes we selected based on their implication on gonadal sex differentiation. The major insights of this study are: 1) We found that the percentage of male- and female-enriched genes that were either upregulated or downregulated during ovarian differentiation and testicular differentiation greatly varied across the different vertebrate species analyzed, including the two fish species; 2) Sex differentiation can be achieved with different degrees of upregulation of pro-male or pro-female genes, depending on the sex, with or without concomitant downregulation of genes of the opposite sex; 3) In some cases, rather than different patterns across sexes, differences were observed in the degree of upregulation vs downregulation of the pro-male and pro-female genes. Taken together, these results show that there are important differences in the temporal expression dynamics between representative species of the major groups and that sex differentiation is achieved by different regulatory mechanisms.

Acknowledgments

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SEX-SPECIFIC SUBFUNCTIONALIZATION OF GSDF DUPLICATES IN EARLY GAMETOGENESIS OF SEA BASS

Alessia Mascoli, C. Zapater, J. Pizarro, B. Crespo, A. Gómez

Email: alessia.mascoli@iats.csic.es

[1] *Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (CSIC) 12595 Ribera de Cabanes, Castellón, Spain*

Abstract

Puberty is the developmental period during which an individual becomes sexually mature for the first time and its regulation is not completely known in teleosts. The gonadal soma-derived factor (Gsdf) was found to be downregulated in precocious testis of male European sea bass (*Dicentrarchus labrax*) and proposed as an early gonadal marker of puberty. The genome of sea bass contains two gsdf duplicates, gsdf1 and gsdf2, whose encoded proteins share 87% identity and have gonad-specific expression. In the present study, we have confirmed that expression of gsdf1 and gsdf2 decreases in testis of 1-year old sea bass males that enter precocious puberty compared with their siblings that remained immature. In adult males, gsdf genes showed high expression levels only in premeiotic testis, and gsdf2 expression was 5-fold lower than that of gsdf1. This expression profile matched with the presence of Gsdf protein in testis extracts, and with its location in Sertoli cells surrounding type A spermatogonia. In adult females, gsdf1 and gsdf2 are expressed to a much lower level than in testis, and the highest expression found corresponds to gsdf2 in post-ovulatory ovaries and also in isolated follicular cells. Indeed, the protein was located in follicular cells surrounding previtellogenic oocytes. To functionally characterize this gonadal factor, a sea bass recombinant Gsdf has been produced in CHO cells, exhibiting the same size as the native protein.

Sea bass gsdf1 and gsdf2 are located in the same chromosome, and their coding sequences are placed in different strands and transcription directions. The functional data obtained so far point to common regulatory elements for both genes, but also a sex-specific regulation for gsdf1 and gsdf2 connected to males and females respectively.

Acknowledgments

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COMBINED EFFECTS OF REARING TEMPERATURE REGIME AND THERMAL TREATMENT ON THE SEX DIFFERENTIATION PROCESS OF NILE TILAPIA

Gonzalo de Alba, F.J. Sánchez-Vázquez, J.F. López-Olmeda

Email: gonzalode.alba@um.es

[1] *Department of Physiology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain*

Abstract

Temperature is the main environmental factor that influences sex differentiation in most teleost fish. In nature, fish experience daily thermocycles (warming up during day and cooling down at night). In addition, the thermal treatments in the thermosensitive periods of development induce changes on sex differentiation of Nile tilapia. We investigated the combined effects of the rearing temperature regimes (thermocycle vs constant temperature) during the early development and a posterior thermal shock during the sex differentiation period (9-23 dpf; day post-fertilization) of Nile tilapia. To this end, fertilized eggs were divided into two groups: thermocycle (TC) of 31ºC:25ºC day:night vs. constant temperature of 28ºC (CTE). Photoperiod was set at 12:12h light/dark cycle. Embryos and larvae were kept under these conditions from 0 to 11 dpf. At the end of this period, larvae from each temperature regime were subjected to a thermal treatment (36ºC for 12 days) or maintained at the same rearing temperatures from 11 to 23dpf. Then, all groups were maintained under CTE until 270 dpf. In larvae, we studied the expression of genes involved in female (cyp19a1a, era) and male (amh, sox9, ara) sex differentiation and gonad development (fshb, lhb). In addition, sex ratio was evaluated at juvenile stage. At 11 dpf, the expression of cyp19a1a, era and lhb were greater in TC group while those reared under CTE presented higher expression of ara. At 23dpf, the thermal treatment decreased the expression of cyp19a1a and no differences were found for sox9, amh and ara in TC group. Tilapia juveniles from TC showed higher percentage of females than those reared under CTE. These findings indicated that the implementation of daily thermocycles during larval development increases the expression levels of female sex differentiation genes and the percentage of females.

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MALE SEX REVERSAL IS NUTRITIONALLY AND GENETICALLY REGULATED IN SEA BREAM: INSIGHTS IN WELFARE AND REPRODUCTIVE RESILIENCE

Paul George Holhorea [1], F. Naya-Català [1], E. Perera [1], J.À. Calduch-Giner [1], A. Felip [2], J. Pérez-Sánchez [1]

Email: paul.holhorea@csic.es

[1] *Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal. Ribera de Cabanes, Castellón, Spain*

[2] *Fish Reproductive Physiology Group, Institute of Aquaculture Torre de la Sal, Ribera de Cabanes, Castellón, Spain*

Abstract

Gilthead sea bream is a highly cultured marine fish in all the Mediterranean area, but new and strict criteria of welfare are needed to assure that the intensification of production has no negative effects in animal farming. Most welfare indicators are specific of a given phase of the production cycle, but others such as the timing of puberty and/or sex reversal are of retrospective value. This is of particular relevance in the protandrous gilthead sea bream, in which the sex ratio is highly regulated at the nutritional level. Social and environmental factors (e.g. contaminant loads) also alter sex ratio, but the contribution of genetic component remains unclear. To assess this complex issue, five sea bream families representative of slow/intermediate/fast growth were grown out with control or a plant-based diet in a common garden system from early life to completion of their sexual maturity in three-year old fish. The plant-based diet enhanced the male-to-female sex reversal by more than 50% in average. This occurred in parallel with the progressive impairment of growth performance, which was indicative of changes in nutrient requirements as the result of the different energy demand for growth and reproduction through development. The effect of a different nutritional and genetic background on the reproductive performance was also assessed by measurements of circulating levels of sex steroids during two consecutive spawning seasons, varying plasma levels of 17β-estradiol (E2) and 11-ketotestosterone (11-KT) with age, gender, diet and genetic background. Principal component analysis of three-year-old fish displayed a gradual increase of the E2/11-KT ratio from males to females with the improvement of nutritional/genetic background. Altogether, these results support the use of a reproductive tract scoring system for leading farmed fish towards their optimum welfare condition, contributing to improve the productivity of the current gilthead sea bream livestocks.

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NEW ROLE OF AMH AS A STEROIDOGENESIS PROMOTING HORMONE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Cinta Zapater, A. Mascoli, S. Ibáñez, A. Ferrer, A. Gómez

Email: cinta.zapater@csic.es

[1] *Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (CSIC) 12595 Ribera de Cabanes, Castellón, Spain*

Abstract

In higher vertebrates, Anti-Müllerian Hormone (AMH) is required for involution of the Müllerian ducts during male sexual differentiation and for negatively regulating gonadal development in both sexes. AMH signals through a transmembrane AMH type II receptor (AMHR2) with serine-threonine kinase activity. Despite the absence of Müllerian ducts in teleosts, orthologues of mammalian AMH have been described in fish, and a role of this hormone in sex determination and gonad differentiation has been demonstrated. In adult teleost gonads, Amh exerts a role at early stages of germ cell development in both males and females, however, the exact mechanism of Amh signaling and its implication in gonad development are poorly investigated. Recently, in sea bass, we have demonstrated that Amh signals through Amhr2, and that Amh is localized in Sertoli cells surrounding early germ-cell generations. In addition, information available of amh and amhr2 expression during sea bass ovarian development suggest a role of Amh during vitellogenesis. However, still knowledge is lacking about the function and the specific mechanisms of Amh signaling during gametogenesis. As tool for studying the mechanisms of Amh action, we have produced specific recombinant mature forms of this hormone, and have used them in in vitro transactivation experiments to study the intracellular signaling pathways of sea bass Amhr2. In addition, we have performed in vitro tissue cultures of ovaries and testis treated with recombinant Amh, and in vivo experiments through Amh injection in fish, to study the impact of this hormone in steroidogenesis, and its interaction with Fsh, both by analyzing steroid production and gene expression in gonads. We finally concluded that Amh promotes steroid production in immature testis and early vitellogenic ovaries, showing a clear involvement of sea bass Amh in gametogenesis.

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CHANGES IN BODY WEIGHT AND PLASMA LEVELS OF REPRODUCTIVE HORMONES IN PREPUBERTAL SEA BASS FEMALES WITH ADVANCED GONADAL GROWTH

Laura Sempere, S. Ibáñez, C. Marín, G. Molés, J. Pérez-Sánchez, A. Felip

Email: laurasb@iats.csic.es

[1] *Instituto de Acuicultura Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Ribera de Cabanes, Castellón, Spain*

Abstract

European sea bass (*Dicentrarchus labrax L.*) is a teleost fish species that presents sexual dimorphism affecting growth. Under culture condition, a substantial proportion of fish develop as males and those exhibiting larger size usually mature earlier during its first year of age (i.e., precocious males). This situation presents economic problems for producers and thus the production of highly female-biased stocks is considered as an opportunity to benefit its aquaculture. Females grow faster than males and mature later, around 3 years old, reaching a marketable size (400 g) after 2-year production cycle. Nevertheless, there is limited information about relationships among body weight in females and how this may affect the rate of oocyte development. The objective of this work is to determine those factors that are important links in the mechanisms by which growth influences ovarian development. To this aim, the evolution of growth and reproductive performance of prepubertal females has been determined in a total of seven batches of fertilized eggs that were reared over the first two years of age. Firstly, results show that there are significant differences of growth in females. Secondly, prepubertal females with slower growth remain immature while those fish with faster growth display advanced oocyte development. It evidences that sea bass females are able to undergo advanced vitellogenesis at 2 years of age although the rate of fish entering into advanced puberty varies among sea bass batches. Significant changes among body weight and plasma Fsh, E2, and Igf-1 levels are also observed between immature and advanced females. These findings suggest that these factors are important links that signals growth and nutritional status to the reproductive axis. The present results show that growth performance in female sea bass has indeed some interesting features that merit to be investigated to face its profitability in aquaculture.

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MOLECULAR PATHWAYS INVOLVED IN OVARIAN FOLLICULAR ATRESIA IN EUROPEAN HAKE (*Merluccius merluccius*)

A. Nzioka, M. Ortiz-Zarragoitia, O. Diaz de Cerio, I. Cancio

Email: anthony.nzioka@ehu.eus

[1] *CBET Research Group, Dept. Zoology & Animal Cell Biology; Faculty of Science & Technology and Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country. Areatza Hiribidea 47, 48620 Plentzia*

Abstract

Environmental stressors have been shown to interfere with the normal progression of oocyte development in many fish species through the disruption of the hypothalamus-pituitary-gonadal (HPG) axis. Under some circumstances such as food deprivation or under exposure to xenobiotics ovarian follicles can suffer atresia, an energy-saving process in which oocyte materials are reabsorbed and maturation is not completed. However, the molecular mechanisms that regulate atresia in teleosts are still unclear. European hake (*Merluccius merluccius*) is a species where seasonal episodes of increased atresia have been reported and we have previously identified that molecular markers of apoptosis and autophagocytosis show differential transcriptional regulation in and among previtellogenic and vitellogenic atretic follicles. In the present study, we analyse at the protein level some of those apoptosis markers involved in 5S rRNA nuclear traffic and p53 activation during atresia in the European hake. Western blot analysis using commercial antibodies allowed quantification of p53, p53 ubiquitin ligase (Mdm2), and 5S rRNA binding ribosomal proteins (Rpl5 and Rpl11) in hake ovaries. Densitometry analysis did not show significant differences in immunoreactive bands between ovaries with atretic and non-atretic oocytes for p53, Mdm2 and Rpl11. Instead significant differences between atretic and non-atretic ovaries were observed for Rpl5, with increased Rpl5 protein levels in atretic ones. Western blotting for p53 showed increased protein levels in previtellogenic (atretic and non-atretic) ovaries in comparison to vitellogenic ones, whereas Mdm2, Rpl5 and Rpl11 protein levels decreased in previtellogenic ovaries. Our protein results confirm the transcriptional variations seen in apoptosis marker genes related to 5S rRNA dynamics in oocytes, being atresia, during early previtellogenesis, marked by an increased p53 protein level.

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EXPRESSION OF ESTROGENIC BIOMARKERS IN THE LIVER OF BROWN TROUT MALES IN DISTINCT REPRODUCTIVE PHASES

Diana Santos, E. Rocha, C. Lopes, F. Malhão, J. F. Gonçalves, T. V. Madureira

Email: dianapss@live.com.pt

[1] *Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal*

Abstract

Vitellogenin (Vtg) and zona radiata proteins (Zrp) are well recognised as estrogenic biomarkers among distinct fish species. In fish, those proteins can be synthetised in the liver either after exposure to natural estrogens or environmental xenoestrogens. Vtg and Zrp are considered female-specific proteins. However, exposure to estrogens can induce an altered expression of hepatic Vtg and/or Zrp in both males and females. Also, we recently found out that hepatocytes exposed in vitro to androgens demonstrate an increased expression of Vtg. Since there is a natural variation in androgen levels in fish throughout the reproductive cycle, the question that arises is whether the baseline levels of Vtg and Zrp could also vary in males. Currently, there is no information available regarding the Vtg and Zrp seasonal changes in the liver of brown trout male adults. Thus, the aim here was to perform a first quantitative evaluation of the immunohistochemistry of Vtg and Zrp proteins in cultured brown trout males, in distinct reproductive phases. Sampling was performed at different stages: spawning capable (December), regressing (March), regenerating (July) and developing (November). Three 3-year-old males were sampled throughout the different seasons. Liver fragments were fixed and processed for paraffin. Antigen retrieval was done, and incubation with primary antibodies (Vtg – 1:2500 and Zrp – 1:3500) was performed overnight (4 ºC). Negative controls ensured immunostaining specificity. Slides were analysed under a light microscope, and photographs were obtained to proceed with a staining intensity analysis translated into scores using the software ImageJ. Immunophenotypic location of Vtg and Zrp demonstrate expression of both proteins in hepatocytes at all stages. Qualitative observations suggest inter-individual and seasonal nuances, but the analysis is not finished to allow statistics. The current data backs the notion that Vtg and Zrp expression in fish males may also be influenced by androgens.

Acknowledgments

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DECREASED ACTIVITY OF THE MELANOCORTIN SYSTEM IN ZEBRAFISH ENHANCES GROWTH DURING EARLY ADULTHOOD BUT HAS NO IMPACT IN PUBERTY

Sandra Navarro [1], R. Guillot [2], D. Crespo [3], R.W. Schulz [4], W. Ge [5], J. Rotllant [6], J.M. Cerdá-Reverter [2], A. Rocha [7]

Email: sannavarro@gmail.com

[1] *Centro de Investigación en Recursos Naturales y Sustentabilidad (CIRENYS), Universidad Bernardo O'Higgins, Avenida Viel 1497, Santiago de Chile*

[2] *Control of Food Intake Group, Department of Fish Physiolgy and Biotechnology, Instituto de Acuicultura de Torre la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain*

[3] *Reproduction and Developmental Biology Group, Institute of Marine Research, Bergen, Norway*

[4] *Reproductive Biology Group, Division Developmental Biology, Department Biology, Science Faculty, Utrecht University, Utrecht, The Netherlands*

[5] *Centre of Reproduction, Development and Aging (CRDA), Faculty of Health Sciences, University of Macau, Taipa, Macau, China*

[6] *Aquatic Biotechnology-ACUABIOTEC Group, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas (CSIC), Vigo, Spain*

[7] *Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal*

Abstract

In two swordtail species of the genus Xiphophorus, onset of puberty in males and females, fecundity in females, and adult size in males are modulated by sequence polymorphism and gene copy-number variation at the P locus affecting the type 4 melanocortin hormone receptor (Mc4r). The involvement of Mc4r in regulating the onset of puberty outside the genus Xiphophorus remains unclear. Mc4r binds the melanocyte-stimulating hormones (Mshs) and two inverse agonists, agouti-related protein (Agrp) and agouti-signalling protein (Asip). Both Agrp and Asip inhibit the constitutive activity of Mc4r and antagonistically compete with α-Msh. We used a transgenic line overexpressing asip1 (asip1-Tg), to investigate the relevance of the melanocortin system on the onset of puberty and adult reproductive performance in the zebrafish (Danio rerio). By comparing wild-type (WT) and asip1-Tg zebrafish we show that a decreased activity of the melanocortin system does not change the timing of puberty but significantly delays early growth of transgenic animals. Hatching time is postponed in asip1-Tg fish and they are significantly smaller than their WT siblings at 75 dpf, despite showing enhanced linear growth after having completed puberty. Even though asip1-Tg females spawn less frequently they produce 1.38 times more eggs that are smaller in diameter but render larvae with a 1.04-fold increase in body length at hatching. We demonstrate that the decreased activity of the melanocortin system induced by asip overexpression does not accelerate the puberty timing but significantly delays early growth of transgenic animals. Once asip-tg animals have outperformed a threshold length, when puberty has already been reached, the transgene rapidly promotes linear growth in absence of obesity.

Acknowledgments

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BRAINS IN METAMORPHOSIS. SPATIOTEMPORAL TRANSCRIPTOME DYNAMICS IN HATCHERY-REARED FLATFISHES

Laura Guerrero-Peña, P. Suarez-Bregua, L. Méndez-Martínez, P. García-Fernández, R. Tur, J.A. Rubiolo, J.J. Tena, J. Rotllant

Email: lguerrero@iim.csic.es

[1] *Instituto de Investigacións Mariñas (IIM-CSIC)*

Abstract

Metamorphosis is a captivating process of change by which the morphology of the larva is reshaped into a completely different one to face the new challenges related to adult life. In fish, including paradigmatic species with commercial value such as flatfish, this process initiated in the brain has traditionally been considered to be a critical rearing point and despite the pioneering molecular work carried out on the metamorphosis of the Japanese flounder and Atlantic Halibut the underlying molecular basis of flatfish metamorphosis is still relatively poorly characterized.

The key point for understanding this phenomenon is to know how the information encoded in the DNA is accurately used by cells to perform the physiological functions that are required during the different phases of metamorphic remodeling process. We already know that the access to this information is tightly regulated by an intricated system of different regulatory layers. These determine, for instance, which genes need to be turned on or off at different developmental times. So, the tight regulation of gene expression in time and space is crucial to understand how the complexity and variation within an organism can arise from a relatively simple DNA blueprint.

We investigated the whole-genome transcriptional profile of turbot brain at three key points in their metamorphic remodeling process (pre-metamorphic, climax and post-metamorphic). We found that gene expression dynamics are largest at pre and post metamorphic stages. Biological pathway enrichment analysis revealed that genes more highly expressed at metamorphosis climax are associated with innate immune system, while genes more highly expressed in pre-metamorphic and post-metamorphic stages are associated with cell differentiation and oxygen carrier activity, respectively. Additionally, our results confirm the importance of the thyroid-stimulating hormone in the metamorphosis process by showing an increase in its gene expression at climax.

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REPROGRAMMING OF EPIGENETIC MARKS DURING EARLY DEVELOPMENT ACROSS VERTEBRATES

Silvia Beato [1], N. Sánchez Baizan [1], A. Felip [2], F. Piferrer [1]

Email: silviabeato@icm.csic.es

[1] *Institut de Ciències del Mar (ICM), Consejo Superior de Investigaciones Científicas (CSIC), Passeig Marítim, Barcelona, Spain*

[2] *Instituto de Acuicultura de Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Ribera de Cabanes, Castellón, Spain*

Abstract

Epigenetics concerns mechanisms modifying gene expression that do not imply changes to the DNA sequences. Epigenetic marks, also to be maintained during mitotic division, can be transmitted from parental germ cells to offspring. DNA methylation, one of the most studied epigenetic modifications, is commonly associated with gene expression silencing. In vertebrates, it mostly occurs in CpG sites. Promoters and other regulatory regions are usually enriched with CpGs. DNA methylation has important roles in vertebrates, being associated with phenomena such as genome imprinting and reprogramming, the latter playing an important role in embryonic development. Reprogramming during early development is well studied in mammals. However, much is still to be done in fish. Here we review the situation concerning epigenetic reprogramming events that occur during embryo development across vertebrates. In mammals is well demonstrated that reprogramming occurs during primordial germ cell development and early embryogenesis. In the second process, paternal and maternal methylomes experience active demethylation during the early cleavage stages, reaching the lowest level upon specification to the inner cell mass. Nevertheless, in fish, information is available only for a couple of species. In zebrafish (*Danio rerio*) methylation changes were mostly detected in oocyte-specific CpGs, to resemble paternal methylome before zygotic genome activation. In contrast, in medaka (*Oryzias latipes*), strategies similar to mammals seem to be adopted, since in both parental genomes methylation levels were decreased up to 20%, earlier, by the 1st cell cycle. This scarce data seems to suggest that epigenome reprogramming could be species-specific in fish. Consequently, our objective is to determine what is the situation during European sea bass (*Dicentrarchus labrax*) embryo development, firstly because it is phylogenetically distant from medaka (*Cyprinodontiforme*) and zebrafish (*Cyprinid*), and second, to improve our knowledge about epigenetic inheritance to devise breeding strategies able to maintain certain favorable traits in cultured species.

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IGF2BPS AS NOVEL RNA-BINDING PROTEINS THAT MODULATE EARLY FISH DEVELOPMENT

Lisen Li, L. Anjos, D.M. Power

Email: a62808@ualg.pt

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

Abstract

Insulin-like growth factor II (IGF II) is a member of the insulin family of polypeptide growth factors that regulate development and growth. Insulin-like growth factor binding proteins (IGFBPs) bind IGF I and II and regulate their bioavailability and function. To better understand the role of Igf2bp's in developing fish embryos, we carried out comparative proteomics to investigate how their abundance changes from 24 hours before hatch to hatching in three Mediterranean fish species. The proteome profiles of eggs in different developmental stages from the white sea bream (*Diplodus sargus*), the meagre (*Argyrosomus regius*), and the gilthead sea bream (*Sparus aurata*) were analyzed by SDS-PAGE and by quantitative SWATH-MS (Sequential Window data independent Acquisition of the Total High-resolution-Mass Spectra) proteomics technology. Comparative genomics revealed three igf2bps in the well annotated genome of the sea bass (*Dicentrarchus labrax*), and three homologue genes in the sea bream genome, igf2bp1, igf2bp2b, and igf2bp3. Sea bass homologues Igf2bp1, Igf2bp2, and Igf2bp3, were confidently identified/quantified in the eggs of the three perciform fish species under analysis. Of note, was the significant increase in Igf2bp1 in all species when the egg content 24h before hatch and after hatch was compared. In the meagre but not any of the other fish, Igf2bp3 significantly increased at hatch, while Igf2bp2b was not significant altered between the developmental stages in any of the species studied. Our results indicate that fish eggs under analysis contain the three Igf2bps, but only Igf2bp1 was altered in between the developmental stages in all fish species, making this an interesting candidate for future studies and suggesting regulation of the availability of Igf II during egg development is important in teleosts.

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ONTOGENY OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBP) DURING GILTHEAD SEA BREAM, *Sparus aurata*, DEVELOPMENT

Babak Najafpour, A.V.M. Canário, D.M. Power

Email: bnajafpour@ualg.pt

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

Abstract

Lineage-specific genome duplication events in teleosts, including the gilthead sea bream and has given rise to fish-specific IGFB isoforms. The gilthead sea bream is an important Mediterranean aquaculture species and despite the importance of the IGF system for growth there is a surprising lack of detail about its role in larval development. To track the gene expression pattern of IGF/IGFBP during early development of the seabream, transcriptomes of whole larvae collected from different aquaculture sites were analysed. A diversity of IGFBPs were identified in the transcriptomes and their evolutionary origin and relationship was determined through phylogenies. The expression of members of the IGF system changed significantly between larvae at 24 dph (average weight = 4.6 mg; beginning of flexion stage) and 51 dph larvae (average weight = 43.8 mg; mid-metamorphosis stage). Specifically, the expression of igfbp1a, igfbp1b, igf1a receptor, igf2b, and ghb receptor were significantly up-regulated in 24 dph larvae compared to 51 dph larvae. The glycoprotein (acid-labile subunit, als) involved in the formation of the tertiary complex of IGF and IGFBP (generally IGFB3/ IGFBP5), was the only gene down-regulated in 24 dph larvae compared to the mid-metamorphosis stage. Overall, most of the IGFBPs (including igfbp2a, igfbp2b, igfbp3a, igfbp3b, igfbp5a, igfbp5b, igfbp6a, igfbp6b) had a constant expression pattern in early developmental stages and generally one isoform of each duplicated gene had a higher expression level (igfbp2a, igfbp5b, igfbp3a, igfbp6b) in the early developmental stage before mid-metamorphosis. The IGFPBs with the highest level of expression were igfbp5b and igfbp5a, and igfbp6a and igbp3b had the lowest levels of expression. The variation in the level of expression of IGFBPs may reflect their tissue-specific function in growth and development, although since pools of larvae were analysed, this could not be established in the present study and will require future work.

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TRANSCRIPTOME PROFILE OF GH/IGF SYSTEM SHOWS AN EARLY TISSUE-SPECIFIC DIFFERENTIATION AND HYPOXIA RESPONSIVE GENES IN SEA BREAM

Fernando Naya-Català, P. Simó-Mirabet, J. À. Calduch-Giner, J. Pérez-Sánchez

Email: fernando.naya@iats.csic.es

[1] *Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellón, Spain*

Abstract

A customized PCR-array of 28 selected genes was used for assessing the simultaneous expression of the Gh/Igf system and related markers of muscle growth, and lipid and energy metabolism during early life stages of gilthead sea bream (60-127 days posthatching). Also, transcriptional reprogramming by mild hypoxia was assessed in fingerling fish with different history trajectories on O2 availability during the same time window. In normoxic fish, the expression of almost all the genes in the array varied over time with a prompted liver and muscle tissue-specific differentiation, which revealed temporal changes in the relative expression of markers of the full gilthead sea bream repertoire of Gh receptors, Igfs and Igf-binding proteins. Results supported a different contribution through development of ghr and igf subtypes on the type of action of GH via systemic or direct effects at the local tissue level. This was extensive to Igfbp1/2/4 and Igfbp3/5/6 clades that clearly evolved through development as hepatic and muscle Igfbp subtypes, respectively. This trade-off is however very plastic to cope changes in the environment, and ghr1 and igfbp1/3/4/5 emerged as hypoxic imprinting genes during critical early developmental windows leading to recognize individuals with different history trajectories of O2 availability and metabolic capabilities later in life. How this knowledge can contribute to establish robust criteria of larval and juvenile fish quality requires more research, though changes in the expression and the quotient expression ratio of duplicated ghr/igf/igfbp genes can serve as a measure of developmental progression and metabolic disturbances of the offspring. Besides, growth, health and well-being also depend on the daily synchronization of endogenous biological rhythms, and the expression of selected genes from antiphase clusters can serve to find synchrony indicators of gilthead sea bream during early life.

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FIRST RESULTS ON GENE TRANSFECTION IN FISH MUSCLE CELLS

Sheida Azizi, C. Muller-Sanchez, A. Otero-Tarrazón, M. Lladonosa, J. Chillarón, M. Reina, J. Gutiérrez

Email: sheidaazizi.60@gmail.com

[1] *Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Avinguda Diagonal, 643, 08028 - Barcelona, Spain*

Abstract

Cell transfection is a commonly used protocol in cell biology and a powerful tool to study different biological aspects. While transfection has been standardized for mammalian cells, and despite some success using fish cell lines, the transfection of fish cells from primary cultures remains a challenge. In the present study we tested different transfection reagents and adenovirus in trout myoblasts in order to determine and optimize a working protocol. Commercially available transfection systems Lipofectamine, X-tremeGene HP DNA transfection, K4 system transfection, METAFECTENE-PRO and Jet OPTIMUS were used to transfer plasmids (pEGFP, pEGF-Actin) into primary cell cultures of trout myoblasts. Similar experiments were performed with adeno-associated virus serotype 2 (AAV2). Cells were seeded in 24 well plates and transfected with the different methods 2 days after seeding when they reached 40-50% confluency. The efficiencies of the transfection were estimated on the 2th and 5th day of transfection. Moreover, LDH assay as a high-throughput screening method was performed to determine the cytotoxicity rate of the reagents and immunofluorescence studies to demonstrate myocyte specificity. We observed fluorescent cells after 48 hours in myoblasts transfected with both plasmids DNA using all methods that were correlated with muscle-specific immunofluorescence staining. Based on the results of the transfection efficiency, neither type of the plasmids nor stages of the culture development revealed the significant differences. In addition, Lipofectamine® 2000 showed the highest potential for gene transferring into trout myoblasts up to 10% without any cytotoxicity impacts in the muscle cells. The trout myoblasts were not transduced with AAV2. Thus, our first results for intercellular delivery of plasmids DNA will open the possibility to improve our knowledge of the myogenesis regulation.

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DOPAMINE, A CONTROVERSIAL PLAYER IN THE FEEDING REGULATION IN TELEOST FISH

Mauro Chvite, F. Naderi, M. Conde-Sieira, M. Alborja-Valado, M. A. Lopez-Patiño, J. M. Miguéz

Email: mchivite@uvigo.es

[1] *Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo*

Abstract

The goal of feed regulation is to achieve a state of energy balance, ensuring sufficient energy to keep internal requirements satisfied over time. In nature, food availability is variable, with periods of absence of food forcing the animal to develop strategies to ensure intake when food is available and to discriminate between different food sources to improve energy intake. In mammals, the neurotransmitter dopamine (DA) has been implicated in the regulation of several physiological processes related to the control of food intake. In particular, DA appears to be a central player in the reward system, activating neural circuits that promote positive reinforcement on appropriate stimuli, allowing the animal to associate between neutral stimuli and rewards. However, in fish, information on the role of dopamine in feeding regulation is lacking. Data provided by a few studies using pharmacological tools indicate that DA promotes appetite inhibition in teleost fish, but there is no evidence for the existence of a DA-mediated reward system. With this in mind, several experiments were conducted to evaluate the role of dopamine in feeding regulation in rainbow trout. First, we studied the effect of feeding per se on dopaminergic turnover in several brain regions. Next, we evaluated the role of DA and its receptors on several feeding parameters, including total food intake, time to intake, and hypothalamic neuropeptide expression. Our results indicate that the dopaminergic system is differentially regulated in the brain during prandial times, showing specific feeding-related changes. Furthermore, D1 and D2 agonist treatments promote an impact on several hypothalamic neuropeptides, supporting an anorexigenic role of DA. Taken together, it appears that DA is an important player in the regulation of teleost feeding, allowing for the existence of a food-related reward system in fish.

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LAT1 AND SNAT2 CARRIERS ARE INVOLVED IN LEUCINE SENSING IN BRAIN OF ATLANTIC SALMON (*Salmo salar*).

Sara Comesaña[1], F. Lai [2], A-E. O. Jordal [2], T. Verri [3], M. Espe [4], I. Rønnestad [2], J.L. Soengas [1]

Email: saracomesana@uvigo.es

[1] *Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, Vigo, Spain*

[2] *Department of Biological Sciences, University of Bergen, Bergen, Norway*

[3] *Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy*

[4] *Institute of Marine Research, Bergen, Norway*

Abstract

Leucine is the main amino acid involved in the regulation of food intake. This is achieved through specific mechanisms in brain sensing leucine levels. However, there is scarce information about the role of amino acid carriers as a putative mechanism of sensing. For instance, certain carries of the SLC family (LAT1, SNAT2, and SNAT9) are key regulators of intracellular leucine concentration in mammals, but their involvement in amino sensing remains unknown. Phylogenetic analysis amongst sequences of these carriers revealed a complex pattern of paralogues in Atlantic salmon, for LAT1 (slc7a5aa, slc7a5ab, slc7a5ba, slc7a5bb, slc7a5ca, slc7a5cb), SNAT2 (slc38a2a, slc38a2b) and SNAT9 (slc38a9). After that, an experiment was carried out in which Atlantic salmon individuals were fed two commercial diets with different levels of leucine: high leucine (35 g/kg) and control leucine (27.3 g/kg). Feed intake was decreased with high leucine diet and an increase in mRNA abundance of specific paralogues of LAT1 (slc7a5aa, slc7a5ab, slc7a5bb) and SNAT2 (slc38a2a, slc38a2b) was observed in brain areas like hypothalamus and telencephalon, which are involved in feed intake regulation. The results obtained suggest a role for LAT1 and SNAT2 in the anorectic effect of leucine, but apparently not for SNAT9. Moreover, this evidenced that these carriers might be an amino acid sensing mechanism not characterized before in fish regulation of feed intake.

Acknowledgments

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FIRST EVIDENCE FOR THE PRESENCE OF FATTY ACID SENSING MECHANISMS IN THE RAINBOW TROUT GASTROINTESTINAL TRACT

Jessica Calo, A.M. Blanco, S. Comesaña, M. Conde-Sieira, J.L. Soengas

Email: jessica.calo.rodriguez@uvigo.es

[1] *Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo*

Abstract

Lipids are major nutrients in fish and they are responsible for numerous physiological processes. Recent studies have demonstrated that fish detects changes in the levels of specific fatty acids (oleic and octanoic acid) through fatty acid sensing mechanisms in hypothalamus, and also, a food intake reduction was shown after these treatments. However, there is lack of information about how these nutrients are sensed at the gastrointestinal tract (GIT) level. In this study we aimed to characterize, for the first time in fish, the mechanisms underlying fatty acid sensing systems in the GIT, using rainbow trout as a teleost model. We intragastrically administered saline solution alone (control) or containing 50 µmol/mL of octanoic acid, oleic acid, linolenic acid or butyric acid. In a first experiment we evaluated changes in food intake levels at 2, 6 and 24h after treatments administration. In a second experiment, samples of blood, GIT (stomach, proximal intestine, middle intestine and posterior intestine) and brain (hypothalamus, telencephalon and hindbrain) were collected at 20 min (blood and GIT) and 2 hours (blood, GIT and brain) after treatments. Gastrointestinal levels of mRNAs encoding fatty acids receptors as well as mRNA and protein levels of key gut hormones (GHRL, CCK, PYY, GLP-1) were measured by RT-qPCR and Western Blot, respectively. Moreover, we assessed neuropeptide expression (NPY, AgRP, CART and POMC) and mRNA and protein expression of transcription factors (CREB, BSX and FoxO1) at central level. Results show that the rainbow trout gastrointestinal tract has mechanisms able to sense and respond to fatty acids in a way comparable (in general) to those described in mammals.

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BENEFITS OF DIETARY HYDROXYTYROSOL ON MUSCULOSKELETAL GROWTH AND HEPATIC METABOLISM IN GILTHEAD SEA BREAM (*Sparus aurata*)

Sara Balbuena-Pecino [1], M. Montblanch [1], S. Balcells-Quintana [1], I. García-Meilán [1], R. Fontanillas [2], J. Gutiérrez [1], I. Navarro [1], E. Capilla [1]

Email: sara.balbuena@ub.edu

[1] *Department of Cell Biology, Physiology and Immunology, University of Barcelona, Barcelona 08028, Spain*

[2] *Skretting Aquaculture Research Centre, Stavanger 4016, Norway*

Abstract

In the last few years, hydroxytyrosol (HT), the major polyphenolic compound found in olive oil, has aroused increasing interest due to its health promoting abilities, such as its antioxidant, anti-inflammatory, and anti-obesogenic properties. Besides, optimization of aquafeeds including plant-based ingredients is recognized as a priority for the demanded more sustainable aquaculture. The aim of this study was to evaluate the effects of the inclusion of HT as an additive (0,5 g HT/Kg feed) in a high-fat (24% lipids) diet in gilthead sea bream juveniles. Moreover, the high-fat diet, in the absence or presence of HT, was administered at a standard ration (3% of total biomass in the tank) and at restricted conditions (40% daily reduction) for 8 weeks. Growth and biometric parameters and mRNA levels of growth hormone/insulin-like growth factors (GH/IGFs) axis, and myogenic- and osteogenic-related genes in white muscle and/or bone and lipid-metabolism-related genes in liver were analyzed. The ration factor was found to be significant in body weight, body length and the body fat percentage measured with a fatmeter. In muscle, a significant interaction between diet and ration regime was observed in the binding protein igfbp-5b, and the receptors ghr-1 and igf1-ra, and the diet factor in dock5 (a marker of muscle fibers fusion). Particularly, fish fed with the diet containing HT at standard ration had higher igfbp-5b and dock5 transcript levels than the group without HT, suggesting enhanced growth and muscle condition. At hepatic level, according to transcriptional data, fish fed HT-supplemented diets appeared to have reduced fatty acid uptake and lipolytic and β-oxidation activities in agreement with the anti-obesogenic potential of HT. Overall, this study provides new insights into the beneficial role of HT as a dietary additive in aquafeeds.

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DIETARY PROTEIN/CARBOHYDRATE RATIO AND FEEDING FREQUENCY AFFECT GILTHEAD SEABREAM (*Sparus aurata*) APPETITE REGULATION MECHANISM

Catarina Basto-Silva [1,2], A. Oliva-Teles [1,2,] I. Navarro [3], E. Capilla [3]; I. Guerreiro [1]

Email: bastosilva.C@gmail.com

[1] *CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal*

[2] *FCUP - Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal*

[3] *Department of Cell Biology, Physiology, and Immunology, Faculty of Biology, University of Barcelona, Av. Diagonal, 643, 08028 Barcelona, Spain*

Abstract

Appetite regulation is a complex mechanism that involves several hormones, central and peripheral organs, and can be influenced by diet composition and fish feeding. To evaluate the effects of dietary protein/carbohydrate (P/CH) ratio and feeding frequency (FF) on gilthead seabream appetite regulation, two practical diets were formulated to include high protein and low starch content (diet P50/CH10), or low protein and high starch content (diet P40/CH20). Each diet was fed at three FF (1, 2, or 3 meals a day) until visual satiation to triplicate groups of fish (9.1±0.1g) for 60 days. Feed intake (FI) was higher in fish fed P40/CH20 diet and, independently of diet composition, growth and FI were higher in fish fed 2 or 3 meals a day. The expression of cocaine-amphetamine-related transcript, corticotropin-releasing hormone, ghrelin receptor-a (ghrr-a), leptin, and neuropeptide y in the brain; cholecystokinin (cck) in the intestine; and leptin and ghrelin in the stomach was not affected by dietary P/CH ratio or FF. However, fish fed P40/CH20 diet presented higher hepatic leptin expression. Fish fed 3 meals a day presented lower brain cck expression than fish fed 2 meals a day, and higher hepatic ghrr-b expression than fish fed 1 meal a day. In conclusion, in an independent way, a P40/CH20 diet and feeding 3 meals a day seem to decrease the satiation feeling of gilthead seabream juveniles through transcriptional regulation of hypothalamic and hepatic factors related to FI control.

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IGFBP1 AND IGFBP2 IN GOLDFISH: DISTRIBUTION, RHYTHMICITY AND INFLUENCE OF ENVIRONMENTAL ZEITGEBERS

Aitana Alonso-Gómez, D. Madera, A.L. Alonso-Gómez, M.J. Delgado, A.I. Valenciano

Email: aitanaalonsogomez@gmail.com

[1] *Department of Genetics, Physiology and Microbiology. School of Biology, Complutense University, Madrid, Spain*

Abstract

Insulin-like growth factor-1 (IGF-1) is an anabolic hormone with a marked pleiotropic profile in fish. The bioavailability and half-life of the IGF-1 is regulated by its binding to the insulin-like growth factor binding proteins (IGFBPs). Previous studies demonstrated that IGFBP1 and IGFBP2 are the most abundant binding proteins present in the liver of salmonids. The aim of this work was to investigate the igfbp1 and igfbp2 paralogs in goldfish (*Carassius auratus*), their tissue distribution, the possible daily rhythms of expression in the liver, and the entrainment by the two main zeitgebers, the light/dark cycle and a feeding scheduled. The phylogenetic analysis demonstrates the existence of four paralogs for each family: igfbp1a-a, igfbp1a-b, igfbp1b-b, igfbp1b-a, igfbp2a-a, igfbp2a-b, igfbp2b-b and igfbp2b-a. All these paralogs are expressed in neural tissues, being igfbp1a-b, igfbp1b-b, igfbp1b-a, igfbp2a-a and igfbp2b-a the most abundant paralogs in the liver. Only the igfbp1a-b shows a daily rhythm of expression in the liver, mainly entrained by the last mealtime, with the acrophase peaking 4-h post-feeding. The analysis of the periprandial profile of expression of these paralogs in the liver shows an increase of igfbp1a-b transcripts in fed fish, and of igfbp1b-b in unfed fish, at 4-h after food ingestion. Overall, these results support a possible functional specialization of IGFBPs paralogs, and highlight the relevance of feeding time in the regulation of igfbp1a-b expression, suggesting a key role of this paralog in the metabolic actions of the IGF-1 in fish.

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POSTER LIST

Poster	Presenting author	Institution	Presentation Title
P1	**Sven David Arvidsson Hernández**	*Grupo de Investigación - Neuroendocrinología de Peces, Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense de Madrid, España*	THE GOLDFISH AS A MODEL TO MEASURE ANXIETY: OPTIMIZATION OF BEHAVIORAL TESTS.
P2	**João P. S. Inácio**	*Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*	EVOLUTION AND COMPARATIVE ANALYSIS OF THE ALLATOSTATIN (AST) PEPTIDES AND RECEPTORS IN MOLLUSCA
P3	**Elisa Samorì**	*Department of Animal Physiology, Faculty of Biology, University of Murcia, Murcia, Spain*	HOW SEASONALITY AND FEEDING TIME AFFECT DAILY RHYTHMS OF FOOD INTAKE AND DIGESTIVE FACTORS IN THE EUROPEAN SEA BASS
P4	**Thomas Knigge**	*Normandie Univ, UNILEHAVRE, UMR-I02, Environmental Stress and biomonitoring of aquatic environments (SEBIO), 76600 Le Havre, France*	PHYSIOLOGICAL COLOUR CHANGES IN TOXICOLOGY
P5	**Josep Rotllant**	*Acuabiotec Lab, Department of Biotechnology & Aquaculture. Institute of Marine Research, (IIM-CSIC), 36208 Vigo*	GENETIC ABLATION OF ACTH RECEPTOR INDUCES SKIN HYPERPIGMENTATION IN FISH
P6	**Ana Patrícia Mateus**	*[1] Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal [2] Escola Superior de Saúde, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal*	MODULATION OF SKIN REGENERATION BY EGG INCUBATION TEMPERATURE AND PROLACTIN IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)
P7	**Cármen Sousa**	*Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*	ENDOCRINE RESPONSE UNDER TWO PATHOGEN CHALLENGES IN N. rossii
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THE GOLDFISH AS A MODEL TO MEASURE ANXIETY: OPTIMIZATION OF BEHAVIORAL TESTS

Sven David Arvidsson, N. Saiz, L. Herrera-Castillo, N. de Pedro, M.J. Delgado, E. Isorna

Email: svenarvi@ucm.es

[1]*Grupo de Investigación - Neuroendocrinología de Peces, Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense de Madrid, España*

Abstract

Some food intake regulators (as NPY) are also involved in an anxiolytic-like behaviour on fish, although mechanisms beyond such central functions are unknown. Behavioural tests to measure scototaxis (dark-light preference) and tigmotaxis (open field) are typically used in mammal studies to evaluate anxiety (and the neural mechanisms that control it), however such tests are not fully standardized on teleost species other than zebrafish. This study focuses on the optimization and validation of the dark/light test and the open field test for goldfish (*Carassius auratus*) to measure anxiety and its connection with food intake and feeding regulators. To this aim, under standardized conditions of light and temperature, we analysed multiple variables such as time spent in each zone, distance travelled, speed or mobility state in control animals and in animals treated intraperitoneally with the anxiolytic GABAA modulator diazepam (5 and 10 µg/g body weight). The effect of different fasting periods (1-7 days) and the link between "fish anxiety" and food intake were also analysed. Both diazepam doses produced a significant increase of time spent in the white side over the dark side (scototaxis) and on time in the open zone (open field). The lower studied dose did not affect total distance travelled and average swimming speed, that were reduced with the higher dose as expected. These results ascertain the validity of our scototaxis and tigmotaxis tests to evaluate anxiety-like behaviour on goldfish. In addition, we find a direct relation between the time spent on the white side and the food intake measured after the dark-light test, suggesting that anxiety decreases food intake. Fasting (1 and 7 days) did not affect the studied behavioural parameters. Relationship between food intake and anxious behaviour remains to be deeper explored.

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EVOLUTION AND COMPARATIVE ANALYSIS OF THE ALLATOSTATIN (AST) PEPTIDES AND RECEPTORS IN MOLLUSCA

João P.S. Inácio, J.C.R. Cardoso, Z. Li, M.X. Peng, D.M. Power

Email: a61206@ualg.pt

[1]*Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

Abstract

The molluscs belong to the Lophotrochozoan phylum and are the second most diverse and specious animal group after insects. They are important models for understanding the evolution and function of neuroendocrine gene families in metazoans as their genomes possess a more similar organisation and gene repertoire to deuterostomes than other invertebrates (insects and nematodes). The Allatostatin (AST) system is composed of a large group of physiologically important small pleotropic alloregulatory neuropeptides and their receptors that are vastly studied in insects but poorly characterized in other invertebrates. In this study we explored mollusc genomes to identify and understand (AST) system evolution. In insects and other arthropods, three peptide groups (AST-A, AST-B and AST-C) that are unrelated in sequence have been described and they activate G-protein coupled receptors (GPCRs). Using sequence similarity searches, insect AST peptide precursors and receptors homologues were characterised in several molluscs (bivalves, gastropods, and cephalopods) and other lophotrochozoans (*annelids*). Genome and transcriptome searches identified homologues of the three arthropod AST-type peptide precursors and receptors in molluscs. The number of peptide precursors and receptors identified in molluscs were different across species and the predicted AST mature peptides were also distinct and the gastropods precursors produced the largest peptide diversity. The number of AST-A, B and C receptors were also distinct across molluscs and phylogenetic analysis revealed that lineage and species-specific duplications explained the difference. Short-range gene linkage analysis revealed that the protostome AST-A systems may be more related to the deuterostome GALR than KISSR. Overall, our study revealed that the AST-system emerged prior to the ecdysozoans-lophotrochozoan divergence and and the greater number of putative mature AST peptides and receptor genes found in molluscs suggest that it is more complex than in insects.

Acknowledgments

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HOW SEASONALITY AND FEEDING TIME AFFECT DAILY RHYTHMS OF FOOD INTAKE AND DIGESTIVE FACTORS IN THE EUROPEAN SEA BASS

Elisa Samorì [1], I. Rodríguez [1], J.A. Paullada-Salmerón [2], L. Moreno Zapata [1], J.A Muñoz-Cueto [2], F.J. Sánchez-Vázquez [1], J.F. López-Olmeda [1]

Email: elisa.samori@um.es

[1] *Department of Animal Physiology, Faculty of Biology, University of Murcia, Murcia, Spain*

[2] *Department of Biology, Faculty of Environmental and Marine Sciences, University of Cádiz, Cádiz, Spain*

Abstract

The regulation of food intake is a complex process regulated by positive and negative loops, in which the hypothalamus plays a crucial role in integrating information of different nature like hormones, nutrients level and circadian input. The same circadian input acts as a modulator of endocrine rhythms. The hormones produced and released will become synchronizing inputs for the hypothalamus and peripheral clocks, providing feedback signal. The purpose of the present experiment was to understand the effects of the season of the year, and feeding time, on the daily rhythms of factors involved in appetite control and digestion in a teleost fish, the European sea bass (*Dicentrarchus labrax*). For this purpose, fish were kept under natural photoperiod and fed either at mid-light (ML) or at mid-dark (MD). Samples were collected in December (10:14 LD) and June (15:9 LD). At each season, samples of the hypothalamus, intestine and liver were collected every 4h during a 24h cycle, with 7 sampling points, to investigate the presence of rhythmicity in anorexigenic and orexigenic factors that affect food intake, and enzymes involved in digestion. The gene expression of agrp, npy, orexin and mch, were assessed in hypothalamus, while gpr, grhelin and cck were assessed in the liver. In addition, in the liver, the expression of trypsinogen, chymotrypsinogen, carboxypeptidase, lipase, amylase and phospholipase were also evaluated. The data obtained in the present study should help to deepen into the knowledge of the regulation of food intake in the European sea bass, to understand the rhythmicity of the factors involved and how they synchronize with different photoperiod and feeding conditions.

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PHYSIOLOGICAL COLOUR CHANGES IN TOXICOLOGY

Thomas Knigge [1], A. Chabenat [1,2], A. Gouveneaux [1,2], C. Bellanger [2], A. Ford [3]

Email: thomas.knigge@univ-lehavre.fr

[1] *Normandie Univ, UNILEHAVRE, UMR-I02, Environmental Stress and biomonitoring of aquatic environments (SEBIO), 76600 Le Havre, France*

[2] *Normandie Univ, UNICAEN, Univ Rennes, CNRS EthoS UMR 6552, 14000 Caen, France*

[3] *Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Portsmouth, Hampshire PO4 9LY, UK*

Abstract

Rapid physiological colour changes allow animals to conceal themselves and to adapt to their surrounding environments. Colour changes in cephalopods, such as the European cuttlefish, Sepia officinalis, provide an particularly impressive example of camouflage. But physiological colour changes can also be found in many other species, such as juvenile green crabs, Carcinus maenas, or sand shrimp, Crangon crangon. These colour changes can be controlled either nervously, as in cephalopods, or neurohormonally, as in crustaceans. Both, neuronal and neurohemal control are influenced by neurotransmitters, such as serotonin or dopamine. Environmental pollutants have the potential to interfere with this signalling cascade, thus disturbing physiological colour changes and cryptic behaviour. Consequently, chromatophore movements and colour changes may serve as indicators of environmental pollution and could point to detrimental effects on behavioural ecology that are important for the animals' survival. Colour change bioassays, therefore, provide a promising tool for environmental testing with the advantage of being non-invasive, inexpensive and relatively straightforward.

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GENETIC ABLATION OF ACTH RECEPTOR INDUCES SKIN HYPERPIGMENTATION IN FISH

L. Guerrero [1], L. Mendez [1], J.M. Cerda-Reverter [2], L. Tort [3], Josep Rotllant [1]

Email: rotllant@iim.csic.es

[1] *Acuabiotec Lab, Department of Biotechnology & Aquaculture. Institute of Marine Research, (IIM-CSIC), 36208 Vigo*

[2] *Control of Food Intake Lab, Department of Fish Physiology and Biotechnology, Institute of Aquaculture from Torre la Sal (IATS-CSIC), 12595 Castellon*

[3] *Department Biologia Cel·lular, Fisiologia i Immunologia. Facultad de Biociéncies. Universitat Autónoma de Barcelona, 08193 Bellaterra*

Abstract

The melanocortin system is a complex neuroendocrine signaling mechanism involved in numerous physiological processes in vertebrates, including pigmentation, steroidogenesis and metabolic control. In humans, five melanocortin receptors (MCR) have been cloned, identified and shown to have a wide distribution throughout the body and likely many diverse functions. It has also been shown that a high degree of identity and conservation in structural characteristics and pharmacology exists between Mcrs from fish and mammals. However, in fishes, the number, affinity, specificity, tissue distribution and physiological roles are far from defined and appear to be species-specific. A clear example is the Mcr2 subtype. In humans, it is well known that Mc2r is expressed in the adrenal gland and controls steroidogenesis and the same function has also been described in fish. In human, ACTH overproduction results into hyperpigmentation because of pharmacological effects on MC1R. Using CRISPR/Cas9, we demonstrated that zebrafish lacking Mc2r shows hyperpigmentation. Ongoing studies are exploring potential direct/indirect roles for Mc2r in fish skin pigmentation.

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MODULATION OF SKIN REGENERATION BY EGG INCUBATION TEMPERATURE AND PROLACTIN IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Ana Patrícia Mateus [1,2], R.A. Costa [1], A.V.M. Canário [1], D.M. Power [1]

Email: apmateus@ualg.pt

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[2] *Escola Superior de Saúde, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal*

Abstract

The skin in fish is a living epithelium with an important role in osmoregulation and regeneration is rapid after scale loss. The skin in mammals is an important non-pituitary source of prolactin (PRL), which regulates skin appendage growth. In the present study the effect of early temperature imprinting on skin regeneration and the potential involvement of PRL in this process in young adult sea bass was determined. Sea bass eggs were incubated at 11, 13 and 16ºC, until hatching and then were reared at a common temperature until 9 months when their skin was damaged and samples were collected 0, 1 and 3 days after scale removal. Molecular analysis of prlr and transcripts associated with reepithelialisation/ tissue remodelling, innate immunity, and antioxidant defence, revealed that the response of both damaged and intact skin was modified by incubation of eggs at lower temperatures. On day 0, prlr was significantly more abundant in damaged and intact skin of fish reared at 16ºC compared to lower temperatures but was not modified at any other time points. Transcripts associated with reepithelialisation/ tissue remodelling were significantly more abundant at day 1 and 3 after scale removal in damaged and intact skin of fish reared at 11ºC and 13ºC compared to 16ºC. Gene transcripts associated with antioxidant defence were significantly more abundant at day 0 in fish from eggs reared at lower temperatures (11 and 13 ºC) compared to fish at 16ºC. No substantial change in gene transcripts associated with innate immunity were observed. Overall, the results indicated that thermal imprinting of eggs modified the skin response to damage in young adults and that prlr may be involved in modulating the response, supporting the idea that prolactin may be involved in skin repair in fish.

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ENDOCRINE RESPONSE UNDER TWO PATHOGEN CHALLENGES IN *N. rossii*

Cármen S.V. Sousa [1], M. Peng [1], P.M. Guerreiro [1], L. Chen [2], A. V.M. Canário [1,2] D.M. Power [1,2]

Email: csvsousa@ualg.pt

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[2] *International Research Center for Marine Biosciences, Ministry of Science and Technology, Shanghai Ocean University (SHOU), 201306 Shanghai, China*

Abstract

The crosstalk between the immune and endocrine system is well established in mammals but has been less studied in teleost fish. In the present study the unique Antarctic notothenioid fish are studied with a view to establishing the response of cortisol and gene expression of elements of the endocrine system under a bacterial endotoxin and viral challenge. Experiments were performed at the Great Wall Station in King George Island and exposed Notothenia rossii (30 ± 4.2 cm length and 312 ± 124.7 g weight) to a bacterial endotoxin (LPS) or viral agonist (Poly I:C). Cortisol was measured in plasma and four tissues (skin, duodenum, spleen, and liver) were collected 8 h after the challenge for transcriptome analysis. Plasma cortisol was not significantly different between the control and any of the experimental groups indicating they were all experiencing a similar stress response. The response to the immune challenge differed between tissues with the duodenum most enriched in endocrine related transcripts (FDR < 0.05) namely in the hormone receptor transcripts for insulin-like growth factor I (igf1) receptor, androgen receptor (ar), estrogen receptor beta-2 (esr2), prolactin receptor (prlr), thyroid receptor interacting proteins (trip11) and insulin-like growth factor-binding protein 5 (igfbp5) that were up-regulated in Poly I:C-treated fish. In the skin endocrine-related transcripts were scarce but included growth hormone receptor (ghr) and thyroid hormone receptor beta (thrb), which were downregulated after the LPS challenge. The results of the study suggest that endocrine factors play a role in modulating a tissue-specific and challenge-specific response during an immune challenge. Further experiments will be required to establish if this is a direct or indirect action.

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IMMUNE AND ANTIOXIDANT RESPONSES TO POLY (I:C) IN ZEBRAFISH (*Danio rerio*) DEPEND ON THE TIME OF ADMINISTRATION

Costanza Guidi [1], M.A. Esteban [2], F.J. Sánchez-Vázquez [1], L.M. Vera [1]

Email: costanza.guidi@um.es

[1] *Department of Physiology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia, Spain*

[2] *Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia, Spain*

Abstract

The circadian clock plays regulatory roles in many biological processes, including the immune system, which shows daily rhythmicity in both mammals and fish. The aim of this work was to study whether responsiveness to Poly(I:C), a synthetic analogue of double stranded RNA able to mimic a viral infection, exhibited day/night differences in zebrafish (Danio rerio). To this end, either PBS or Poly(I:C) (10µg/µL in PBS) was administered by intra-peritoneal (IP) injection to fish at two different times of the day: ZT4 (4 h after lights on) and ZT16 (4 h after lights off). Then, fish were returned to their tanks for 6 h, after which they were euthanized and sampled (at ZT10 and ZT22, respectively) (n=12/group/time point). In addition, a control group (intact fish) was also sampled at the same times. Skin, liver and kidney samples were taken to measure the expression of a set of genes involved in the immune function and the response to oxidative stress. Our results showed that some genes were up-regulated by Poly(I:C) IP injection, and in general, the relative expression levels were higher when zebrafish were injected at ZT4 than at ZT16 (e.g. gpx7, tlr3, in skin and liver, ifnphi3 in skin and ifih1 in liver). For other genes, day/night differences were found in the control group, although these differences were abolished in the PBS and Poly(I:C) groups (e.g. cata, tlr22 in skin). In addition, other genes were down-regulated by both PBS and Poly(IC) injections, such as sod, which showed lower expression levels and day/night differences in these groups but not in the control fish. Taken altogether, our results showed the existence of day/night differences in the expression of several genes involved in the immune and antioxidant defences of zebrafish in response to Poly(I:C) administration.

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EFFECT OF COLD TEMPERATURE PRE-TREATMENTS ON THE EUROPEAN EEL SPERMATOGENESIS AND SPERMIATION

Leonor Ferrão [1], M. Morini [1], V. Gallego [1], A. Felip [2], A. Gómez [2], L. Pérez [1], J.F. Asturiano [1]

Email: mleonor.pferrao@gmail.com

[1] *Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Camino de Vera s/n, 46022 València, Spain*

[2] *Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal (IATS), CSIC, Ribera de Cabanes, 12595 Castellón, Spain*

Abstract

The crosstalk between the immune and endocrine system is well established in mammals but has been less studied in teleost fish. In the present study the unique Antarctic notothenioid fish are studied with a view to establishing the response of cortisol and gene expression of elements of the endocrine system under a bacterial endotoxin and viral challenge. Experiments were performed at the Great Wall Station in King George Island and exposed Notothenia rossii (30 ± 4.2 cm length and 312 ± 124.7 g weight) to a bacterial endotoxin (LPS) or viral agonist (Poly I:C). Cortisol was measured in plasma and four tissues (skin, duodenum, spleen, and liver) were collected 8 h after the challenge for transcriptome analysis. Plasma cortisol was not significantly different between the control and any of the experimental groups indicating they were all experiencing a similar stress response. The response to the immune challenge differed between tissues with the duodenum most enriched in endocrine related transcripts (FDR < 0.05) namely in the hormone receptor transcripts for insulin-like growth factor I (igf1) receptor, androgen receptor (ar), estrogen receptor beta-2 (esr2), prolactin receptor (prlr), thyroid receptor interacting proteins (trip11) and insulin-like growth factor-binding protein 5 (igfbp5) that were up-regulated in Poly I:C-treated fish. In the skin endocrine-related transcripts were scarce but included growth hormone receptor (ghr) and thyroid hormone receptor beta (thrb), which were downregulated after the LPS challenge. The results of the study suggest that endocrine factors play a role in modulating a tissue-specific and challenge-specific response during an immune challenge. Further experiments will be required to establish if this is a direct or indirect action.

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ARGININE VASOTOCIN AND ISOTOCIN PITUITARY CONTENTS AND HYPOTHALAMIC NEUROTRANSMITTERS IN MALES OF RAINBOW TROUT DURING SPAWNING AND OUT OF SPAWNING

Arnau Rodríguez-Illamola A.[1], M. Chivite[2], M. Alborja-Valado[2], J.M. Míguez[2], V. Kholodnyy[1], M. Rodina[1], B. Dzyuba[1], S. Boryshpolets[1]

Email: arodriguez@frov.jcu.cz

[1] *Laboratory of Reproductive Physiology. Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses. University of South Bohemia in České Budějovice. Zátiší 728/II, 389 25 Vodňany, Czech Republic*

[2] *Laboratory of Animal Physiology, Department of Functional Biology and Health Sciences. Faculty of Biology. University of Vigo. 36310 Vigo, Spain*

Abstract

Arginine vasotocin (AVT) and isotocin (IT) are involved in the fish reproductive physiological and behavioural processes. These peptides act on specific gonadal receptors as endocrine and/or as paracrine signals, regulating the production/release of sexual steroids. Hypothalamic mRNA levels of both peptides and circulating AVT levels fluctuate during the seasons and so, they are probably key hormones for the seasonal synchronization of the fish reproductive physiology. The dopaminergic and the serotoninergic systems in hypothalamus are believed to be involved in the modulation of the production/release of these neurohormones. In this study, the AVT and the IT endocrine systems, and the dopaminergic and the serotoninergic neurotransmission systems in the hypothalamus were evaluated in mature males of two rainbow trout strains during and out of their spawning periods. Results showed that in both strains, pituitary AVT levels were higher at the beginning of their spawning seasons than at the end and beyond these periods. We have also found an inverse correlation in the hypothalamus between the dopaminergic system and the AVT pituitary content and in parallel, an inverse correlation between the serotoninergic system and the IT pituitary content. Results suggest that: i) AVT system may advance the onset of the spawning period while the role of IT remains uncertain, and ii) dopamine and serotonin may act on the AVT and on the IT, probably inhibiting their functions at certain reproductive phases.

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CHARACTERIZATION OF GENES RELATED TO ADVANCED GONADAL GROWTH IN PREPUBERTAL EUROPEAN SEA BASS FEMALES

L. Sempere, S. Ibáñez, Alicia Felip

Email: afelip@iats.csic.es

[1] *Instituto de Acuicultura Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Ribera de Cabanes, Castellón, Spain*

Abstract

Several studies suggest that body weight in teleost during critical periods affects the proportion of fish that reach maturation in a given reproductive season. Although there is a large variation in the age of maturity, this trait depends on genetic, physiological and environmental factors that contribute to gonadal growth. While interactions between growth and reproductive competence in male fish have been reported, much is still to be resolved in females. In the sea bass a substantial proportion of males mature precociously during the first year of life suggesting that it is only allowed in those fish which have reached a certain threshold of development or accumulated enough lipid reserves. Precocious males, which are usually larger in size, exhibit a significant increase in 11-KT presumably induced by Fsh that is able to block amh expression, a meiosis inhibiting factor, thus evoking spermatogenesis progression. Little is known about the oocyte growth in prepubertal female sea bass and its relationships with body growth; however it has been reported that advanced puberty can also affect captive-reared females. Recently, we have monitored the growth history of female sea bass and the degree of advancement in oocyte development during the first two years of life. Some body indexes and circulating vitellogenin served as markers of advanced ovarian growth. Over the second year of age, females could be divided into four distinct groups: females that remained at perinucleolar stage, females with oocytes in varying degrees of cortical alveoli incorporation and no lipid droplets, females with oocytes in secondary growth including oocytes in varying degrees of lipid droplet incorporation and no yolked droplets and females with oocytes with both lipid and yolked droplets inside. Consequently, our objective is to determine changes in ovarian gene influencing the rate of oocyte development in this aquaculture finfish species.

Acknowledgments

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IN VIVO EFFECTS OF ESTRADIOL ON THE PITUITARY AND TESTIS OF MALE EUROPEAN SEA BASS AT DIFFERENT STAGES OF SPERMATOGENESIS

G. Molés, C. Zapater, S. Ibáñez, A. Escorza, C. Lluch, Ana Gómez

Email: a.gomez@csic.es

[1] *Department of Fish Physiology and Biotechnology, Instituto de Acuicultura Torre de la Sal (CSIC) 12595 Ribera de Cabanes, Castellón, Spain*

Abstract

The pituitary gonadotropins, follicle stimulating hormone (Fsh) and luteinizing hormone (Lh) are central endocrine regulators of gametogenesis in vertebrates. Their synthesis and secretion are regulated by brain and local factors, such as gonadotropin-releasing hormone (Gnrh). In addition, gonadal sex steroids have a feedback effect modulating the availability of gonadotropins. All these actions at the level of the pituitary have a direct impact on gametogenesis progression. Previous studies in sea bass showed that the three estrogen receptors have different annual expression profiles and localization in male pituitaries, and that circulating levels of estradiol are maximum in pre-gametogenesis. In addition, estradiol could reduce the stimulatory effect of Gnrh on gonadotropin production in primary cultures of pituitary cells. In this study, we have focused on the differential in vivo action of estradiol in males in pre-gametogenesis, early gametogenesis before meiosis, and initial stages of gametogenesis including phases after meiosis entry.
We have observed that the effect of estradiol on the gonado-somatic index was different depending on the gonadal stage. It increased in pre-gametogenesis due to proliferation of type A spermatogonia but decreased at later stages due to inhibition of spermatogenesis progression.
Estradiol had a general inhibitory effect on fshb gene expression and Fsh content in pituitary and plasma at all stages analyzed. However, the effects on Lh were different depending on the spermatogenesis phase. The effect of estradiol on androgen production by the testis was inhibitory both in pre-gametogenesis and initial steps of gametogenesis, however, the outcome on gonad physiology was very different depending on the stage.

Acknowledgments

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ASSESSMENT OF OSMOTIC PUMPS AS A METHOD TO INDUCE SEXUAL MATURATION OF EUROPEAN EEL MALES AND FEMALES

Marta Blanes-García [1], P. García-Salinas [1], M. Morini [1], A. Gómez [2], A. Felip [2], L. Pérez [1], J.F. Asturiano [1], V. Gallego [1]

Email: marblaga@upv.es

[1] *Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Camino de Vera s/n. Edificio 7G 46022, Valencia, Spain*

[2] *Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal (IATS), CSIC, Ribera de Cabanes, 12595 Castellón, Spain*

Abstract

The sexual maturation of the European eel (A. anguilla) has been traditionally induced by hormonal treatments, requiring weekly handling and causing fish stress. The osmotic pumps (OP) were tested as an alternative method for induction of maturation of eel males and females. Males were divided in Control, OP-100 and OP-200 groups (n=10/group). The control group received weekly injections of recombinant hCG (1.5 IU/g fish) for 15 weeks. The induction of sexual maturation of OP groups was carried out by implanted hCGrec-loaded osmotic pumps (ALZET 1004 and 2006). Sperm samples were weekly collected by stripping and several sperm parameters were evaluated by CASA. Biometric parameters such as fin color and gonadosomatic index were evaluated as key indicators of maturation. Blood samples were taken and 11-ketotestosterone levels determined by a specific EIA. Osmotic pumps induced the maturation and spermiation in males, but without the required intensity to produce a sufficient quantity of gametes (sperm volume, density) or quality parameters (motility) for aquaculture purposes. Eel females were divided in Control (n=11) and OP-2ML4 group (n=10). The control group received weekly injections carp pituitary extract (CPE; 20 mg/kg fish) for 15-20 weeks. The OP-2ML4 group was implanted with a CPE-loaded OP ALZET 2ML4. The females were weekly monitored and those riped were induced to spawn and artificial fertilization was performed. The gonadosomatic index was also evaluated. The results showed that OP evoked an unusual early maturation in some females, evidencing that this method might become a viable alternative for eel hatchery procedures.

Acknowledgments

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5S rRNA AND tRNA IN FISH OVARIES: A HISTORY OF DUPLICATIONS

Joyanta Bir, O. Diaz de Cerio, G. Garcia, G. Chowdhury, M. Ortiz-Zarragoitia, I. Cancio

Email: joyanta.bir@ehu.eus

[1] *CBET Research Group, Dept. Zoology & Animal Cell Biology; Faculty of Science & Technology and Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country. Areatza Hiribidea 47, 48620 Plentzia*

Abstract

The transcription of 5S rRNA along with transfer RNAs (tRNA) in eukaryotes is commanded by RNA polymerase III (Pol III) and controlled by specific general transcription factors (Gtf3a, b and c). 5S rRNA is strongly expressed and accumulated in teleost oocytes constituting an effective molecular marker of sex in teleost fish gonads. Gtf3a is formed by a single protein and it is involved in transcription and accumulation of only 5S rRNA, not tRNAs. It is coded by a gene duplicated in teleost fish, with gtf3ab exclusive expression in ovaries while gtf3aa is expressed in all somatic tissues. Transcription of 5S rRNA and gtf3ab is especially high during early oogenesis (previtellogenic oocytes) and such transcript levels can be used to infer oogenic stage in fish. Hereby, we demonstrate that tRNAs are also strongly produced in fish ovaries. We have additionally studied the duplication of genes involved in the production of the polypeptidic Gtf3b. Genomes of teleosts, especially the most primitive ones, show duplications in brf1 (brf1a & b), brf2 (brf2a & b) and bdp1 (bp1a & b). Additionally, a duplication in the Pol II inhibitor maf1 is present in Danio rerio. All such genes have been sequenced in the pollution sentinel species Chelon labrosus (thicklip grey mullet) using degenerate primers and through a RNA-Seq of mullet gonads. We are studying the tissue specific transcription levels of these genes, plus TATA binding protein like-2, in D. rerio and C. labrosus (also intersex testis) and preliminary results are showing ovary specific transcription of one of the paralogues in each couple. Therefore, it can be concluded that activation of Pol III for production of 5S rRNA and tRNAs in fish ovaries is tightly controlled during oocyte differentiation and it is important in fish reproduction.

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UNBIASEDLY MEASURING OOCYTE VOLUME IN FISH USING STEREOLOGY: CAN WE USE A FASTER "CHEATED" PROCEDURE INSTEAD OF A GOLD STANDARD?

Paula Inês Costa, M. J. Rocha, E. Rocha

Email: paulainescosta.pc@gmail.com

[1] *Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal*

Abstract

Stereological methods unbiasedly measure quantitative aspects of sectioned particles. The size of fish ovarian follicles is often of interest, namely in endocrinology, but measuring their diameters in histology is biased. Unbiased follicular volumes estimate are obtainable using stereology by sampling cells with physical disectors (gold standard - GS) and computing volumes with the nucleator. Yet, because oocytes easily exceed dozens of µm in diameter, the (time-consuming) physical disector must be used. In addition, the multinucleolated nature of oocytes makes difficult the use of the traditional nucleator. We aim to find "short cuts" for estimating oocyte volumes using (theoretically biased) single-section "disectors" (SSD), avoiding serial sectioning and physical section pairing, empirically validating our methodology against the GS. We hypothesized that the GS and SSD methods provide comparable data. The goldfish was chosen for this work due to its handy size. Ovaries were processed for histology. Preliminarily, 14 serial sections (5 µm-thick) were stained with Goldner's trichrome. Six sites with follicles of different sizes were analyzed. Physical disectors were made with a distance between sections pairs of 5, 20 and 35 µm, depending on the oocyte size. The fields were randomly sampled, and section pairing was made in images taken from whole-digital slides. The unique feature for unbiased sampling and measurement of oocytes was the presence (in one paired section) of a nucleus with the envelope in sharp focus. The sampled cells were subjected to the nucleator probe (via ImageJ software). The cell sampling using the SSD was based on the same criteria. The average volumes per sampled zone and per oocyte type were analyzed with t-tests for paired samples. Both strategies produced cell and nuclear volumes that did not differ significantly. Therefore, preliminary data supports the possibility of safely using the SSD strategy instead of the GS, providing enormous time savings.

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IMPACT OF POLLUTANT FLUOXETINE EXPOSURE ON FISH ESTROGEN RECEPTORS EXPRESSION AND SIGNALLING

Catarina Moreira [1], M.A. Korzeniewska [2], P. Lima [2], C. Zapater [3], M. Dulce Estêvão [2,4], T. Knigge [1], T. Monsinjon [1], A. Gomez [3], D.M. Power [2], P. Pinto [2]

Email: catarina92moreira@gmail.com

[1] *FR CNRS 3730 SCALE, UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO), Université Le Havre Normandie, F-76600 Le Havre, France*

[2] *CCMAR-Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[3] *IATS - Instituto de Acuicultura Torre la Sal, Ribera de Cabanes, 12595 Castellón, Spain*

[4] *Escola Superior de Saúde, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal*

Abstract

There are increasing concerns regarding the accumulation of pharmaceuticals in the environment as even low dose exposures can interfere with the endocrine system of fish and other non-target aquatic organisms. In this study, we carried out different in vitro approaches using European sea bass tissues or estrogen receptors to investigate possible estrogenic-disrupting effects of fluoxetine (FLX), a widely used antidepressant and emerging environmental contaminant. Using an in vitro scale bioassay, a slight but significant increase in the enzymatic activity of osteoclast marker TRAP was detected upon exposure to FLX or to the natural estrogen 17b-estradiol (E2) for 24h, while the activity of osteoblast marker ALP was unaffected. The transcript expression of different estrogen receptor subtypes was detected in sea bass scales (nuclear receptors esr2a and esr2b and membrane receptor gperb) but this was not affected by the in vitro exposure to both compounds. Using an in vitro estrogen-response element reporter gene assay, FLX alone did not activate any of the three sea bass nuclear estrogen receptors (Esr1, Esr2a, Esr2b) but when combined with E2 showed antiestrogenic effects on the transactivation through Esr1 and Esr2b. Using an in vitro cAMP response element-luciferase reporter gene assay, significant induction of both membrane estrogen receptors (Gpera and Gperb) was achieved with FLX or E2 alone, but no synergistic effects were detected using the mixture of the two compounds. This combination of in vitro assays suggests different estrogenic and antiestrogenic mechanisms through which FLX may interfere with the scales and other fish tissues and provides promising tools for risk assessment of other potential endocrine disrupting compounds.

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DOES WARMING MODEL THE WAY XENOESTROGENS IMPACT FISH LIVER MASS, CELL SIZE AND CELLULARITY? LET'S FIND OUT USING THE GUPPY FISH!

Margarida Vilaça [1], M.J. Rocha [1], S. Tantiwisawaruji [2], E. Rocha [1]

Email: up201804847@edu.fc.up.pt

[1] *Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal*

2 Learning Institute, King Mongkut's University of Technology Thonburi, Bangkok, Thailand

Abstract

Aquatic ecosystems and organisms that inhabit them are affected by the ubiquitous presence of pollutants, which include endocrine disruptors. These compounds, metabolized by the liver, can act on and cause morphological and functional changes in this organ. Apart from that, the increase of water temperature due to global warming has inevitable consequences to ectothermic animals, like fishes, namely disturbances of metabolic processes, reproduction, growth and survival rates. However, our knowledge of the eventually combined effects of endocrine-disrupting compounds and temperature is still limited. Thus, this study aims to investigate if a warmer temperature and exposure to 17α-ethinylestradiol (EE2) influence (independently/interactively) the fish liver mass and cellularity. To test our goal, we made a stereological analysis at light microscopy. For this, we used 25 µm-thick paraffin sections of livers obtained from an assay with adult male guppies. The animals were divided into two (solvent) control groups, each kept for 45 days either at 26°C or 29°C (simulating warming), and two other groups, in the same conditions, but in which fishes were exposed to EE2 (at a nominal concentration of 50 ng/L). The results showed that EE2 and temperature independently impacted body biometry, with EE2 negatively affecting body mass and length. Preliminary stereological data suggested that fishes from control groups had larger livers, which would be in line with body biometry. In addition, control fish seem to have larger or more numerous hepatocytes (under study). The results appear to indicate that the higher temperature impacted the fundamental structure of the liver. Yet, at this point, we can not infer if the temperature modelled the eventual effects of EE2 on the liver parameters. Whatever the outcome at the end of the study, the new data will contribute to disclosing the still dubious mechanisms of fish liver responses to both factors.

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A TRIPLE MIXTURE OF ONE ESTROGEN AND TWO PROGESTINS TRIGGER MORE HISTOLOGICAL EFFECTS IN ZEBRAFISH GONADS THAN SIMPLER SCENARIOS

Rodolfo Miguel Silva, M.J. Rocha, J. F. Gonçalves, E. Rocha

Email: rmsilva@icbas.up.pt

[1] *Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal*

Abstract

Environmental pollution of water bodies by endocrine disrupts (EDCs) and particularly by the ubiquitous compounds able to mimic natural oestrogens, termed xenoestrogens, has been a hot research topic. However, there is a gap in investigating the effects of oestrogens and progestins mixtures. Experimental evidence over the years points to relevant risks for species of various taxa and significant ecological impact. This study was conducted to access effects on gametogenesis kinetics of zebrafish adults exposed to a progestins mix and ethinylestradiol (EE2). Sixty-four zebrafish (32 females and 32 males, in groups of 4) were exposed for 28 days to solvent (ethanol at 0.1%), progestins mix (100ng/L of levonorgestrel and of megestrol acetate), progestins mix + EE2 (100ng/L+10ng/L) and EE2 alone (10ng/L). We focused on three parameters of gonadal evaluation: maturity grading (1 to 5, where grade 5 corresponds to fully mature), gonadal volume and GSI values. Although the gonadal volume and GSI did not vary significantly among groups, there is a marked trend towards a lower gonadal volume and GSI in females, in groups exposed to the mixtures and EE2 alone (particularly in the latter group). This scenario generally agrees with the published studies. Regarding the grading of the ovary maturity, the control group shows a median score of 3, while the EE2 and progestin mix groups tend to have less developed maturity scores (1.5); however, without showing a significant difference. The progestin+EE2 mix group has a more clear decrease in ovary maturation, significantly differing from the control. The outcome indicates that the substances used have a direct influence on gametogenesis, as hypothesised. The most striking data comes from the mixture of progestins + EE2, which suggests a cumulative impact on the ovary maturation, likely via a delay in oocyte progress. A refined quantitative approach is underway to offer mechanistic insights.

Acknowledgments

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SKELETAL MINERALIZATION IN FISH: MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF PTH4 NEURONS

Luis Méndez-Martínez [1], L. Guerrero-Peña [1], P. Suarez-Bregua [1], S. Naranjo [2], J.J. Tena [2], J. Rotllant [1]

Email: lmendez@iim.csic.es

[1] *Acuabiotec Lab, Department of Biotechnology & Aquaculture. Institute of Marine Research, (IIM-CSIC), 36208 Vigo, Spain*

[2] *Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas/ Universidad Pablo de Olavide, Sevilla, Spain*

Abstract

Skeletal development and mineralization are essential processes driven by the coordinated action of neural signals, circulating molecules and local factors. The PTH family of peptides consists of at least six genes and plays a crucial role in the process of bone mineralization in fish by regulating calcium-phosphate homeostasis. The Pth4 peptide, recently isolated and characterized in zebrafish, has been shown to be involved in bone metabolism through the regulation of phosphate in adult zebrafish. By creating a transgenic Pth4:eNTR zebrafish line we performed a nitroreductase-mediated cell ablation of the Pth4:eNTR-expressing neurons in zebrafish larvae. This resulted in impaired mineralization and altered expression of phosphate regulation and osteoblast differentiation genes. To specifically characterize the single-Pth4 cells transcriptional landscape, we devised a workflow, including tissue disaggregation, sorting of single cell populations by fluorescence activated cell sorting (FACS), total RNA isolation/purification and construction of libraries followed by high-throughput sequencing (CNAG-CRG). Bioinformatics analyses are underway and results will be presented at the conference.

Acknowledgments

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THE MYOMAKER/MYOMIXER COMBO IN GILTHEAD SEA BREAM (*Sparus aurata*): CHARACTERIZATION AND INVOLVEMENT IN THE MYOGENESIS AND MUSCLE

Miquel Perelló-Amorós, A. Otero-Tarrazón, V.J. Pedraza, I. García-Pérez, F. Moshayedi, J.C. Gabillard, J. Blasco and J. Gutiérrez

Email: miquelperelloamoros@gmail.com

[1] *Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona. Avinguda Diagonal, 643, 08028 - Barcelona, Spain*

Abstract

The skeletal muscle is a locomotor and endocrine tissue representing up to 60% of the body weight in vertebrates formed by multinucleated myofibers originated by episodes of hyperplasia and hypertrophy during the myogenesis. Furthermore, injuries trigger a process of regeneration that involves inflammation, removal of damaged myofibers, cell migration, myogenesis and muscular remodeling. The fusion of myoblast implicates a large number of molecules, among them, the recently discovered fusogens Myomaker and Myomixer have been demonstrated to be indispensable for myoblast fusion. As the information about these proteins in fish is very scarce, the present work aimed to characterize these proteins in gilthead sea bream and their role in in vitro myogenesis and muscle regeneration. Phylogenetically, Myomaker, a 285 aa transmembrane protein is highly conserved among vertebrates, while the Myomixer, a 75 aa micropeptide, presented moderate conservation but maintaining its crucial AxLyCxL motif. In primary myocytes, myomaker, myomixer and myogenin were upregulated from day 4 to 8, to then decrease progressively. The downregulation of myods before day 8 suggests that in gilthead sea bream, the expression of myomaker and myomixer during the late myogenesis is independent of the Myods. In contrast, in the muscular regeneration in vivo, the expression of all the MRFs, myomaker and myomixer was overlapped (as observed in mammals), being upregulated 16 days post injury to then decrease progressively. Hence, in gilthead sea bream, the myogenesis is arrested during the first two weeks of regeneration where inflammation and proteolysis takes place. In conclusion, Myomaker and Myomixer are excellent markers of muscle growth that together with other regulatory molecules, such as the Gh/Igf axis, the proteolytic systems and other muscle growth markers can provide a deeper understanding of the myogenesis regulation.

Acknowledgments

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MUSCLE REGENERATION IN GILTHEAD SEA BREAM: IMPLICATIONS OF AUTOCRINE GH-IGF AXIS, PROTEOLYTIC SYSTEMS AND NEW REGULATOR FACTORS

Aitor Otero-Tarrazón, M. Perelló-Amorós, V.J. Pedraza, F, Moshayedi, I. García-Pérez, E. Capilla, I. Navarro, J. Fernández-Borràs, D. Garcia de la Serrana, J. Blasco, J.Gutiérrez

Email: aoterota7@alumnes.ub.edu

[1] *Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Avinguda Diagonal, 643, 08028 - Barcelona, Spain*

Abstract

Myogenesis is the process of skeletal muscle formation in which quiescent satellite cells activate and differentiate into myoblasts that will proliferate and fuse together to generate new muscle fibres or to pre-existing fibres to increase their size. Such complex process is mainly orchestrated by the myogenic regulatory factors (MRFs) as well as other molecules from GH-IGF axis, components of the proteolytic systems, and recently discovered molecules, such as myomaker and myomixer, among others. In fish, muscle regeneration has been previously studied in few species, but many aspects of its molecular basis are still unknown and this is the reason why this study was carried out in which an injury was done into the left anterior epaxial muscle of seventy 15 g gilthead sea bream (*Sparus aurata*) juveniles to evaluate the expression of the aforementioned genes, at days 0, 1, 2, 4, 8, 16 and 30 post injury. Results showed an early upregulation of igf-2 and a pax7 and igf1 downregulation, suggesting a possible igf2-induced satellite cell activation and proliferation. Other molecules from the GH-IGF axis did not appear to be involved in regeneration. MRFs presented a pattern of expression that fit well in myogenesis 16 days after the injury, and after the recovery of the igf1 expression. Caveolin-3, as myomaker and myomixer, followed MRFs expression as they are implicated in the later fusion stage. Proteolytic systems played crucial roles before myogenesis in cell migration (capn2) and in the subsequent muscle remodelling process (mafbx) in accordance with the revascularization suggested by the vegf expression in this final stage. The present model appears as a useful approach to study roles of the molecules involved in muscle regeneration in fish.

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ELECTRICAL PULSE STIMULATION AS A TOOL TO SIMULATE IN VITRO EXERCISE IN GILTHEAD SEA BREAM CULTURED MUSCLE CELLS

Isabel García-Pérez, A. Molsosa, M. Perelló-Amorós, A. Otero-Tarrazón, D. Garcia de la Serrana, J. Gutiérrez

Email: isabelgarcia@ub.edu

[1] *Departament de Biologia Cel·lular, Fisiologia i Immunologia. Facultat de Biologia. Universitat de Barcelona. Barcelona, Spain*

Abstract

Electrical pulse stimulation (EPS) of cultured primary myoblasts has gained special attention during the last 10 years as an in vitro exercise model in mammals. However, EPS has scarcely been tested in fish cells, so it may be an innovative approach for profiling the exercise-induced effects on muscle development and growth. Hence, the present study aimed to investigate the possible mechanism underlying the molecular effects of in vitro exercise in gilthead sea bream cultured muscle cells, and to compare the results with an in vivo model. Satellite cells from white skeletal muscle were isolated and seeded at a density of 2 × 106 cells/well in a 6-well plate. At days 3 and 7 of culture, cells were electro-stimulated for 24 h following a chronic low frequency protocol (pulse trains of 1 Hz and 30 V for 2 ms, given every 5 s) using a C-Pace EP Cell Culture Stimulator in conjunction with the C-Dish Electrode Assemblies (IonOptix). After 8 h of EPS the medium was changed to remove harmful byproducts of electrolysis. Cell survival was monitored throughout the culture to check whether EPS parameters were harmful to cells, and they turned out to be suitable EPS conditions. RNA extraction was performed after each EPS for further analysis, including the gene expression of proliferation and apoptosis related markers, myogenic regulatory factors and mitochondrial proteins that will be discussed in order to establish the equivalence of such a treatment with the in vivo exercise effects.

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THYROID HORMONES REGULATE THE EXPRESSION OF NEURODEVELOPMENTAL FACTORS IN FLATFISH DURING METAMORPHOSIS

Aurora Olvera [1], C. Carballo [2], M. Manchado [2], D.M. Power [1]

Email: aurora.olvera.vidal@gmail.com

[1] *Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[2] *IFAPA el Toruño, Puerto de Santa María, España*

Abstract

Vertebrate metamorphosis is an spectacular post-embryonic transformation of a larva into a juvenile, regulated by thyroid hormones (THs). In flatfish metamorphosis, the symmetric pelagic larva transforms into an asymmetric benthic juvenile. One enigmatic and non-obvious consequence of flatfish metamorphosis is the remodelling of the central nervous system (CNS), and although it is well known that THs have a critical and determinant role in regulating CNS development in all vertebrates, during flatfish metamorphosis TH regulation of CNS remodelling remains unexplored. We previously identified 6 transcripts (tubb6, sox9-like, notch-like, ahnak, cdnf, klf9) related to CNS development which in absence of THs their transcription was altered. With the aim to analyse the involvement of THs in CNS remodelling during flatfish metamorphosis we designed an experimental paradigm setting 3 groups; a standard normal larvae group; a hypothyroid, MMI-treated pre-metamorphic larvae; and a third group TH-treated with tetraiodothyronine (T4); sampling in 3 different stages up until the end of metamorphosis. Analysing head and body gene expression we found that CNS related factors are regulated by THs in a tissue-specific fashion. T4 treatment altered the expression of sox9-like, cdnf and klf9 in the body; tubb6 and notch-like in the head. While the blocking of TH synthesis altered the expression of notch-like in the body and head, and cdnf in the body. CNS remodelling during sole metamorphosis is far from clear, however the present work provides hints about how THs are involved in this complicated process.

Acknowledgments

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GH/IGF AXIS GENE EXPRESSION PROFILE IN DEVELOPING ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*)

G. Martínez-Rodríguez [1], N. Gilannejad [1,2], C. Johnstone [3], J.M. Quintanilla [3], A. Ortega [4], F. de la Gándara [4], A. García [3], Juan Miguel Mancera [5], R. Laiz-Carrión [3]

Email: juanmiguel.mancera@uca.es

[1] *Institute of Marine Sciences of Andalusia (ICMAN), Spanish National Research Council (CSIC), Puerto Real, Cadiz, Spain*

[2] *NORCE Norwegian Research Centre AS, Uni Research Environment, Nygårdsgaten 112, Bergen, 5008, Norway*

[3] *Spanish Institute of Oceanography Español de Oceanografía (IEO-CSIC), Málaga O.C., 29640 Fuengirola, Málaga. Spain*

[4] *Spanish Institute of Oceanography (IEO-CSIC), Murcia O.C. 30860., Mazarrón, Murcia. Spain*

[5] *Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Puerto Real, Cádiz, Spain.*

Abstract

Atlantic bluefin tuna (ABFT), Thunnus thynnus (Linnaeus, 1758), is a large migratory oceanic top predator, considered as an important worldwide fishery source and a key species in pelagic ecosystems. Survival during the early life stages is crucial for future recruitment success, with larval growth being a determining process. Fish growth and development are mainly controlled by the GH/IGF axis, being involved in skeletal and soft tissue growth, as well as in immune function, appetite control, behavior (including foraging, aggression, and predator avoidance). To characterize the ontogenetic development profile of the GH/IGF axis at the level of gene expression, an ABFT larval rearing experiment (under controlled feeding conditions) was performed in the aquaculture facilities of the Spanish Institute of Oceanography (IEO), in Mazarrón during June 2019. Eggs and larvae from 3 replicates were collected regularly every 2-3 days from 0 until 30 days post-hatching (dph). In a total of 14 sampling points (n = 6-12 larvae) along the ontogeny, growth hormone (gh) and two forms of insulin growth factor (igf1 and igf2) were analyzed by real-time RT-PCR. A sigmoidal gh expression profile was observed, with higher values at 5 and 23 (maximum) dph, and lower values at 0 (minimum), 12 and 30 dph. Nevertheless, igf1 and igf2 showed a gradual increase from early days, also with lower values at 0 and 12 dph, but with maximum levels at 30 dph. Results are discussed considering growing rates and transition from larvae to juvenile, underlining the importance of gh/igf axis during the ABFT early development and growth.

Acknowledgments

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COMPARATIVE ANALYSIS OF ENDOCRINE FACTORS DURING THE ONTOGENY OF THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Soraia Santos [1], K. Moutou [2], G. Koumoundouros [3], D.M. Power [1]

Email: soraias89@gmail.com

[1] *Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[2] *University of Thessaly, Biopolis, 41500 Larissa, Greece 3 Biology Department, University of Crete, 70013 Heraklion, Crete, Greece*

Abstract

European sea bass (*Dicentrarchus labrax*) is an important aquaculture species in the Mediterranean. The hatchery production stage of this species is of crucial importance as it supplies the juveniles and determines the productivity and quality of the commercialized fish. Growth is a trait of particular importance in aquaculture since bigger and more robust juveniles have a higher market value. The growth axis, which includes factors released from the pituitary gland, liver and locally in tissue has an important role in determining skeletal growth, which is intimately linked with somatic growth. The present study aimed to determine the relative importance of different endocrine factors in determining growth performance during the hatchery stage by studying sea bass larvae collected under different production regimes from several different hatcheries. Total RNA was extracted from larvae and used to produce cDNA that was used for quantitative PCR. A panel of genes encoding endocrine factors linked to skeletal and somatic growth performance were quantified from first feeding (4dph) until mid-metamorphosis (~50dph) from pre-selected and classified fish batches based on size and skeletal quality. The study provided insight into the profile of hormones that regulate production traits of interest, and these were correlated with skeletal quality and size. Despite differences in the genetics of broodstock, management and production regimes at the different participating hatcheries there was surprising constancy of endocrine profiles.

Acknowledgments

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FIRST LOOK INTO lncRNA REGULATING MUSCLE DEVELOPMENT AND FUNCTION IN GILTHEAD SEA BREAM (*Sparus aurata*)

A. Molsosa [1], I. García-Pérez [1], E. Sarropoulou [2], J. Gutiérrez [1], Daniel Garcia de la Serrana [1]

Email: garciadelaserrana@ub.edu

[1] *Departament de Biologia Cel·lular, Fisiologia i Immunologia. Facutat de Biologia. Universitat de Barcelona. Barcelona, Spain*

[2] *Helenic Center for Marine Research, Crete, Greece*

Abstract

Long non-coding RNAs (lncRNAs) are an emerging group of diverse non-coding RNAs (ncRNAs) with important regulatory functions at different levels. While several lncRNAs have been characterized in humans and some mammalian models, their role in fish still very much unknown. In the present work we have used existent data for the gilthead sea bream (*Sparus aurata*) fast skeletal muscle to identify lncRNAs expressed in the tissue and with a potential regulatory role. Using this approach, we have identified a total of 290 lncRNAs expressed in the fast skeletal muscle from juveniles and/or mature gilthead sea bream. Based on their relative abundance and distribution between adults and juveniles we decided to further test 20 lncRNAs. We successfully amplified 12 lncRNAs and their coding potential, cellular localization, secondary structure and potential interactions with mRNA of muscle related genes were bioinformatically predicted. LncRNAs were studied at transcription level at different ontological stages, during myogenesis, in response to nutrition, regeneration, exercise and electrostimulation. We found that lncRNAs were differentially regulated in response to the experimental conditions, suggesting different roles in regulating fast skeletal muscle response, that deserve future research to better understand their function in fish.

Acknowledgments

This work has been funded by the Agencia Estatal de Investigación by the Research Ministery of the Spanish Goverment. Grant RTI2018-100757-B-100.

AQUAFEED SUPPLEMENTATION WITH THE GHRP-6 PEPTIDE, A GHRELIN ANALOGUE, IMPROVES GROWTH PERFORMANCE IN *Sparus aurata*

L. Rodríguez-Viera [1], I. Martí [2], R. Martínez [3], E. Perera [4], J.M. Mancera [2], Juan Antonio Martos-Sitcha [2]

Email: juanantonio.sitcha@uca.es

[1] *Center for Marine Research, University of Havana, Cuba*

[2] *Department of Biology, Faculty of Marine and Environmental Sciences, Instituto de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), University of Cádiz, Spain*

[3] *Aquatic Biotechnology Department, Biotechnology Animal Division, CIGB, Havana, Cuba*

[4] *Instituto de Ciencias Marinas de Andalucía, Consejo Superior de Investigaciones Científicas (ICMAN-CSIC), Puerto Real, Cádiz, Spain*

Abstract

In the present study, the effects of the addition of different concentrations of the GHRP-6 peptide, a ghrelin analogue, to a commercial diet of gilthead seabream (*Sparus aurata*) on growth performance and intermediate metabolism of cultured animals were analysed. Juvenile fish (~20 g initial mean body mass) were fed in a medium/long term feeding period with three different diets: i) Control, non-supplemented (CTRL); ii) D100, supplemented with 100 µg GHRP-6/kg feed; and iii) D500, supplemented with 500 µg GHRP-6/kg feed. Sea breams were maintained in the experimental facilities of the Servicios Centrales de Investigación en Cultivos Marinos (SCI-CM, University of Cádiz) in an open system with constant water temperature (19 ºC) and salinity (36 ‰), and fed ad libitum during 97 days. At the end of the trial, different biometric, somatic and feed efficiency indexes were concomitantly analysed with Gh plasma levels, as well as several parameters related to the metabolic status of the animals. Our results demonstrated the existence of an overall improvement of growth performance in fish fed with diets supplemented with both doses of GHRP-6, and these effects were slightly mirrored by plasma Gh levels. Moreover, the GHRP-6 peptide does not generate physiological or metabolic alterations in fish fed the supplemented diets, although promotes a better aerobic management by the specimens fed the D100 diet as denoted by the lowering effect observed in plasma lactate. Taking together, our results strongly suggest the potential beneficial impact of GHRP-6 peptide on the aquaculture industry, as well as in fed producers for this and others cultured species.

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CORTISOL AND DEXAMETHASONE PROMOTE GLUCOCORTICOID ACTIONS IN THE LESSER SPOTTED CATSHARK (*Scyliorhinus canicula*)

J. Cabrera-Busto [1], J.M. Mancera [1], Ignacio Ruiz-Jarabo [1,2],

Email: ignaru02@ucm.es

[1] *Departament of Biology, Faculty of Marine and Environmental Sciences, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Puerto Real, Cádiz, Spain*

[2] *Department of Physiology, Faculty of Biological Sciences, University Complutense, Madrid, Spain*

Abstract

Glucocorticoids (GC), a group of corticosteroids, are hormones produced in vertebrates that are involved in physiological processes such as carbohydrate metabolism management mediated by GC receptors (GR). In sharks, the major circulating corticosteroid is 1α-hydroxycorticosterone (1α-OHB), but its effects as a GC are not entirely clear. The lack of commercially purified 1α-OHB prevents a thorough understanding of the GC actions mediated by corticosteroids. In the present study, we evaluated GC actions of corticosteroids in the lesser spotted catshark (Scyliorhinus canicula) through a set of in vivo and ex vivo approaches. In a first experiment, slow-release implants of dexamethasone (DEX, a synthetic corticosteroid) in vivo decreased plasma 1α-OHB levels after 7 days, increased liver glycogen stores and consumed those of the muscle. These results highlight that DEX affects carbohydrate homeostasis, but not if it acts as a GC. To confirm the GC actions of corticosteroids, ex vivo culture of liver and white muscle explants were exposed to cortisol (the major corticosteroid of teleost fish) and DEX. These hormones increased glucose secretion from these tissues, which is the paradigmatic response of GCs. Moreover, the use of a specific GR inhibitor (mifepristone or RU-486), confirmed the involvement of GR mediating glucose secretion induced by both cortisol and DEX. This study provides, for the first time, strong evidences supporting the influence of corticosteroids in the glucose balance of S. canicula, though the role of 1α-OHB as a GC hormone in sharks should be further confirmed.

SUB-CHRONIC TREATMENT WITH REV-ERBA AGONIST MODIFIES GLUCOSE AND LIPID METABOLISM IN GOLDFISH LIVER

Lisbeth Herrera-Castillo [1], M. Conde-Sieira [2], N. Saiz [1], E. Isorna [1], J.L. Soengas [2], M.J. Delgado [1], N. De Pedro [1]

Email: lisbethh@ucm.es

[1] *Grupo de Investigación "Neuroendocrinología de Peces", Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense de Madrid, España*

[2] *Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía and Centro de Investigación Mariña, Universidade de Vigo, España*

Abstract

REV-ERBα is a nuclear receptor that, unlike other nuclear receptors, is unable to activate transcription, since it lacks the carboxy-terminal activation function (AF2), necessary to recruit transcription co-activators. It has been proven crucial in the circadian clocks functioning as well as in the metabolic homeostasis in mammals, but few studies have been performed in fish. To determine the implication of REV-ERBα in glucose and lipid metabolism in fish, we carried out a sub-chronic treatment (7 days) with an agonist of REV-ERBα, the SR9009 (100 mg/kg, intraperitoneal administration), using the goldfish (*Carassius auratus*) as a teleost model. At the end of the experimental period, liver enzymatic activities related to glucose and lipid metabolism, and circulating glucose and triglycerides levels were quantified. The pyruvate kinase (PK) activity was significantly raised by the REV-ERBα agonist treatment, and an increase trend in the activity of the hexokinase (HK) was noticed. In agreement, a significant decrease in plasma glucose levels was observed. Sub-chronic SR9009 administration caused a pronounced reduction of plasma triglycerides, aligning with a reduction trend in the liver ATP-citrate lyase (ACLY) and fatty acid synthase (FAS) activities. Overall, these results are a novel approach for the understanding of REV-ERBα function in the metabolism of fish and point to an involvement of this receptor in the glucose and lipid homeostasis in fish. Particularly, the REV-ERBα agonist activates the glycolytic pathway and reduces the lipogenesis in goldfish, matching previous results in mammals. Nevertheless, further studies are needed to get deeper insights into REV-ERBα's metabolic role in fish, investigating other important metabolic target, such as the adipose tissue.

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A SINGLE CRITICAL SPEED SWIMMING TEST PROMOTES MITOCHONDRIAL REORGANIZATION IN TWO DIFFERENT STRAINS OF EUROPEAN SEA BASS

Adrià Robles, A. Sánchez-Moya, B. Campo Mendizabal, M. Perelló-Amorós, L. Serra-Sarró, M. López, M. Herlin, J. Gutiérrez, J. Fernández-Borràs, J. Blasco

Email: roblesbri16@hotmail.com

[1] *Department of Cell Biology, Animal Physiology and Immunology, Faculty of Biology, University of Barcelona*

Abstract

Sea bass is one of the most produced fishes in European marine aquaculture. This species naturally carries out a sustained aerobic swimming and punctual anaerobic burst in wild; however, the domestication and selection process has prioritized growth rate in detriment of swimming capacities. Repeated moderate training promotes numerous adaptations such as muscular and mitochondrial reorganization, angiogenesis and a more aerobic phenotype; nevertheless, the effects of a single extenuating exercise on these parameters are less known. The aim of our study was to identify the mitochondrial changes after a single critical speed swimming test in two selected sea bass strains of different Mediterranean producers. Firstly, fish were placed individually in a 10 L Loligo® Systems swim tunnel and rested for 4 hours at 0.5 BL/s; then, the water flow was increased in 0.5 BL/s each 15' until reaching total fatigue. Immediately, fish were recovered for 40' and then sacrificed for sampling. Fish not subjected to the test were used as a control groups. Relative expression of mitochondrial biogenesis- (pgc1α, pgc1ß, tmem20a, tmem20b, tfam), fusion- (mit1, mit2) and fission- (fis1), metabolism- (cs, cox4, ucp2a, ucp3), angiogenesis- (vegfa) and hypoxia (hif)-related genes were analysed by qPCR in red muscle, heart and liver. A single extenuating exercise generally increased the relative gene expression of pgc1α, pgc1ß, mit1, ucp2a, vegfa and hif and reduced mit2, fis1, tfam and ucp3 through tissues. However, few differences were attributable to the different strains, suggesting that breeding selection process scarcely affected to the short-term mitochondrial response.

In conclusion, a single critical speed swimming test promoted angiogenesis, hypoxia adaptation and mitochondrial reorganization, as fusion, in order to optimize energy generation and management. Furthermore, these results showed for the first time in sea bass the quick plasticity of these tissues in response to an extenuating exercise.

Acknowledgments

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