8ISFE

8th International Symposium
on Fish Endocrinology
Gothenburg, Sweden
June 28th to July 2nd 2016

CONFERENCE BOOK

8th International Symposium on Fish Endocrinology

Gothenburg, Sweden
June 28th – July 2nd 2016

We thank our generous sponsors

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IUBS

ELSEVIER

European Society of Endocrinology

the European hormone society

International Society for Fish Endocrinology (ISFE)

The mission of the newly formed ISFE is to promote the study of hormones and hormone actions in fishes (including hagfish, lampreys, cartilaginous fishes, lobed-finned fishes and ray-finned fishes). This includes topics in areas such as growth, adaptation, reproduction, stress, immunity, behaviour and endocrine disruption. ISFE will foster all studies aiming at elucidating basic mechanisms of hormone action in any fish model. The ISFE will promote research in conventional models and favor the emergence of new model species for both basic and applied research.

The ISFE website isfendo.com will provide a platform for communication between members of the community.

Through ISFE meetings and participation in meetings of sister societies, it will facilitate exchange of ideas and collaborations among scientists worldwide. The 8ISFE meeting in Gothenburg is the first major meeting organized by the society.

In particular, the Society wants to encourage and foster career development of junior members, and for the current 8ISFE meeting, the Society has provited travel grants to many junior participants.

As such support is derived from member fees, all fish endocrinologists are encouraged to join ISFE.

Welcome to Gothenburg

On behalf of the International Society for Fish Endocrinology (ISFE), we are pleased to welcome you to Gothenburg for the 8th International Symposium on Fish Endocrinology (8ISFE).

The 8ISFE gathers around 230 scientists from 27 countries for a meeting with 5 selected plenary lectures, 14 oral sessions with a total of 84 oral presentations, as well as 2 poster sessions with over 110 posters.

We are particularely happy to see the very active participation of young scientists in the meeting, with about 80 students and postdoctors attending. Our sponsors have made it possible to substantially reduce the registration fee for those participants.

We hope you will enjoy your stay in Gothenburg, both socially and scientifically. If you have any questions, please don't hesitate to ask members of the local organizing committee.

Welcome Reception

On Tuesday June 28th afternoon (17:00-19:00), the City of Gothenburg has the pleasure of inviting you to attend the 8ISFE Welcome reception hosted at the conference venue, where finger food and a glass of wine or beer will be served (pre-registration is mandatory).

Committees

ISFE International Committee

Prof Olivier Kah (Univ Rennes 1, CNRS, France), presiding
Prof Ching-Fong Chang (Nat Taiwan Ocean Univ, Taiwan)
Prof Wei Ge (Univ Macau, China)
Prof Hamid Habibi (Univ Calgary, Canada)
Prof Makito Kobayashi (International Christian Univ, Japan)
Prof Berta Levai-Sivan (The Hebrew Univ Jerusalem, Israel)
Prof Carl Schreck (Oregon State Univ, USGS, USA)
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Prof Yoshio Takei (Univ Tokyo, Japan)
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Prof Robert M. Dores (Univ Denver, USA)
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Prof Thrandur Björnsson (Univ Gothenburg, Sweden)

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Prof Svante Winberg (Uppsala Univ)
Prof Monika Schmitz (Uppsala Univ)
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Dr Eva Andersson (Institute of Marine Research, Bergen)
Dr Geir Lasse Taranger (Institute of Marine Res, Bergen)
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Prof Ivar Rönnestad (Univ Bergen)
Dr Lars Ebbesson (Uni Research Bergen)
Prof Even Jörgensen (Univ Tromsø)
Prof Ian Mayer (Norwegian School of Vet Science, Oslo)
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8ISFE Local Organizing Committee

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Prof Kristina Snuttan Sundell, Univ Gothenburg
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Dr Marcus Johansson, Univ Gothenburg
Dr Linda Hasselberg Frank, Univ Gothenburg
MSc Ida Hedén, Univ Gothenburg
MSc Daniel Morgenroth, Univ Gothenburg

Conference information

Venue
All events except the banquet will take place at Lindholmen Conference Centre, Gothenburg

Address
Chalmers Konferens & Restauranger, Lindholmen Science Park, Lindholmspiren 5, Gothenburg

Registration
The registration desk is located in the "Candela Lobby" on the left hand side after the main entrance to the building (follow the sign).
The desk will usually be manned during the conference hours, if not, find any member of the local organizing committee for assistance

8ISFE Contact person
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Conference bureau
Sweden MEETX AB is the conference bureau handling the 8ISFE, including registration and hotel bookings
E-mail: 8isfe@meetx.se
Phone: +46 31 708 86 90

Conference website:
http://8isfe.se

Getting there

By taxi:
The street address of the conference centre is **Lindholmspiren 5**

By car:
The street address of the conference centre is **Lindholmspiren 5**. There is a parking lot in front of the conference centre. Parking permit can be bought from a machine by credit card.

By bus:
The bus stop in front of the conference centre is "**Lindholmen**". To commute between the conference and down-town (bus stop "**Nordstan**") see details on the #16 bus below.

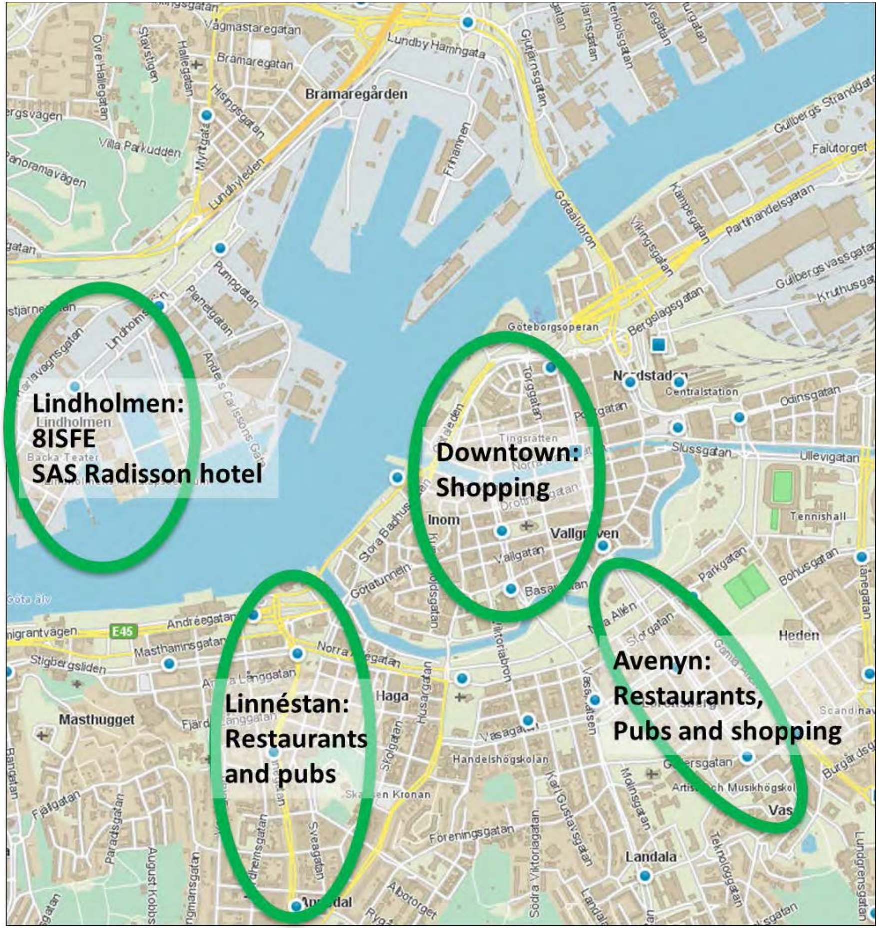
By ferry:
The ferries across the river are a part of the public transport system:

#285 Älvsnabben runs from early morning to about midnight. It stops at **Lindholmspiren** for getting to the conference, and at **Stenpiren** and **Lilla Bommens Hamn**, for getting down-town. You can pay onboard with cash and credit card.

#286 Älvsnabbare runs from 7am-7pm between the stop for the conference centre **Lindholmspiren**, and the down-town stop **Stenpiren**. **It is free of charge!!**

See maps and time-tables below

Gothenburg City overview



Key bus and ferry stops
Lindholmen – Down-town

#285 Älvsnabben (ferry from 6:30am-midnight)

285 Älvsnabben

KLIPPAN–NORRA ÄLVSTRANDEN–LILLA BOMMEN

You can pay onboard with cash or credit card

285 Älvsnabben

Klippan–Norra Älvstranden–Lilla Bommen

MÅNDAG – FREDAG																
Lindholmspiren	06.46	07.16	07.46	08.16	08.46	09.16	09.46	10.16	10.46	11.16	11.46	12.16	12.46	13.16	13.46	14.16
Stenpiren	06.52	07.22	07.52	08.22	08.52	09.22	09.52	10.22	10.52	11.22	11.52	12.22	12.52	13.22	13.52	14.22
Lilla Bommens Hamn	06.57	07.27	07.57	08.27	08.57	09.27	09.57	10.27	10.57	11.27	11.57	12.27	12.57	13.27	13.57	14.27

MÅNDAG – FREDAG															
Lindholmspiren	14.46	15.16	15.46	16.16	16.46	17.16	17.46	18.16	18.46	19.16	20.16	21.16	22.16	23.16	00.16
Stenpiren	14.52	15.22	15.52	16.22	16.52	17.22	17.52	18.22	18.52	19.22	20.22	21.22	22.22	23.22	00.21
Lilla Bommens Hamn	14.57	15.27	15.57	16.27	16.57	17.27	17.57	18.27	18.57	19.27	20.27	21.27	22.27	23.27	

285 Älvsnabben

Lilla Bommen–Norra Älvstranden–Klippan

MÅNDAG – FREDAG																
Lilla Bommens Hamn	06.30	07.00	07.30	08.00	08.30	09.00	09.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00
Stenpiren	06.36	07.06	07.36	08.06	08.36	09.06	09.36	10.06	10.36	11.06	11.36	12.06	12.36	13.06	13.36	14.06
Lindholmspiren	06.42	07.12	07.42	08.12	08.42	09.12	09.42	10.12	10.42	11.12	11.42	12.12	12.42	13.12	13.42	14.12

MÅNDAG – FREDAG																
Lilla Bommens Hamn	14.30	15.00	15.30	16.00	16.30	17.00	17.30	18.00	18.30	19.00	19.30	20.30	21.30		22.30	23.30
Stenpiren	14.36	15.06	15.36	16.06	16.36	17.06	17.36	18.06	18.36	19.06	19.36	20.36	21.36		22.36	23.36
Lindholmspiren	14.42	15.12	15.42	16.12	16.42	17.12	17.42	18.12	18.42	19.12	19.42	20.42	21.41	21.42	22.42	23.42

#286 Älvsnabbare (free ferry from 7am-7pm)

286 Älvsnabbare

LINDHOLMSPIREN–STENPIREN

The trip takes 5 minutes

286 Älvsnabbare

Lindholmspiren–Stenpiren

MÅNDAG – FREDAG

ANMÄRKNING	C		C		C		C		C	C	C	C	C	C
Lindholmspiren	06.55		10		25		40		55	09.10	09.25	09.40	09.55	10.10	10.25
Stenpiren	07.01		16		31		46		01	09.16	09.31	09.46	10.01	10.16	10.31

MÅNDAG – FREDAG

ANMÄRKNING	C	C	C	C	C	C
Lindholmspiren	10.40	55	10	25	40	18.55
Stenpiren	10.46	01	16	31	46	19.01

286 Älvsnabbare

Stenpiren–Lindholmspiren

MÅNDAG – FREDAG

ANMÄRKNING	C		C		C		C		C	C	C	C	C	C	C
Stenpiren	07.04		19		34		49		04	09.19	09.34	09.49	10.04	10.19	10.34
Lindholmspiren	07.09		24		39		54		09	09.24	09.39	09.54	10.09	10.24	10.39

MÅNDAG – FREDAG

ANMÄRKNING	C	C	C	C	C	C
Stenpiren	10.49	04	19	34	49	19.04
Lindholmspiren	10.54	09	24	39	54	19.09

#16 Bus [from 7am to 1am]

16 Eketrägatan–Lindholmen–Högsbohöjd och omvänt

The bus ride between **Lindholmen** and **Nordstan** by **bus #16** takes 8-9 minutes. It departs every 10 min between 07:00-21:00 and every 15-20 min after that.

If you're going to the conference, take bus #16 with destination "**Eketrägatan**".

If you're going down-town, take bus #16 with destination "**Högsbohöjd**".

You find time tables and other details on public traffic at http://www.vasttrafik.se

You can buy public transport tickets with the app **Västtrafik To Go.** However, this works only with cards connected to banks in the Nordic countries

You can buy public transport tickets at Västtrafik shops, Pressbyrån or 7-Eleven. A single ticket is 28 SEK and good for 90 minutes. You can also buy 1-day (85 SEK) or 3-day tickets (170 SEK).

You cannot pay at the bus.

Special Honors at the 8ISFE

– LIFETIME ACHIEVEMENT AWARDS –

At each ISFE meeting, Lifetime Achievement Awards are given to a select number of retiring scientists. The recipients are truly distinguished in the field of fish endocrinology, and have made a highly significant, enduring impact on the field through research, education, or other activities.

We are proud to present the 8ISFE recipients of the ISFE Lifetime Achievement Awards as:

– The Richard E Peter lectureship –

At each ISFE meeting, a prominent scientist is recognized for significant contributions to the field of fish endocrinology, and invited to give the honorary RE Peter Lecture.

At the 8ISFE, the recipient of the Richard E Peter Lectureship is **Professor Carl B Schreck**

– The Yoshitaka Nagahama lectureship –

The Yoshitaka Nagahama lectureship is awarded to a young investigator in recognition of significant and novel scientific contributions to the area of fish endocrinology.

At the 8iSFE, the recipient of the Y Nagahama Lectureship is **Professor Suraj Unniappan**

Programme

Morning sessions Wednesday June 29

08:30-09:00	**Opening ceremony (Main Lecture Hall)** **Olivier Kah** (FRA): President of the International Society of Fish Endocrinology (ISFE) **Thrandur Björnsson** (SWE): Head of the 8ISFE organizing committee	
09:00-10:00	**The Richard E Peter Lecture (Main Lecture Hall)** **Carl Schreck (USA) Stress, haruspication and why the endocrine system is so similar and also dissimilar amongst fishes (OR-01)**	
10:00-12:30	**Session 1: Neuroendocrinology of behaviour** **Main Lecture Hall** Chairs: **Makito Kobayashi** (JPN), **Rosemary Knapp** (USA), **Svante Winberg** (SWE)	**Session 2: Endocrine control of growth** **Lecture Hall Pascal** Chairs: **Elisabeth Jönsson** (SWE), **Takashi Yada** (JPN)
10:00-10:20	**OR-02 Matías Pandolfi** (ARG) Brain aromatase expression levels and its relationship with agonistic behaviour in a Neotropical cichlid	**OR-08 Beth M Cleveland** (USA) Specific roles for 17β-estradiol versus gonad development in nutrient partitioning and regulation of nutrient- and growth-related mechanisms during sexual maturation in rainbow trout
10:20-10:40	**OR-03 Rosemary Knapp** (USA) Brain transcriptional profiles of male alternative reproductive tactics in bluegill sunfish	**OR-09 Munetaka Shimizu** (JPN) Production of recombinant salmon insulin-like growth factor binding protein-1 subtypes
10:40-11:00	**OR-04 Øyvind Øverli** (NOR) Association between skin melanin patterns and coping style: A novel role for pigmentation genes in the stress response?	**OR-10 Shunsuke Moriyama** (JPN) Regulation of growth and seawater adaptability by insulin-like growth factors in chum salmon (*Oncorhynchus keta*) fry
11:00-11:30	**Coffee / Tea break**	**Coffee / Tea break**
11:30-11:50	**OR-05 Yukitoshi Katayama** (JPN) Thirst accompanied by water drive is induced by angiotensin II in the mudskipper	**OR-11 Miranda Marvel** (USA) Examining the functional roles of gonadotropin-releasing hormone II (Gnrh2): Insights from the zebrafish model
11:50-12:10	**OR-06 Malin Rosengren** (SWE) Rainbow trout with high and low HPI-axis reactivity possess different metabolic rates and intestinal integrity	**OR-12 Joaquim Gutiérrez** (ESP) GH effects in growth and metabolism of fingerlings and juveniles of gilthead sea bream
12:10-12:30	**OR-07 Kouhei Matsuda** (JPN) Observation of swimming behaviour in the mineralocorticoid receptor-knockout medaka fish	**OR-13 Peggy Biga** (USA) Growth hormone differentially regulates myostatin in Danio species
12:30-14:00	**Lunch / Poster viewing**	

OR-01

Stress, haruspication and why the endocrine system is so similar and also dissimilar amongst fishes

Carl B Schreck
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This lecture is dedicated to Dick Peter for his wisdom, friendship, and the major influence he had on how we think about and practice our science. In keeping with that theme, I will present information from my work, most of which is new and unpublished, that supports the contention that the generalities concerning the endocrine system across the great diversity of fishes is remarkably similar, yet at the level of the individual, population and species, is remarkably dissimilar. I use examples of research on stress and the brain, pituitary, interrenal axis in this regard. The main attributes in terms of factors involved in response to stressors are generally the same amongst the fishes, as are the generalities concerning the patterns of the responses. However, marked differences are apparent in the details of such responses, both temporally and in magnitude. It is well established that this variation is driven by the diversity in genetics and environments. However, the variation is also influenced by unsuspected, subtle differences within a population. Such variation is attributable to effects of: ontogenetic stage (e.g., transitional stages are more sensitive to stressors), life history tactics (e.g., fish seemingly sympatric may in actuality not be), energetic status [e.g., how fish cope energetically with stressors (allostasis) is context specific], temperature (e.g., a fraction of a degree decrease in temperature can affect directionality of fish movement), the nature of rearing tanks used in experiments (e.g., minute changes in lighting, flow and density can affect the physiological nature of the fish; a small amount of cover can affect brain development), the social environment (e.g., individually housed fish may be stressed if they are a gregarious species), and diet (e.g., not simply diet quality but also feeding tactics employed directly affect fish quality).

OR-02

Brain aromatase expression levels and its relationship with agonistic behaviour in a Neotropical cichlid

Martín Ramallo[1], Leonel Morandini[1], Agustina Birba[1], Gustavo M Somoza[2], Matías Pandolfi[1]
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Historically, there has been a supported notion that male to male aggressive behaviour is mainly regulated by androgens. However, in the past decades estradiol has emerged as a key element in the regulation of male reproductive and aggressive behaviour. The enzyme aromatase (CYP19A1) is the main element involved in estrogen biosynthesis through the conversion of C19 androgens to C18 estrogens. While a single cyp19a1 gene is present in non-teleost vertebrates, two copies are found in teleosts: cyp19a1a mostly expressed in the gonads, referred as gonadal aromatase, and cyp19a1b, mostly expressed in the brain, accordingly known as brain aromatase. In this work we aimed to analyze the variation in cyp19a1b expression in brain and pituitary of males of a highly social Neotropical cichlid, Cichlasoma dimerus, and its relationship with inter-individual variability in agonistic behaviour. We first began by characterizing C. dimerus specific cyp19a1b mRNA, and predicted amino acid sequence, and found a high degree of conservation when compared to other teleost brain aromatases, and its tissue expression pattern. Using an antibody raised against a conserved region of teleost aromatases, we studied CYP19A1B location within C. dimerus brain. Immunoreactivity was solely observed at putative radial glial cells of the forebrain, lining the walls of the ventricles, at the dorsal and ventral telencephalon, with the highest numbers of immunoreactive cells found at the preoptic area and hypothalamus. We then studied the relative expression levels of cyp19a1b by Real Time PCR in the brain and pituitary of males of different social status (territorial vs. non-territorial males), and its relationship with an index of agonistic behaviour. We found that even though, brain aromatase expression in whole forebrain and pituitary samples did not differ between types of males, pituitary cyp19a1b expression levels correlated with the index of agonistic behaviour ($r=0.86$, $p=0.007$), with a higher frequency of aggressive behaviour associated to a greater aromatase expression. This indicates an effect of the social environment on pituitary cyp19a1b expression, which might, in turn, result in the regulation of social behaviour by local estrogen synthesis.

OR-03

Brain transcriptional profiles of male alternative reproductive tactics in bluegill sunfish

Rosemary Knapp[1], Charlyn Partridge[2,3], Matthew MacManes[4], Bryan Neff[3]
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Understanding the molecular mechanisms influencing variation in behavior can provide insight into how different behavioral phenotypes are mediated. One type of behavioral variation that has not yet received much attention in this respect are the distinct phenotypes that comprise male alternative reproductive tactics (ARTs), which are found in a wide array of taxa, especially among fishes. One of the classic systems for studying male ARTs are bluegill sunfish, *Lepomis macrochirus* (Eupercaria: Centrarchidae). This species has two distinct life histories: parental and cuckolder, encompassing three reproductive tactics, parental, satellite, and sneaker. Parental males provide sole parental care to offspring, including any sired by the cuckolder males, which leave the nest immediately after spawning. The parental tactic is fixed, whereas individuals who enter the cuckolder life history transition from the sneaker to the satellite tactic as they grow. We used RNAseq to characterize the brain transcriptome of each male tactic during spawning to identify gene categories associated with each tactic and identify potential candidate genes influencing their different spawning behaviors. We found that sneakers had higher levels of differential gene expression compared to the other tactics, suggesting that life history is not the main factor driving differential gene expression. Sneakers had high expression in ionotropic glutamate receptor genes, specifically AMPA receptors, which may be important for increased working spatial memory while attempting to cuckold nests on bluegill colonies. We also found significant expression differences in several candidate genes involved in ARTs that were previously identified in other species of fish. Several endocrine pathways were prevalent among the differentially expressed genes between two or more male phenotypes. These genes included several in the glucocorticoid and thyroid hormone signalling pathways, genes involved in the control of feeding, and brain aromatase and several other steroidogenic enzymes. We will also discuss how these differences correlate with previously documented differences in plasma steroid hormone levels during spawning. The results of this study open new avenues of research into the neuroendocrinology underlying profound within-sex differences in reproductive behavior.

OR-04

Association between skin melanin patterns and coping style: A novel role for pigmentation genes in the stress response?

Øyvind Øverli
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In a range of vertebrate species, dark melanin-based pigmentation patterns are major characteristics of life history strategy. Typically, distinctly pigmented individuals are socially dominant, stress- and disease resistant. Yet, causative mechanisms for this widespread trait association have remained elusive. In salmonid fishes the skin area covered by melanin-based spots is directly correlated to post-stress cortisol levels. Selection studies have demonstrated that this consistent trait association is inherited, not acquired. Specifically, selection divergent post-stress cortisol production over several generations has yielded high- (HR) and low-responsive (LR) lines of rainbow trout *Oncorhynchus mykiss* which also differ in pigmentation. This association is also seen in non-selected outbred populations where contrasting stress coping styles differ in hormone dynamics, response to novelty, boldness, and parasite resistance. A single nucleotide polymorphism (SNP) in the coding region of melanocortin 1 receptor (MC1R) paralog_2 in *O. mykiss* potentially links behavior, physiology and skin pigmentation. Structural modeling and expression studies suggest a novel regulatory function for MC1R protein variants in affecting steroidogenic MC2R activity through interaction with its accessory protein (MRAP). The resulting variation in plasma cortisol levels affect several traits associated with stress coping, including expression of the MC1R antagonist agouti-signaling protein (ASIP) in skin. These novel molecular-genetic mechanisms confirm dermal melanin as a reliable indicator of heritable variation in stress responsiveness. In fishes particularly, understanding the association between colour polymorphisms and other physiological-behavioral trait clusters (i.e. coping styles and animal personalities) can illuminate the evolution of alternative life history strategies, as visual markers are are easily recognised and may provide information about other traits without requiring extensive physiological, behavioural and genetic analysis.

OR-05

Thirst accompanied by water drive is induced by angiotensin II in the mudskipper

Yukitoshi Katayama[1], Makoto Kusakabe[1], Tatsuya Sakamoto[2], Yasuhisa Kobayashi[2], Kazuhiro Saito[2], Yoshio Takei[1]
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Amphibious mudskipper was used to study the relationship between thirst and behavior and we have demonstrated both general thirst (hormone-induced) and local thirst (neuron-induced) for the first time in teleosts. Thirst can be defined as a conscious sensation of a need for water and a desire to drink, thus to search for water can be interpreted as the evidence of thirst. Two types of thirst are known in mammals: 1) General thirst that is mainly induced by high angiotensin II (Ang II), and 2) Local thirst that is induced by neural activities that sense water deprivation in local tissue such as dried mouth. Aquatic fishes can drink surrounding water only by swallowing reflex without thirst as removal of the forebrain did not inhibit Ang II-induced drinking. The mudskipper, *Periophthalmus modestus*, reserves water in their opercular and buccal cavities on land and is supposed to migrate to water to drink. We first examined general thirst and showed that intracerebroventricular injection of Ang II induced copious drinking and increased the staying time in water as in tetrapods. *In situ* hybridization showed that Ang II type-1 receptor was located in area postrema (AP), which is the site where Ang II induces swallowing reflex in teleosts. Intracerebroventricular Ang II increased immunoreactive c-Fos in AP, but not in the site of forebrain implicated in thirst in mammals. Ang II induced water-going behavior, but the behavioral control by forebrain neurons required further studies. Secondly, we tested whether the water-going behavior was induced by drainage of opercular cavities as a stimulus of local thirst. Mudskipper opercula were pierced to drain the reserved water from the cavities and pierced individuals stayed in water longer. The treatment effect was immediate, which implies that neural activity, rather than hormones, was mediating this behavior. Water drive, motivation for migration into water and ingestion of water, was induced by water deprivation in the cavities. In nature, it is supposed that Ang II induces swallowing, leading to a decrease of water in the opercular cavities, and subsequently stimulates the water-going behavior to replenish the reserved water. These results demonstrate the existence of local thirst in mudskipper and how it may integrate with general thirst to maintain osmotic balance.

OR-06

Rainbow trout with high and low HPI-axis reactivity possess different metabolic rates and intestinal integrity

Malin Rosengren[a], Fredrik Jutfelt[b], Svante Winberg[c], Per-Ove Thörnqvist[c], Erik Sandblom[a], Kristina Snuttan Sundell[a]
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Within a given population, inherent differences in HPI-axis reactivity during stress are evident. Some individuals consistently show a higher plasma cortisol response than others. Since cortisol regulate many bodily functions, this can result in a range of downstream effects and links between HPI-axis reactivity and stress coping styles has been found. The aim of this study was to examine if individuals, with high- and low cortisol responses, differ in traits linked to fitness and disease resistance that are generally affected by cortisol. Effects on intestinal barrier function, metabolic rate, expression of stress related genes in the brain and behaviour was examined during both basal and stress conditions. A population of rainbow trout were assigned as high (HR) or low (LR) responders based on repeated confinement stress tests and plasma cortisol analyses. In, contrast to our hypothesis (based on earlier research on stress coping styles), the HR group exhibited a higher routine metabolic rate compared to the LR group, possibly linked to cortisol boosting metabolic activity. There was no difference in aerobic scope or hematology measurements. While no major differences in general activity or boldness was found during an open field trial, the LR group exhibited a higher level of burst swimming. The HR group had higher intestinal integrity during basal levels (measured as low paracellular permeability and high electrical resistance), which could be positive from a disease resistance perspective. However this pattern was reversed during stress conditions with the HR group showing a decreased intestinal integrity, possibly mediated by cortisol, while the LR group instead showed an increase. An increase in intestinal integrity, indicating a strengthened barrier, has never been reported during stress conditions and the mechanisms behind remains unclear. Furthermore, during basal conditions a negative correlation between plasma cortisol and intestinal barrier function were found for the LR group, possibly revealing a higher sensitivity to the stress hormone during basal conditions for this group. This study provides insight into inherent differences between salmonids with diverging HPI-axis reactivity and how they react to stress and handling. It also gives insight into the possible origin of the large individual variation in data sets assessing metabolism and intestinal barrier function.

OR-07

Observation of swimming behaviour in the mineralocorticoid receptor-knockout medaka fish

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In teleost fish, it has been held that cortisol carries out both glucocorticoid and mineralocorticoid actions via glucocorticoid receptor, since teleost has been considered to lack aldosterone. Recently, the counterparts of tetrapod mineralocorticoid receptors and their specific endogenous ligand, 11-deoxycorticosterone, have been identified. However, the effects of treatment with mineralocorticoid receptor (MR) and glucocorticoid receptor agonists or antagonists have pointed out the minor role of mineralocorticoid signalling in the osmoregulation in fish. In mammals, mineralocorticoid signalling has been implicated to control central actions such as locomotor activity in addition to the osmoregulation. Thus, we have generated the first MR-knock-out (KO) model in medaka fish (*Oryzias latipes*) using transcription activator-like effector nuclease technique. Hyper- and hypo-osmoregulation were normal in MR-KO medaka compared to wild-type (WT) controls. These findings reinforce previous results showing a minor role for mineralocorticoid signalling in fish osmoregulation. Therefore, in this study, we observed swimming behaviour in the MR-KO medaka. In comparison to WT of fish, the MR-KO fish failed to track the moving dots on the screen, and decreased locomotor activity, whereas showed normal thigmotaxis. Subsequently, we used a preference test (upper/lower test) for measuring anxiety-like behaviour in fish. In comparison to WT fish, MR-KO fish prolonged the time spent in the lower area. These results indicate that MR-KO induces behavioural changes in medaka, and suggest the involvement of mineralocorticoid signalling in psychomotor action.

OR-08

Specific roles for 17β-estradiol versus gonad development in nutrient partitioning and regulation of nutrient- and growth-related mechanisms during sexual maturation in rainbow trout

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The contribution of sex steroids to nutrient partitioning and energy balance during gonad development was studied in rainbow trout (Oncorhynchus mykiss). Nineteen month old triploid (3N) female rainbow trout were fed a diet supplemented with 17β-estradiol (E2) at 30 mg steroid/kg diet for a 1 month period. Growth performance, nutrient partitioning, and expression of genes central to growth and nutrient metabolism were compared to 3N and age-matched diploid (2N) female fish consuming a control diet. Only 2N fish exhibited active gonad development, with gonad weights (GSI) increasing from 3.7% to 5.5% of body weight throughout the study while GSI in 3N fish remained at 0.03%. Consumption of E2 in 3N fish reduced fillet growth and caused lower fillet yield compared to 2N and 3N controls ($P < 0.05$). In contrast, viscera fat somatic index was least in maturing 2N ($P < 0.05$) but was not affected by E2 in 3N. Gene transcripts associated with physiological pathways were identified in maturing 2N and E2-treated 3N fish that differed in abundance from 3N control fish ($P < 0.05$). In liver these mechanisms included the growth hormone/insulin-like growth factor (IGF) axis (*igf1*, *igf2*), IGF binding proteins (*igfbp1b1*, *igfbp2b1*, *igfbp5b1*, *igfbp6b1*), and genes associated with fatty acid binding (*fabp3*, *fabp4*), synthesis (*acc*), fatty acid oxidation (*cpt1a*), and the pparg transcription factor. In muscle these mechanisms included reductions in myogenic gene expression (*fst*, *myog*) and the proteolysis-related genes, *ctsl* and *gabarapl1*, suggesting an E2-induced reduction in the capacity for muscle growth. These findings suggest that increased E2 signaling in the sexually maturing female rainbow trout alters physiological pathways in liver, particularly those related to IGF signaling and lipid metabolism, to reduce de novo fatty acid synthesis, increase lipid oxidation, and partition nutrients away from muscle growth towards support of maturation-related processes. In contrast, the mobilization of viscera lipid stores appear to be predominantly driven by energy demands associated with gonad development.

OR-09

Production of recombinant salmon insulin-like growth factor binding protein-1 subtypes

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Insulin-like growth factor (IGF)-I is a growth promoting hormone exerting its actions through endocrine, pracrine and autocrine manners. Local IGF-I is essential for normal growth whereas circulating IGF-I plays a crucial role in supressing production and secretion of growth hormone (GH) by the pituitary gland. These actions of IGF-I are modulated by six insulin-like growth factor binding proteins (IGFBPs). In teleosts, there are subtypes of each member of IGFBP due to an extra round of whole genome duplication. IGFBP-1 is generally an inhibitor of IGF-I actions and two IGFBP-1 subtypes of zebrafish exhibit overlapping yet distinct inhibitory actions during development. In salmon, IGFBP-1a and -1b are two of three major circulating IGFBPs and increased under catabolic conditions. Despite supporting evidence of their inhibitory actions on IGF-I, exact functions of the IGFBP-1 subtypes on growth regulation are not known due to the lack of enough amount of purified or recombinant protein. We expressed recombinant salmon (rs) IGFBP-1a and -1b with a fusion protein (thioredoxin, Trx) and His-tag in *E. coli*. Recombinant proteins were fractionated and purified by Ni-affinity chromatography. Trx.His.rsIGFBP-1s were then enzymatically cleaved to remove the fusion protein. As results, rsIGFBP-1a without fusion protein was obtained while rsIGFBP-1b was degraded. Therefore additional adjustments are required for rsIGFBP-1b. We next added Trx.His.rsIGFBP-1a and -1b in combination with IGF-I to the medium of the primary pituitary cell culture of 2-year-old immature masu salmon to see how these IGFBPs modulate the IGF-I action on GH secretion. IGF-I alone inhibited GH secretion at high dose and the combination with either Trx.His.rsIGFBP-1a or Trx.His.rsIGFBP-1b enhanced the inhibitory effect of IGF-I. However, such effect was not seen in the culture of pituitary cells from 1-year-old masu salmon smolts. These results suggest that IGFBP-1s could potentiate IGF-I action on the pituitary under certain stage/condition in salmon. We are currently preparing larger amounts of rsIGFBP-1s without fusion protein and His-tag to validate their effects. Availability of rsIGFBP-1s should also be useful for antibody production and assay development.

OR-10

Regulation of growth and seawater adaptability by insulin-like growth factors in chum salmon (*Oncorhynchus keta*) fry

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Chum salmon (*Oncorhynchus keta*) is one of the most important fishery products in Hokkaido and Tohoku areas of Japan. Over four hundred million salmon fry are released from hatcheries every year to maintain and increase salmon resources in Iwate Prefecture. The homing rate of chum salmon, however, has gradually decreased during the past two decades. This may be due to high mortality and predation on salmon fry staying inside the bay after being released from the hatchery. To enhance the salmon resources, the regulation of somatic growth and seawater adaptation in the salmon fry need to be investigated. It is well established that the growth hormone (GH) and insulin-like growth factor (IGF) axis plays an essential role in the regulation of growth and seawater adaptation in euryhaline fish, including salmonids. The function(s) of the GH/IGF axis in salmon fry, however, are still unclear. Therefore, the objective of this study was to examine growth and physiological variables associated with growth and seawater adaptability, by monitoring IGF-I and IGF-II mRNA expression in the gills and liver, as well as the major endocrine regulators of these processes in chum salmon fry reared for several weeks in FW and SW. The present study demonstrated the rising activity of the GH/IGF axis seems to promote the hypoosmoregulatory ability first, and then the axis involving liver organ achieves the somatic growth. This study also investigated the growth-promoting effect by feeding pellets containing salmon GH, Isada krill (*Euphausia pacifica*) extract, and salt to the salmon fry.

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OR-11

Examining the functional roles of gonadotropin-releasing hormone II (Gnrh2): Insights from the zebrafish model

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Gonadotropin-releasing hormone (Gnrh) is a decapeptide found in the brain of all vertebrates, with up to three isoforms existing in teleosts. The hypothalamic population of Gnrh (Gnrh1 or 3) is well known to be the main regulator of the reproductive brain-pituitary-gonad axis. However, the Gnrh2 isoform, located in the midbrain tegmentum, has been much less studied. Gnrh2 is evolutionarily conserved in all vertebrates except rodents, suggesting an important role of this peptide. Prior studies suggest Gnrh2 has roles in inhibiting feeding in fish and stimulating reproductive behaviors in female musk shrews and sparrows, but the exact function and mechanism of action of this peptide in the feeding and reproductive pathways are largely unknown. With mice lacking Gnrh2, zebrafish has emerged as an ideal organism to study Gnrh2. We developed a line of Gnrh2 knockout zebrafish (gnrh2-/-) with a targeted, heritable mutation within the coding region resulting in a frameshift and subsequent loss of the peptide. Using this line of fish to conduct loss-of-function studies, we show that Gnrh2 may have a central role in controlling feeding behavior and could be a downstream signal of satiation in zebrafish. At early life stages, gnrh2-/- fish display significantly increased food intake, mobility, growth rates, and dry weights compared to wild-type fish. This was associated with alterations of gene expression profiles of several feeding-related peptides, such as the downregulation of agrp and upregulation of mch. Gnrh2-/- fish did not exhibit any differences in reproductive performance despite higher levels of fsh and lower levels of lh expression levels in embryos and adult pituitaries and gonads. Interestingly, the double knockout (gnrh2-/-; gnrh3-/-) fish, that completely lack the Gnrh system, were also reproductively normal, suggesting that compensation may play a role in the reproductive pathway. Overall, these findings strongly suggest that Gnrh2 is an important player in the feeding pathway, controlling the intake of food in zebrafish by potential interactions with feeding neuropeptides, and may be involved in the integration of feeding and reproductive activities.

OR-12

GH effects in growth and metabolism of fingerlings and juveniles of gilthead sea bream

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The optimization of growth in commercial fish species is an important objective for aquaculture. Gilthead sea bream is greatly appreciated in European markets and one of the most cultured species in the Mediterranean. The objective of this study was to know how far from an optimal growth we are in the culture of this species. For that, we administered a prolonged-release recombinant bovine growth hormone (rBGH) formulation (Posilac®, Elanco) to either fingerlings (2 g body weight) or juveniles (16 g) with a single injection of 4 or 6 mg/g body weight, respectively, and then we followed the growth during 6 or 12 weeks. Six weeks post-injection, liver, vertebra bone, muscle tissue and blood samples were collected in the case of fingerlings, and at times 0, 6, 9 and 12 weeks for juveniles. The gene expression levels of insulin-like growth factors (IGF-I and II), growth hormone receptors (GHR-I and II), IGF-I receptors (IGF-IRa and Rb), as well as some IGF binding-proteins (IGFBP1, 2, 4 and 5) were measured by quantitative PCR. Furthermore, the IGF-I plasma levels were analysed. In fingerlings and juveniles rBGH injection provoked significantly higher body weight and an increase in Standard Growth Rate in comparison to control fish injected with vehicle (i.e. sesame oil). The hepato-somatic and mesenteric-fat indexes diminished in rBGH-treated fish. Moreover, mRNA analyses indicated an enhanced expression of several genes of the GH/IGFs system in fingerlings liver, while variable differences were observed in the skeletal tissues. In addition, the plasma levels of IGF-I increased significantly as an effect of the rBGH treatment in fingerlings and juveniles at 6 and 12 weeks, respectively. The metabolic changes showed that the rBGH treatment acted in the fish saving dietary proteins for muscle growth by promoting the use of lipids and carbohydrates for energy demands. In summary, these findings reveal the important role of the hepatic IGFs mediating the effects of rBGH in gilthead sea bream, and demonstrate the possibility to improve growth and quality of cultured fish looking for those conditions that will generate the best GH/IGF ratio and IGF-I response. Thanks to Elanco Animal Health for kindly providing the rBGH. Supported by MINECO (AGL2012-39768) and Catalonian Government (2014SGR-01371and XRAq).

OR-13

Growth hormone differentially regulates myostatin in Danio species

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Zebrafish (*Danio rerio*) and giant danio (*Devario aequipinnatus*) exhibit different growth paradigms, determinate-like (zebrafish) and indeterminate (giant danio), allowing for an in depth investigation into pathways regulating muscle growth. Recent studies in our lab have demonstrated that myogenic precursor cells (MPCs) isolated from these two species exhibit different proliferation capacities *in vitro* and biomarkers that represent different phenotypic stages in myogenic cell lineage commitment. Giant danio (indeterminate growth) MPCs exhibit greater proliferation *in vitro* compared to zebrafish MPCs. Consistent with the proliferative data, zebrafish MPCs express higher levels of the myogenic lineage marker myf5, while giant danio MPCs express low levels of myf5 but high levels of the early myogenic stem cell markers Pax-3 and -7. In addition, growth hormone induces proliferation to a greater extent in giant danio MPCs *in vitro* compared to zebrafish MPCs. These data are consistent with increased overall giant danio body mass observed *in vivo* following growth hormone injection, while zebrafish fail to exhibit a maintained body mass increase in response to growth hormone injections *in vivo*. Corresponding to these changes (or lack thereof) in growth in response to growth hormone are changes in myostatin expression. Growth hormone reduced myostatin in giant danio, but increased myostatin expression in zebrafish muscle *in vivo*. These data suggest that muscle tissue receptivity to growth hormone and/or insulin-like growth factor 1 may be important in overall growth potential in actively growing fishes.

Afternoon sessions Wednesday June 29

14:00-16:30	**Session 3: Endocrine control of ion- and osmoregulation** **Main Lecture Hall** Chairs: **Steve D McCormick** (USA), **Kristina S Sundell** (SWE), **Gordon Grau** (USA)	**Session 4: Evolution of endocrine systems** **Lecture Hall Pascal** Chairs: **Dan Larhammar** (SWE), **Robert Dores** (USA), **Sylvie Dufour** (FRA)
14:00-14:20	**OR-14 Yoshio Takei** (JPN) Molecular physiology of active esophageal desalination in seawater eels	**OR-20 José Miguel Cerdá Reverter** (ESP) A journey in time across the melanocortin system
14:20-14:40	**OR-15 Paula Suarez-Bregua** (ESP) *In vivo* genetic evidence for PTH-mediated regulation of systemic phosphate homeostasis in fish	**OR-21 Dan Larhammar** (SWE) Evolution of the growth hormone pathway in relation to the vertebrate genome doublings
14:40-15:00	**OR-16 Juan Miguel Mancera** (ESP) Osmoregulatory role of vasotocinergic and isotocinergic systems in the gilthead sea bream (*Sparus aurata* L)	**OR-22 Deborah M Power** (PRT) Mapping thyroid axis evolution through metamorphosis
15:00-15:30	**Coffee / Tea break**	**Coffee / Tea break**
15:30-15:50	**OR-17 Tom O Nilsen** (NOR) Androgens up-regulate freshwater ion transporters in seawater acclimated Atlantic salmon (*Salmo salar* L.)	**OR-23 Josep Rotllant** (ESP) A new model for the evolution of the role of the PTH family in bone formation during the vertebrate transition from an aquatic to terrestrial lifestyle
15:50-16:10	**OR-18 Makoto Kusakabe** (JPN) Identification of genes responsible for adaptation to different salinities in three-spined stickleback	**OR-24 Gersende Maugars** (FRA) Evolution of the corticotropin-releasing hormone paralogs in teleosts
16:10-16:30	**OR-19 Andre P Seale** (USA) Recent advances in the tilapia prolactin cell model for osmoreception	**OR-25 Robert M Dores** (USA) Analysing the evolution of melanocortin-2 receptor ligand selectivity: studies on rainbow trout MC2R
16:30-18:30	**Poster session I: Presenting authors requested to stand by odd numbered posters**	

OR-14

Molecular physiology of active esophageal desalination in seawater eels

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Marine teleosts can absorb more than 80% of imbibed seawater (SW) by the intestine, which is in contrast to terrestrial mammals that lose water by drinking SW. This extraordinary ability of marine teleosts is enabled by the esophageal desalination that specifically removes Na^+ and Cl^- from luminal SW without osmotic loss of water. In the sac experiment, desalination was enhanced 10 folds in SW eel esophagus than that of freshwater (FW) eels. The facilitated desalination was abolished by the removal of either Na^+ or Cl^- from the luminal fluid, showing that Na^+ and Cl^- absorption are coupled. Various inhibitors were applied on either mucosal or serosal side of the sac to assess the transporters involved in the desalination, and the results suggest that Na^+/H^+ exchanger (NHE) and Cl^-/HCO_3^- exchanger (AE) on the apical membrane and Na^+/K^+-ATPase (NKA) and Cl^- channel (CLCN) on the basolateral membrane are responsible for transcellular NaCl transport. Transcriptomic analyses (RNA-seq) were performed in the esophagus of FW and SW eels to identify candidate genes expressed in the esophagus and upregulated in SW fish. The responsible genes for low water permeability of SW eel esophagus were also sought. The changes in gene expression were validated by real-time qPCR. The expression of the candidate genes in the esophageal epithelial cells was also confirmed by in situ hybridization. Among NaCl transporting genes upregulated in the epithelial cells of SW eels, we suggest that (1) NHE3 and AE2 combined by a scaffolding protein (NHERF1) are involved in the NaCl uptake at the apical membrane of epithelial cells, which are facilitated by the upregulated cytosolic carbonic anhydrase (CA2) that supplies H^+ and HCO_3^-, and (2) NKA
□1c1/NKAiβ3a and
CLCN2 are responsible for NaCl transport on the basolateral membrane. The low water permeability may be due to the prominent downregulation of aquaporin genes (apical AQP1 and basolateral AQP3) and upregulation of the CLDN15a gene after SW acclimation. These results allow us to depict a molecular mechanism of active desalination via transcellular route and low water permeability in the esophagus of marine teleosts, which is essential for adaptation to high salinity SW environment. We have shown that guanylins, natriuretic peptides, and vasoactive intestinal peptides are important for NaCl and water absorption by the intestine of SW eels, and some of the hormones and their receptors are also expressed in the esophagus. Investigations are underway to examine the effects of these hormones on the regulation of esophageal desalination in SW eels

OR-15

In vivo genetic evidence for PTH-mediated regulation of systemic phosphate homeostasis in fish

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The mineralized bone is one of the most distinctive features of vertebrates that provide the supportive framework for the body but also the protection for the internal organs. Besides this evident structural role, the mineralized skeletal tissue performs an essential metabolic function as ion reservoir for homeostasis maintenance. Thereby, bone mineralization is dependent on calcium and phosphate minerals availability which are laid down on the bone matrix as hydroxyapatite crystals. The mineralization process takes place first during early development but also throughout the entire life and underlies to a complex multifactorial regulation. We have recently identified in zebrafish the parathyroid hormone 4 (Pth4), an ancestral member of the PTH family that has been lost in placental mammals during the vertebrate lineage evolution. Gain of function analysis in adult transgenic zebrafish showed Pth4 as a neuropeptide acting in bone mass accrual through the phosphate homeostasis regulation. Here, we generated knock out mutants for the Pth4 gene. We used the CRISPR/Cas9 system to target the exon 1 of the gene and recovered a mutant with a 276bp insertion, leading to a frame shift resulting in a truncated protein. Fish homozygous for this mutation showed impaired bone mineralization while the formation of cartilage was unaffected. This shows that the absence of Pth4 leads to decrease in bone mineralization. The results, therefore, provide clear evidence that Pth4 is an essential brain regulator of bone formation and mineral homeostasis.

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OR-16

Osmoregulatory role of vasotocinergic and isotocinergic systems in the gilthead sea bream (*Sparus aurata* L)

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Gilthead sea bream, *Sparus aurata* L., is an important fish species for the Mediterranean aquaculture and is considered a good model for the study of osmoregulatory processes due to its capacity to cope with great changes in environmental salinity (5-60 ppt). Our group studied the osmoregulatory role of different endocrine systems in this species, focussing on the vasotocinergic and isotocinergic systems during the last years. For this purpose, cDNAs for pro-AVT, pro-IT, two AVT receptors (V1a2- and V2-types) and one IT receptor (ITR) were cloned. Acclimation to different environmental salinities induced a direct lineal relationship between plasma AVT levels and salinity, with no changes in plasma IT values. In addition, higher values in hypothalamic pro-VT and pro-IT mRNA expression, correlated with changes in plasma cortisol levels, as well as with pituitary AVT and IT storage levels in both hypo- and/or hyper-osmotic transfers, suggesting an interaction between cortisol and AVT/IT pathways. Moreover, mRNA expression of specific receptors demonstrated an important osmoregulatory orchestration in different organs. The use of different *in vitro* techniques indicated: i) a modulatory role of AVT and IT in Cl^- secretion across the opercular epithelium mediated by different ion transporters; ii) a stimulatory role of AVT in the regulation of intestinal ion absorption; and iii) a functional involvement of AVT and IT in the stimulation of intestinal water transport *via* AQP1 paralogs, promoting acclimation to high salinity environments. In addition, specimens intraperitoneally injected with AVT and transferred to LSW or HSW enhanced plasma cortisol levels and/or gill Na^+,K^+-ATPase activity. These effects could be related to the energy repartitioning process occurring during osmotic adaptation of *S. aurata* to extreme environmental salinities, which could be mediated not only by plasma cortisol but also by AVT. Finally, our results indicated a very important role of the vasotocinergic and/or isotocinergic systems in both osmoregulatory and non-osmoregulatory organs.

OR-17

Androgens up-regulate freshwater ion transporters in sea-water acclimated Atlantic salmon (*Salmo salar* L.)

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The anadromous lifecycle of salmonids entails migratory transitions between freshwater (FW) and seawater (SW) habitats. Such migrations require a remodeling of key ion transporting mechanisms in osmoregulatory organs. Early puberty is a phenomenon most pronounced in males, as they normally reach puberty at a lower age and size than females. Androgens may compromise ion homeostasis, yet information on potential effects of androgens on hyper-osmoregulatory mechanisms in migratory salmonids is limited. Here we investigated the effects of androgens on osmoregulatory systems by *in vivo* administering testosterone, T (75 µg/g body weight), 11-ketoandrostenedione, OA, (25 µg/g), or T+OA (T; 75 µg/g +OA 25 µg/g) to immature, SW-acclimated Atlantic salmon post-smolts. The fish displayed a transient increase in circulating T and 11-KT levels after 7 days, with levels still elevated above control levels after two weeks. Gill Na^+, K^+ ATPase (Nka) activity, plasma osmolality and Cl^- levels in androgen-treated post-smolts did not differ from sham-injected controls, except for a decrease of Nka activity in OA and T+OA. Androgen-treated post-smolts displayed several-fold increases in gill *nka-*α*1a*, *cftr-II* and *h-atpase* β subunit mRNA levels, all of which are transcripts typically up-regulated in FW gill epithelia. Conversely, the expression of typical SW transporter genes, such as gill *nka-*α*1b, nkcc1a* and *cftr-I,* as well as the number of Nka-α1b positive ionocytes (ICs), and Nka-α1b protein levels were not affected by androgen treatment. Androgen-treated post-smolts displayed 35-65 fold increases in the abundance of gill Nka-α*1a* positive ICs, which were located mostly on the primary filaments. The presence of gill androgen receptors (*ara*1 and *ara*2) and up-regulation of *ara*2 transcripts in gills of T and T+OA treated post-smolts may allow for direct androgen action on gill epithelia. Exposure to androgens does not exert osmoregulatory perturbations in SW acclimated pre-pubertal Atlantic salmon, but up-regulates FW ICs and ion transporters in gill epithelia, possibly mediated via locally expressed androgen receptors.

OR-18

Identification of genes responsible for adaptation to different salinities in three-spined stickleback

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Adaptation to high salinity environments requires precise and sophisticated mechanisms of osmoregulation. Marine and stream ecotypes of three-spined stickleback (*Gasterosteus aculeatus*) are an excellent model to explore the osmoregulatory mechanisms in fishes. To identify factors that are indispensable for adaptation to seawater environments, we employed an integrated approach of genomics and physiology. Wild type, second and third generations of marine and stream stickleback were challenged to 100% seawater to evaluate the osmoregulatory ability in different ecotypes. There was a significant difference between the plasma Na^+ concentrations of marine and stream ecotypes after seawater transfer even in the different generations. These results suggest that the seawater adaptability is regulated by inheritable factors. To identify the crucial osmoregulatory genes, we conducted quantitative trait locus (QTL) analysis. We generated an F_2 intercross between marine and stream ecotypes, and challenged individuals to 100% seawater. Plasma sodium levels were used as a phenotype for QTL mapping to identify loci associated with osmoregulatory ability, which indicated a region on Chromosome 16 underlies this trait. To screen the candidate genes, whole genome sequencing was conducted and six genes occurring within QTL regions were predicted to have functionally important amino acid substitutions. Candidate genes were further investigated by RNA-sequencing of F_2 fish with high and low plasma sodium levels, microarray analysis and quantitative RT-PCR using gill samples of multiple marine and stream ecotypes. This analysis showed that another six genes were expressed significantly higher in marine ecotypes than in stream ecotypes. Genome scan analysis confirmed that some of these candidate genes were located in genomic islands of high differentiation (F_{ST}). Candidate genes include *atp5g3a, calcrla, igfbp5a* and *col5a2a* suggesting that ATP synthesis, calcitonin-gene-related peptide regulation, insulin-like growth factor signaling, or structural protein regulation may be crucial for seawater adaptation.

OR-19

Recent advances in the tilapia prolactin cell model for osmoreception

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Osmoregulation is a foundational prerequisite for life in complex organisms and osmoreception is the first step in osmoregulation. Mozambique tilapia, *Oreochromis mossambicus*, are an excellent model for studying osmoreception and osmoregulation as they are able, with high sensitivity and precision, to modify the function of the cells and tissues that regulate salt and water balance. The native distribution of Mozambique tilapia is characterized by estuarine areas subject to salinity variations between fresh water (FW) and seawater (SW). Adaptation to changes in environmental salinity is largely mediated by prolactin (PRL), a pituitary hormone that is directly stimulated by a fall in extracellular osmolality and acts to promote ion uptake in osmoregulatory tissues, thereby allowing the organism to compensate for large changes in external osmolality while maintaining internal osmolality within a narrow range. Employing the tilapia PRL cell model, our laboratory has studied the mechanisms underlying osmoreception as well as how this sensory modality can be affected by various hypothalamic and extra-hypohtalamic factors and by different salinity regimes. Exposing PRL cells to estradiol (50 nM) for 48 h, augments the baseline release of PRL in hyperosmotic conditions, *in vitro*. Rearing tilapia in tidally-changing salinities, characterized by changes between FW and SW every 6 h, uncouples circulating PRL from physiological variations in plasma osmolality, *in vivo*. We have also recently found that PRL exerts positive feedback on itself, and the extent of this feedback is modulated by extracellular osmolality. Together, these findings support the notion that a full understanding of the multifactorial regulation of PRL can only be obtained when many levels of regulation and their interactions are considered comprehensively.

OR-20

A journey in time across the melanocortin system

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Melanocortin conforms one of the most complex endocrine systems. It integrates different agonists encoded in the multiplex precursor named proopiomelanocortin (POMC). The basic structure of POMC involves three domains, i.e. the N-terminal pro-γ-MSH (melanocyte-stimulating hormone), the ACTH (adrenocorticotropic hormone) and the C-terminal β-lipotrophin domain. Each domain contains an MSH peptide delineated by a core sequence HFRW. The agonist effects are mediated by five different melanocortin receptors (MCR1-MC5R) that positively couple the cAMP production. Atypically, this endocrine system also includes endogenous antagonists that compete with melanocortin agonist for binding to the receptors. The activity of these receptors is modulated also by the interaction with the melanocortin receptor accessory proteins (MRAP) that can drive the intracellular trafficking of the receptors or even change their pharmacological profile. The melanocortin system probably arose prior to the emergence of jawless vertebrates, over 500 MYA ago. The system was diversified during the two genome duplication rounds. Several evolutionary processes are represented within this family including internal tandem duplications within the POMC gene to give rise the different MSH sequence cores or local duplication to generate two different receptors MC2R/MC5R or MC4R/MC5R. New intron acquisition by reverse splicing and, of course, gene loss processes after genome duplication. The evolution of the family is linked also to putative co-evolutionary processes nicely represented by the lack of MC3R and the main receptor ligand γ-MSH in teleost fish and the possible rescue of MC2R function by the functional divergence of the MRAP2 copy, now named MRAP1. Recently, we demonstrated that MC4R/MRAP2 interaction provides ACTH sensitivity to the receptor and we think that these interactive processes have evolutionary importance since the receptor/accessory protein interaction could provide new mechanisms to the GPCRs for modifying the binding spectrum and also result in sensitivity of new target tissues to the peptide hormone. In this talk, we summarize different evolutionary hypotheses proposed for the different melanocortin partners.

OR-21

Evolution of the growth hormone pathway in relation to the vertebrate genome doublings

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The growth-regulating pathway of growth hormone (GH) and insulin-like growth factor 1 (IGF1) consists of multiple components exerting feed-forward and feed-back regulation. We review here the evolution of several of these components in relation the two early vertebrate genome doublings (2R) and the teleost-specific genome doubling (3R). GH release is stimulated by GHRH and inhibited by somatostatin. It has previously been reported that somatostatin as well as two ancestral somatostatin receptors (SSTR) were duplicated in 2R and 3R; SSTR increased from 2 to 6 genes in 2R (Tostivint et al., 2014; Ocampo Daza et al., 2012). Beyond the GH receptor (GHR), the Janus kinases (JAK) constitute a quartet that arose in 2R. These kinases activate STAT5b which resulted from a duplication in 2R (that gave rise to STAT5 and 6) and subsequently underwent a local duplication in the ancestor of placental mammals (5a and 5b). STAT5b stimulates IGF1 which is bound to IGFBP that also increased from 2 to 6 genes in 2R (Ocampo Daza et al., 2011). The GH and GHR family duplications have been considerably more difficult to resolve in relation to 2R. The GH family also contains prolactin (PRL), prolactin-2 (PRL2) and somatolactin (SL). Their relationships are obscured by variable evolutionary rates, differential gene losses in the main vertebrate lineages, and chromosomal rearrangements disrupting conservation of synteny. We propose the following scenario: GH, PRL and possibly also SL arose through local duplications before the 2R events. Subsequently, 2R generated PRL and PRL2. Both are present in elephant shark, ray-finned fish, coelacanth, birds and reptiles, but PRL2 has been lost in mammals. Neither GH nor SL seem to have any surviving duplicates from 2R. The teleost genome doubling (3R) generated SL duplicates, SLa and SLb, both of which are still present in basal teleost lineages, including zebrafish. SLb has seemingly been lost in most teleosts. For the GHR family, a jawed vertebrate ancestor underwent a local duplication generating GHR and PRLR post 2R, and subsequently 3R generated duplicates of both of these. The two GHR genes in teleosts differ in their ability to respond to GH, SLa and SLb. The two PRLR genes have not yet been studied functionally. In conclusion, the 2R events have contributed multiple gene duplicates to the GH cascade overall, but the GH family itself expanded only moderately via 2R while the GHR family expanded exclusively after 2R.

OR-22

Mapping thyroid axis evolution through metamorphosis

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The flatfish (Pleuronectiformes) are a group of teleosts that suffer a profound change in morphology during the larval to juvenile transition. In all flatfish this metamorphosis is associated with a shift from symmetry to asymmetry and this event is driven by the thyroid hormones (THs) and inevitably involves the central hypothalamus-pituitary-thyroid (HPA) axis and also the elements of the thyroid axis in the periphery. There is increasing molecular evidence suggesting that flatfish are polyphyletic and do not all descend from a common ancestor with the Psettodes (eg. Indian halibut, spiny turbot) being clustered with non-flatfish (Campbell et al., Mol. Phylog. & Evol. 2013). Studies of *Amphistium* and *Heteronectes*, extinct, spiny-finned fishes from the Eocene epoch, indicate that acquisition of cranial asymmetry in flatfish was gradual (Friedman, Nature, 2008) and raises questions about when the thyroid hormones were recruited to this process. The evolutionary trajectory of the flatfish makes them an interesting model for investigating how evolutionary pressure has shaped metamorphosis, and presumably also thyroid axis evolution. The recent release of the Chinese tongue sole (*Cynoglossus semilaevis*; Chen, *et al. Nat Genet* 2014) genome and the publicly available genome and transcriptome sequences of other flatfish, such as the Senegalese sole (*Solea senegalensis*), Atlantic halibut (*Hippoglossus hippoglossus*), Japanese flounder (*Paralichthys olivaceus*) and the turbot (*Scophthalmus maximus*) represent a resource that is providing important insights into gene evolution and in particular genes of the thyroid axis. Comparison of the genes of the thyroid axis and accessory factors in the flatfish with those in other actinopterygii including the more ancient cod (gadiformes) and salmon (salmoniformes), the ray-finned fish, the spotted gar (*Lepisosteus oculatus*) and cartilaginous fish (elephant shark), agnatha such as the lamprey and the coelacanth (*Latimeria chalumnae*) are being investigated and are providing revealing insights into thyroid axis evolution in the fishes.

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OR-23

A new model for the evolution of the role of the PTH family in bone formation during the vertebrate transition from an aquatic to terrestrial lifestyle

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Understanding the neuro-regulatory role of the brain on bone development and homeostasis represents an important area of research in skeletal biology. Here, we uncover an ancient parathyroid hormone (Pth4) that was secondarily lost in eutherian mammals, but appears to be a central player in the brain-to-bone signaling pathway in zebrafish. Based on our discovery and characterization of Pth4, we propose a model for evolution of bone homeostasis in the context of the vertebrate transition from an aquatic to terrestrial lifestyle.

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OR-24

Evolution of the corticotropin-releasing hormone paralogs in teleosts

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Corticotropin-releasing hormone (CRH) plays a central role as coordinator of developmental stages and plasticity in response to environmental changes, by controlling not only pituitary-adrenal/interrenal but also thyroidal axes in some vertebrates, including teleosts. Two whole genome duplication events (1R and 2R) took place before the radiation of vertebrates and a third one (3R) in the teleost lineage. Recently a *crh2* gene, paralog of *crh* (*crh1*), and likely resulting from 2R, has been described, first in a holocephalan, then in basal mammalians, various sauropsids and a non-teleost actinopterygian fish. It was suggested that *crh2* was lost in the teleost lineage. We further investigated the impact of genome expansion on CRH evolution in teleosts. Genome analyses revealed the presence of duplicated *crh1* genes, *crh1a* and *crh1b*, in most teleosts, resulting from the 3R. We found *crh2* in basal teleosts, indicating that *crh2* had been inherited by the teleost lineage. The presence of a single *crh2* gene in basal teleosts suggested that one 3R-duplicated *crh2* paralog was lost early after the 3R. Furthermore, the lack of any *crh2* in other teleosts revealed that the other paralog was lost during teleost radiation. To better understand the functional evolution of the *crh* paralogs, we analyzed by qPCR their tissue distribution in two representative teleosts: in a basal teleost, the European eel (Elopomorphes), which possess three *crh* paralogs (*crh1a, crh1b*, *crh2*), and in the Atlantic salmon (Salmoniformes), which possess four *crh* paralogs, but all of the *crh1*-type, resulting from the salmonid genome duplication (4R) of *crh1a* and *crh1b*. The distribution study revealed an expression mainly cerebral and retinal for eel *crh1b* and both salmon *crh1b* 4R-paralogs. In contrast, *crh1a* was found highly expressed in the muscle in both species, and in the ovary specifically in the eels. Salmon 4R-paralogs of *crh1a* could be differentiated by their tissue expression specificity with one duplicate expressed in spleen and the other in retina. Eel *crh2* was weakly expressed in the brain but found to be expressed in kidney, intestine and gonads. Our results highlight functional divergences that may have contributed to the conservation of various *crh* paralogs, which arose from different genome duplication events in teleosts.

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OR-25

Analysing the evolution of melanocortin-2 receptor ligand selectivity: studies on rainbow trout MC2R

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Previous studies have shown that activation of either teleost or tetrapod melanocortin-2 receptor (MC2R) orthologs requires that the receptor recognize two motifs in ACTH; the HFRW motif and R/KKRRP motif. Studies on human MC2R indicate that residues in TM2, TM3, TM6, and TM7 form a hydrophilic binding pocket near the surface on the extracellular space side of the receptor for the HFRW motif of ACTH. In addition, it appears that residues in TM4, EC2 (extracellular loop 2), and TM5 may form the binding pocket for the R/KKRRP motif of ACTH. To determine whether residues in the later regions of the rainbow trout (*Onchorhynchus mykiss*) MC2R ortholog also play a role in activation, a rainbow trout (rt) MC2R cDNA was inserted into a pcDNA3+ expression vector (GenScript) and transiently co-expressed with an MRAP1 cDNA, and a CRE/Luciferase reporter cDNA in Chinese Hamster Ovary (CHO) cells to evaluate the activation of rtMC2R by hACTH(1-24). The EC_{50} value for the wild-type rtMC2R was a robust 4.6 $x10^{-11}$M +/- 0.7. Single alanine substitutions were made at 19 positions in rtMC2R, beginning at V^{153} (TM4) to F^{175} (TM5). Each mutant rtMC2R construct was expressed in CHO cells and the EC_{50} value for each mutant was compared to the EC_{50} value of the wild-type rtMC2R control. Statistically significant increases in EC_{50} values (i.e. drop in activation) were observed for positions V^{157}, M^{158}, and V^{159} in TM4, F^{161}, K^{168} in EC2, and F^{171}, F^{175} in TM5. However, drops in activation of 15 fold or higher (our criterion for a physiological effect) were only observed for V^{159} (TM4), and F^{171}, F^{175} (TM5). These results will be discussed in light of our recent published study on site-directed mutagenesis of human MC2R and recent observations on the ligand selectivity of cartilaginous fish MC2R orthologs.

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Morning sessions Thursday June 30

09:00-10:00	**Plenary Lecture I (Main Lecture Hall)** **Sylvie Dufour (FRA) Gene duplications and the neuroendocrine control of fish reproduction (OR-26)**	
10:00-12:30	**Session 5: Endocrine control of reproduction: brain-pituitary system** **Main Lecture Hall** Chairs: **Gustavo Somoza** (ARG)**, Berta Levavi-Sivan** (ISR)	**Session 6: Endocrinology of stress** **Lecture Hall Pascal** Chairs: **Mathilakath M Vijayan** (CAN), **Patrick Prunet** (FRA), **Peggy Biga** (USA)
10:00-10:20	**OR-27** GnRH receptor in LH cell is a critical red/green signal for LH surge. **Shinji Kanda** (JPN)	**OR-33** Molecular mechanisms of glucocorticoid action in zebrafish. **Marcel J M Schaaf** (NLD)
10:20-10:40	**OR-28** Differential pituitary networks are responsible for the regulation of LH and FSH. **Berta Levavi-Sivan** (ISR)	**OR-34** Maternal cortisol modulates developmental programming of the stress axis in zebrafish. **Mathilakath M Vijayan** (CAN)
10:40-11:00	**OR-29** "Big Data" reveals novel functions and complex neuroendocrine control of stem-like neurosteroidogenic radial glia. **Vance Trudeau** (CAN)	**OR-35** Stress-related hormones in the medaka brain: identification of a new player and sex differences. **Kataaki Okubo** (JPN)
11:00-11:30	**Coffee / Tea break**	**Coffee / Tea break**
11:30-11:50	**OR-30** Targeted mutagenesis of the hypophysiotropic Gnrh3 in zebrafish (*Danio rerio*) reveals no effects on reproductive performance. **Olivia Smith Spicer** (USA)	**OR-36** Involvement of the mineralocorticoid receptor pathway in the regulation of stress responses in rainbow trout. **Patrick Prunet** (FRA)
11:50-12:10	**OR-31** Evidence for Kiss1 hexadecapeptide directly regulates GnRH1 in scombroid fish model, chub mackerel. **Hirofumi Ohga** (JPN)	**OR-37** Stress-resilience differences related to emergence time in rainbow trout. **Manuel Gesto** (DNK)
12:10-12:30	**OR-32** Regulatory mechanisms of growth and reproduction in growth hormone-transgenic common carp. **Wei Hu** (CHN)	**OR-38** RNA-seq reveals involvement of oxidative stress in the hepatic fibrosis and skeletal muscle atrophy in response to handling stress in the red cusk-eel (*Genypterus chilensis*). **Juan Antonio Valdes** (CHL)
12:30-14:00	**Lunch served for those not participating in the boat excursion**	
12:30-18:30	**Excursion participants: Lunch served aboard the excursion boat**	

OR-26

Gene duplications and the neuroendocrine control of fish reproduction

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Two rounds (1R and 2R) of whole genome duplications have occurred in early vertebrates, providing the genomic basis for remarkable morphological and physiological innovations. A third round (3R) of whole genome duplication, specific to the teleost lineage, has further contributed to genome expansion and possibly to the striking diversification of this group, the largest among vertebrates. Further tetraploidization events have also occurred in some specific groups, including teleost and non-teleost actinopterygian fishes. In addition to whole genome duplications, local events of specific gene duplications have also increased the number of paralogs throughout vertebrate evolution. Neuroendocrine axes (brain-pituitary-peripheral glands) represent some of the remarkable innovations of the vertebrate lineage, playing master roles in the integration of environmental and internal cues, and regulation of major physiological functions and life traits. Based on examples from the gonadotropic axis, the review will address the impact of whole genome and specific gene duplications on the numbers and evolutionary histories of neuroendocrine actors' paralogs involved in the control of the reproductive function in extant fishes. Recent investigations benefit from the increasing number of available metazoan genomes, including fish species of key-phylogenetic positions, such as basal sarcopterygian (caelacanth), basal non-teleost actinopterygian (spotted gar), basal teleosts (elopomorphs and osteoglossomophs) and multiple representatives of more recently emerged teleost groups. Evolutionary scenarios reveal that the larger number of paralogs in teleosts as compared to mammals, may not systematically arise from teleost-specific 3R, but may have various origins according to gene families. Also, following duplications, paralogs may have been conserved or lost, according to lineages and species. Conservation of multiple paralogs can be related to amplification or partition of pre-existing functions (subfunctionalization), or to emergence of new functions (neofunctionalization). Paralog identification in extant fish species, and reconstruction of their evolutionary histories, are becoming essential for phylogenetically-supported gene nomenclatures, and relevance of comparisons between research advances on the increasing number of fish models investigated for reproductive basic and applied research.

OR-27

GnRH receptor in LH cell is a critical red/green signal for LH surge

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Studies in mammals have demonstrated that kisspeptin neurons play an essential role in reproduction. However, recently accumulating evidence strongly suggests that such regulation does not exist in fish; e.g., lack of expression of mRNA for kisspeptin receptor, *gpr54*, in GnRH neurons has been demonstrated in several fish species. Here, by using a genome editing technique, TALEN, we generated knockouts for *kiss1* and *kiss2* medaka. Both male and female homozygous knockout of each gene as well as their double knockout reproduced normally, and their expression level of pituitary *lhb* and *fshb* remained intact. Kisspeptin knockouts have also been reported to reproduce normally (Tang et al., 2015) in zebrafish, which is phylogenetically distant from medaka. Therefore, we can conclude that kisspeptin is not directly involved in reproductive regulation in teleosts. We therefore studied mechanisms of kisspeptin-independent regulation of reproduction. Medaka is a seasonal breeder, and their reproductive state can be experimentally manipulated solely by day-length. We compared the firing activity of the hypophysiotropic GnRH1 neurons in females *gnrh1*-EGFP transgenic medaka in the evening when its frequency reaches the daily maximum (Karigo et al., 2012) under two conditions: long-day (LD: breeding) and short-day (SD: non-breeding). Interestingly, there was no significant change in the firing frequency and the number of firing per unit time ($P>0.05$). Interestingly, immature individuals showed much lower firing frequency compared to adults in LD and SD, suggesting that, once the fish reaches their sexual maturity, the GnRH neurons do not change their firing activity according to their gonadal maturation stage. Then, we examined the Ca^{2+} responses of LH neurons to GnRH. By using *lhb*-inverse pericam medaka (Karigo et al., 2014), whose LH cells are labelled with a genetically encoded Ca^{2+} indicator, we compared between LD and SD females the Ca^{2+} response to 1 μ M GnRH peptide. The LH cells in LD condition showed significantly stronger response. In addition, OVX reduced the response, while estrogen treatment caused its recovery. Thus, the GnRH response of the LH cell is suggested to depend on the serum concentration of sex steroids, which show drastic changes between breeding and non-breeding conditions.

OR-28

Differential pituitary networks are responsible for the regulation of LH and FSH

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The function and components of the hypothalamic-pituitary axis are conserved among vertebrates. However, in fish, a neuroglandular mode of delivery (direct contact between axons and endocrine cells) was considered dominant, whereas in tetrapods hypothalamic signals are relayed to their targets via the hypophysial portal blood system (neurovascular delivery mode). By using a transgenic zebrafish and tilapia models, we showed that along with direct innervation by hypothalamic neurons to the pituitary, there is also a neurovascular control of gonadotropes via GnHR3. We further showed that LH cells are arranged in 3D networks which are also functionally active. These networks enable the pituitary to mount large scale coordinated LH responses to GnRH stimuli. FSH cells, on the other hand, act individually and benefit from a better accessibility GnRH signals and to blood-born metabolites and stimuli. We found that LH cells exhibit close cell-cell contacts and form a continuous network throughout the gland, while FSH cells were more loosely distributed and maintained small degree of cell-cell contact by virtue of cytoplasmic processes. Gap-junction uncouplers abolished the LH response to GnRH stimulation while had no effect on FSH response. Dye transfer between neighboring LH cells provides further evidence for functional coupling. The two gonadotropins were also found to be differently packaged within their corresponding cell types. Folliculostellate cells of the teleost pituitary share many common attributes with their mammalian counterparts. In the pars intermedia, stellate cells were arranged around neuronal bundles and their processes extended into the pars distalis. Within the pars distalis, stellate cells formed close associations with FSH cells and, to a lesser degree, with GH and LH cells, suggesting differential paracrine regulation of the two gonadotrope populations. The production of follistatin by stellate cells further corroborates the notion of a paracrine role on FSH release. We also found stellate cells to form gap junctions that enabled dye transfer to neighboring stellate cells, implicating that these cells form a large-scale network that connects distant parts of the pituitary. Our findings suggest new perspective of the teleost pituitary including novel pituitary networks that may attribute to the differential regulation of FSH and LH.

OR-29

"Big Data" reveals novel functions and complex neuroendocrine control of stem-like neurosteroidogenic radial glia

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Radial glial cells (RGCs) are abundant neuronal progenitor cells in the adult teleost brain and are the exclusive site of aromatase B expression and neuroestrogen synthesis. As active participants in the tripartite synapse, RGCs are under neuronal regulation by neurotransmitters and neuropeptides. Our anatomical evidence in goldfish and zebrafish shows a close association between RGCs and neurons synthesizing dopamine (DA) or the granin-derived 34 amino acid neuropeptide secretoneurin A (SNa) in the telencephalon. Both neuronal systems are important for reproduction, being respectively part of major inhibitory and stimulatory pathways controlling luteinizing hormone release. Using an *in vitro* RGC culture preparation with >95% purity, the transcriptome and proteome of female goldfish *(Carassius auratus)* RGCs were characterized. Next generation sequencing and *de novo* assembly generated the first reference transcriptome for fish RGCs that contains 12,180 non-redundant unigenes. Gene ontology analysis revealed a diverse receptor and signaling molecule profile (e.g., >170 G-protein coupled receptors and 50 nuclear receptors), suggesting that RGCs can synthesize and respond to an array of hormones, peptides, cytokines, and growth factors. Our previous data indicated that DA D1 receptor activation in RGCs regulated cell cycle/proliferation, growth, death, and survival. This was predominantly an inhibition of progenitor features in RGCs. Next, we determined which RGC processes may be regulated by SNa *in vitro* by sub-network enrichment analysis of all transcriptomic data. A total of 192 cell processes were identified and include CNS physiology (neurogenesis, synaptic plasticity, transmission of nerve impulses, neuron development, memory), immune responses (B lymphocyte proliferation and NK cell mediated cytotoxicity), and water homeostasis. Quantitative proteomics using iTRAQ and LC MS/MS confirmed that >2000 proteins could be identified in this cell type and that numerous signalling pathways and processes are active in RGCs. Other proteins expressed in RGCs included those related to mitochondria, oxidative stress and biotransformation. These first datasets on the RGC transcriptome and proteome reveal multiplicity of new functions critical to neuronal-glial communication in the neuroendocrine brain.

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OR-30

Targeted mutagenesis of the hypophysiotropic Gnrh3 in zebrafish (*Danio rerio*) reveals no effects on reproductive performance

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Gnrh is the major neuropeptide regulator of vertebrate reproduction, activating the pituitary-gonadal axis that results in reproductive competence. Previous research in mice and humans have demonstrated that *Gnrh*/*GNRH* null mutations result in hypogonadotropic hypogonadism/infertility. Our goal was to eliminate *gnrh3* (the hypophysiotropic form) function in zebrafish to determine how reproductive ontogeny and performance are affected, as well as downstream factors in the reproductive axis. Using the TALEN technology, we developed a *gnrh3*$^{-/-}$ knockout line that harbors a 62 bp deletion in *gnrh3*. With immunohistochemistry, we verified that *gnrh3*$^{-/-}$ fish do not possess Gnrh3 peptide in any brain regions. However, other than changes in mRNA levels of gonadotropin genes (*fshb*, *lhb*, and *cga*) during early development, which are repaired by adulthood, there were no changes in the expression of key genes in the reproductive axis in *gnrh3*$^{-/-}$ fish. Reproductive ontogeny was normal in the *gnrh3*$^{-/-}$ fish: The migration of Gnrh3 neurons during development was not disrupted/misguided, as *gnrh3*$^{-/-}$ *gnrh3:tdtomato* fish did not display different Gnrh3 neuronal routing compared to *gnrh3*$^{+/+}$ *gnrh3*:*tdtomato* fish. In addition, the *gnrh3*$^{-/-}$ zebrafish are fertile, displaying normal and complete oogenesis in females and spermatogenesis in males. The *gnrh3*$^{-/-}$ fish exhibited normal reproductive performance with no differences in fecundity, fertilization rate, and offspring survival. With our previous results that Gnrh3 cell ablation causes infertility, these results indicate that a compensatory mechanism is being activated, most likely primed early on upon Gnrh3 neuron differentiation. In order to determine if Gnrh2 (the only other Gnrh isoform in zebrafish) compensates for reproductive performance when Gnrh3 is absent, we developed a double *gnrh3*$^{-/-}$ *gnrh2*$^{-/-}$ knockout line, which, surprisingly, also undergoes normal and complete gametogenesis and exhibits no differences in fecundity, fertilization rate, and offspring survival. Therefore, a factor that is not an identified Gnrh isoform is responsible for the compensation observed in our *gnrh3*$^{-/-}$ *gnrh2*$^{-/-}$ line. Other potential compensation factors for Gnrh3 and sensitive windows of time for compensation during development will be discussed.

OR-31

Evidence for Kiss1 hexadecapeptide directly regulates GnRH1 in scombroid fish model, chub mackerel

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Here, we report the first evidence of Kiss1 hexadecapeptide (Kiss1-16) which directly regulates the functional GnRH form in the preoptic area (POA) of scombroid fish model. The localization of two kisspeptin (*kiss1* and *kiss2*) neurons and kisspeptin receptors (*kissr1* and *kissr2*) in the brain of adult chub mackerel, *Scomber japonicus* were analyzed using *in situ* hybridization (ISH) method. Further, co-localization of *kissr* transcripts with GnRH1 neurons were examined with dual-fluorescence ISH. The *kiss1* and *kiss2* neurons localized mainly at the nucleus recessus lateralis (NRL) and nucleus of the posterior recess (NRP) in the hypothalamus. The *kissr1* was present at anterior POA and habenular nucleus. The *kissr2* was detected widespread including POA, lateral tuberal nucleus (NLT), NRL, and NRP. A striking result was that GnRH1 neurons were expressed at the POA and their neurons co-expressed *kissr1*. In contrast, a lot of *kissr2* expressed vicinity of GnRH1 neurons but do not seem to have co-expression. Furthermore, we characterized the endogenous mature form of Kiss1 peptide. In chub mackerel, deduced amino acid sequence indicates that mature Kiss1 form is Kiss1-16. The result of reporter gene assay clearly showed that Kiss1-16 had stronger potency for receptor activation compared with any other Kiss1 short forms. These results indicate that Kiss1-16 is the mature Kiss1 form in chub mackerel and may directly regulate reproductive BPG-axis via control of GnRH1 neuronal activity.

OR-32

Regulatory mechanisms of growth and reproduction in growth hormone-transgenic common carp

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Growth and reproduction are closely related and there may be significant hormone cross-talk between the two systems controlling these key processes in fish. Common carp transgenic (tg) for growth hormone (GH) have enhanced growth and delayed reproductive development so are an amenable model to study endocrine interrelations. We found that pituitary and serum LH levels decreased in GH-tg common carp compared with non-transgenic fish. We also found that over-expression of GH directly inhibited pituitary LH production and release through the GH receptor. RNA sequencing was used to compare the transcriptional differences of genes at puberty in male and female fish. We found increased hypothalamic expression of *gnih* (gonadotropin-inhibitory hormone) and pituitary *gnihr* (GnIH receptor). The decreased pituitary expression of *gnrhr2* (gonadotropin-releasing hormone receptor 2) may be linked to reduced pituitary sensitivity of GH-tg carp the LH-inducing injection of salmon GnRH agonist plus a dopamine antagonist. Moreover, we found the expression of *gys* (glycogen synthase), *igfbp1* (insulin-like growth factor-binding protein 1) and blood glucose concentration were lower in GH-tg carp. *Agrp1* (agouti-related protein 1) and *slα* (somatolactin *α*) which are related to appetite and lipid catabolism and are significantly lower in GH-tg carp. Increased appetite and decreased lipid contents, and the associated decrease in liver *leptin* expression indicate disrupted feeding and metabolic status in GH-tg carp. Leptin receptor mRNA was detected by florescent *in situ* hybridization in the pituitary in LHb-positive cells of the proximal pars distalis, suggesting a direct effect of leptin**.** Incubation of dispersed pituitary cells with common carp recombinant leptin increases *gthα*, *fshβ*, and *lhβ in vitro*. The results reveal that the delayed reproductive development of fast-growing GH-tg carp is associated with upsets in the reproductive endocrine system and energy metabolism. In addition to neuroendocrine factors, reduced hepatic leptin signaling to the pituitary may be part of the response to overexpression of GH and the resulting delay in puberty onset.

OR-33

Molecular mechanisms of glucocorticoid action in zebrafish

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Upon a stressful stimulus, vertebrate organisms activate their stress axis and produce glucocorticoid hormones like cortisol. These hormones control the stress response by regulating a wide range of systems, like our metabolism, growth, immune system and behavior. The effects are mediated by the glucocorticoid receptor (GR), which acts as a ligand-activated transcription factor. In our laboratory we use the zebrafish as an in vivo model system to unravel the molecular mechanism of GR action. First, using transcriptome analysis we found two distinct clusters of genes regulated by GR. The first cluster is regulated under basal conditions and contains mainly genes involved in cell cycle control and apoptosis. The second cluster is regulated upon increased activation of GR and consists mainly of genes involved in glucose metabolism and proteolysis. Second, we studied the anti-inflammatory action of GR using tail fin amputation as an inflammation model. Transcriptome analysis revealed the glucocorticoid treatment has a dramatic effect on the amputation-induced gene regulation. Almost the entire transcriptional response was inhibited. Both neutrophils and macrophages migrate towards the wounded site upon amputation, and only the migration of neutrophils is inhibited by glucocorticoids, whereas macrophage migration is unaffected. Further studies showed that the glucocorticoid resistance of macrophages must be due to a transcriptional pathway that was not detected by our (whole body) analysis. Third, we performed a forward-genetic screen using as readout the glucocorticoid-induced decrease in POMC expression in the pituitary gland, which is important for stress axis feedback. As a result of this screen three zebrafish mutants were identified that are resistant to glucocorticoid suppression of the stress axis. Genetic identification of two of the mutants showed mutations in the adenomatous polyposis coli (apc) and the wd repeat domain 82 (wdr82) gene. Both genes have not previously been associated with glucocorticoid feedback of the HPA axis, and we are currently investigating the molecular mechanism behind their involvement.

OR-34

Maternal cortisol modulates developmental programming of the stress axis in zebrafish

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Prenatal exposure to excess glucocorticoid due to maternal stress affects developmental programming in vertebrates, but the mechanisms are far from clear. Using zebrafish (*Danio rerio*) as a model we are testing the hypothesis that excess cortisol in the zygote due to maternal stress affect offspring development. In this presentation I will talk about how manipulation of cortisol content in the zygote affects larval stress response and behaviour. Elevated cortisol level in the zygote was accomplished by microinjecting this steroid into the yolk. Reduced cortisol bioavailability in the zygote was achieved by microinjecting antibodies against this steroid. Manipulation of zygotic cortisol content revealed that maternal corticosteroid deposition into the oocytes is critical for larval development in zebrafish. Specifically, excess cortisol deposition leads to cardiac defects, impaired cortisol stress axis development and altered behavioural phenotype. Elevated cortisol level in the newly fertilized embryo also increased primary neurogenesis in a region-specific manner, and displayed higher transcript abundance of the proneural genes neuronal differentiation 4 (*neurod4*) and orthopedia b (*otpb*) in zebrafish brain. Our results suggest that maternal cortisol is critical for zebrafish development. However, the zygotic cortisol levels have to be tightly regulated for proper development and we propose a control mechanism at the level of ovarian follicles that limits excess cortisol deposition into the embryos. Overall, maternal stress, and the associated elevation in cortisol content, affects developmental programming of the stress axis in zebrafish.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to MMV.

OR-35

Stress-related hormones in the medaka brain: identification of a new player and sex differences

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In vertebrates, including teleost fish, the physiological and behavioral responses to stress are largely mediated by stress-related hormones such as the corticotropin-releasing hormone (CRH) family peptides, adrenocorticotropic hormone (ACTH), and glucocorticoids. In this paper, I describe two of our recent studies on these hormones in teleosts: identification of a novel, teleost-specific member of the CRH family peptides and sex differences in glucocorticoid signaling in the teleost brain. We identified the gene encoding a previously undescribed CRH family peptide in medaka and several other teleost species. It was found exclusively in teleosts and thus designated teleocortin (*tcn*). *tcn* most likely arose from a duplication of an ancestral *crh* early in vertebrate evolution, but was lost in the tetrapod lineage shortly after its divergence from the teleost lineage. In the medaka brain, *tcn*-expressing neurons were found in nuclei of the telencephalon, preoptic area, hypothalamus, tegmentum, and isthmic region. As none of these nuclei have been shown to innervate pituitary ACTH cells, Tcn presumably exert its effects centrally in the brain, rather than via stimulation of the pituitary-interrenal axis. Most *tcn*-expressing neurons also expressed *crh*, and Tcn activated Crh receptors with equivalent or slightly higher potency than Crh, suggesting that Tcn and Crh share common functions. These data identified Tcn as a novel, teleost-specific member of the CRH family peptides that may act centrally together with Crh to mediate physiological and behavioral responses to stress. We also found sex differences in the expression of a glucocorticoid receptor gene (*gr1*) in the medaka brain, with females having higher expression in several preoptic and thalamic nuclei. In addition, *gr1* was found to be expressed more highly in females in preoptic neurons producing neuropeptides that have been implicated in the regulation of neuroendocrine and behavioral functions: vasotocin and gonadotropin-releasing hormone 1. These data suggest that glucocorticoids have a more profound influence on physiology and behavior mediated by these neuropeptides in females than in males, which may contribute to sex differences in the brain response to stress.

OR-36

Involvement of the mineralocorticoid receptor pathway in the regulation of stress responses in rainbow trout

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Glucocorticosteroids play an essential role in the regulation of the stress responses in fish. The control and release of corticosteroids from the HPI-axis in response to stressors is carefully governed by negative feed-back regulation on HPI-axis where corticosteroid receptors are present. The glucocorticoid receptors (GR) have been demonstrated to be active in this feed-back regulation. However, the specific roles of mineralocorticoid receptor (MR) in the fish stress axis still need to be clarified. In the present study, we analysed corticosteroid signalling pathways (GR and MR) in the HPI axis of rainbow trout exposed to stressors. Following chronic confinement stress, we observed an increase of plasma cortisol and 11-deoxycorticosterone which was associated with a negative feed-back on corticosteroid receptors gene expression (rtGR1, rtGR2, rtMR) in the pituitary but not in the hypothalamus or the interrenal. We also analysed expression of the 3 corticosteroid receptors in the HPI axis in juvenile trout exposed to chronic stressful situations, i.e. hypoxia associated with poor water quality or vegetable diet. Finally, using in situ hybridization approach, we co-localized expressions of the 3 receptors in the different pituitary cell types which gave us interesting information on the pituitary functions regulated by corticosteroids. Overall, these results suggest that MR signalling pathways can be mobilized, in parallel to GR signalling pathways, to regulate HPI-axis response to stressors. Whether these effects are direct regulation by MR signalling pathway or interaction between MR and GR will also be discussed.

OR-37

Stress-resilience differences related to emergence time in rainbow trout

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In wild salmonid fish, individual behavioural traits have been suggested to be coupled with the timing of fry emergence form gravel spawning nests, in such a way that early emerging fish have shown to be more aggressive and to have a higher probability to become socially dominant than those fish emerging at a later stage. Besides aggression and dominance, other behavioural and metabolic traits such as boldness, metabolic rate or growth had also been coupled to emergence time. Altogether, early- and late-emerging fish have traits resembling those of proactive and reactive stress coping styles, respectively. Proactive fish are considered to be more resilient to stress. However, it is currently unclear if that coupling is maintained in farmed fish populations, which showed no consistent evidence of a clear relation between emergence time and stress coping style. In this study, fish were hatched and larvae were fractionated according their emergence time (Early fraction: first 20 % of fish to emerge; Intermediate fraction: mid 20 %; Late fraction: last 20 %). Several months later, the resilience against a mild stressor (30 min of high stocking density), along with the stress habituation ability was investigated in 5 g fish from the different fractions. Results showed that fish from different fractions displayed a similar neuroendocrine response to a novel stressor. Interestingly, the capacity of habituation to stress was however better in the fish from the early emergence fraction, which showed no cortisol response to the stressor after being exposed daily for 15 days to another mild acute stressor (1.5 min of air exposure). These results demonstrate that at least some behavioural differences related to emergence time exist in a domesticated trout population. The aquaculture-related implications of these stress resilience differences are currently under study.

OR-38

RNA-seq reveals involvement of oxidative stress in the hepatic fibrosis and skeletal muscle atrophy in response to handling stress in the red cusk-eel (*Genypterus chilensis*)

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Chilean aquaculture is mainly focused on salmon production, evidencing a clear necessity for diversification. In recent years thanks to government initiatives there is a trend towards diversifying breeding species to maintain the sustainability of the Chilean aquaculture industry. One such cultivated marine species is the red cusk-eel (*Genypterus chilensis*). This endemic fish is highly valued in national and international markets due to exceptional flesh quality and high nutritional value. However, this species in captivity is susceptible to stress, showing low growth rates, which could be due to alteration in the compensatory response to stress. In this work we studied the effect of handling stress on the metabolic, immune and growth response of *G. chilensis*. Using Illumina RNA-seq technology, skeletal muscle, liver and head-kidney sequence reads for *G. chilensis* were generated under control and handling stress conditions. Reads were mapped onto a reference transcriptome, resulting in the identification of differential expressed transcripts. Gene ontology enrichment analysis revealed a significant up-regulation of genes associated with liver angiogenesis and skeletal muscle atrophy. Conversely, the down-regulated genes were associated with liver steatosis and skeletal muscle contraction. Moreover analysis of stress oxidative markers revealed that handling stress induces increases in lipid peroxidation, protein carbonylation, and DNA oxidation. Due to the strategic importance for Chile of aquaculture diversification, this work will not only allow consolidating the red cusk-eel commercial cultivation but also lay the foundation of marine aquaculture industry having a significant impact on national economic development.

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Morning sessions Friday July 1

09:00-10:00	**Plenary Lecture II (Main Lecture Hall)** **Stephen D. McCormick (USA) Preparing for the sea: The endocrinology of anadromy (OR-39)**	
10:00-12:30	**Session 7: Endocrine control of reproduction: pituitary-gonad system** **Main Lecture Hall** Chairs: **Rüdiger Schulz** (NLD)**, Wei Ge** (CHN)	**Session 8: Endocrine responses to environmental challenge and change** **Lecture Hall Pascal** Chairs: **Glen van der Kraak** (CAN)**, Ned Pankhurst** (AUS)**, Juan Fuentes** (PRT)
10:00-10:20	**OR-40** An intrinsic mechanism of sexual fate decision in germ cells: Commitment of femaleness independent of estrogenic action. **Minoru Tanaka** (JPN)	**OR-46** Minutes and slight water temperature decrease triggers hormone mediated downstream migratory behavior in Pacific salmon. **Arimune Munakata** (JPN)
10:20-10:40	**OR-41** Regulation of the ovarian IGF system in the zebrafish ovary. **Glen Van Der Kraak** (CAN)	**OR-47** Change the expression profiles of digestive factors in red spotted grouper (*Epinephelus akaara*) by the water temperature. **Eun-Jeong Jeon** (KOR)
10:40-11:00	**OR-42** Genetic evidence for the gatekeeping role of Ybx1 in controlling follicle activation and early folliculogenesis in the zebrafish. **Bo Zhu** (CHN)	**OR-48** Thermo-reception and smoltification in salmonids: a hot topic. **Laura Gabriela Nisembaum** (FRA)
11:00-11:30	**Coffee / Tea break**	**Coffee / Tea break**
11:30-11:50	**OR-43** Expression profiling identifies Sertoli but also Leydig cell genes as Fsh targets in adult zebrafish testis. **Diego Crespo** (NLD)	**OR-49** Stressor- and sex-specific effects of increased temperature and ocean acidification on the reproductive endocrine axis of the cinnamon anemonefish. **Nicholas J Bernie**r (CAN)
11:50-12:10	**OR-44** Targeted gene disruption of secretogranin II in zebrafish reduces reproductive success. **Kimberly Mitchell** (CAN)	**OR-50** Fish hormones and the ocean carbon cycle. **Juan Fuentes** (PRT)
12:10-12:30	**OR-45** Effects of 11-ketotestosterone on the early ovarian development in sterlet, *Acipenser ruthenus.* **Hongxia Hu** (CHN)	**OR-51** Organochlorine pesticides disrupt fish immune responses. **Nancy D Denslow** (USA)
12:30-14:00	**Lunch / Poster viewing**	

OR-39

Preparing for the sea: The endocrinology of anadromy

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All anadromous species must make the transition from freshwater to seawater at least once in their lifetime. Salmon undergo morphological, physiological and behavioral changes that are preparatory and adaptive for seawater entry and are collectively known as the parr-smolt transformation. Smolt development is regulated by environmental factors such as photoperiod and temperature and mediated by the neuroendocrine system. The development of salinity tolerance is the most well-studied developmental change that occurs during smolting. Cortisol, growth hormone (GH), insulin-like growth factor I (IGF-I) and thyroid hormones increase during and promote smolt development, whereas prolactin is inhibitory and decreases late in smolt development. Recent research indicates that the hypothalamus and pituitary are activated during smolt development leading to higher levels of circulating ACTH and cortisol. There are important interactions at several levels between the HPI (hypolthalamic-interrenal-pituitary) and GH/IGF-I axes that promotes salinity tolerance and its underlying mechanisms. Dynamic regulation of GH and IGF-I receptors and IGF-I in the gill during smolting and seawater exposure results in activation of the JAK/STAT second messenger pathways. Comparison of neuroendocrine changes anadromous and landlocked salmon populations indicates that the hormonal control of smolt development is subject to natural selection. We have only a limited understanding of the neuroendocrine control of anadromy in non-salmonid species, offering rich opportunities for further research.

OR-40

An intrinsic mechanism of sexual fate decision in germ cells: Commitment of femaleness independent of estrogenic action-

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It was suggested that germline stem cells are sexually indifferent or unfixed even after they are colonized in testis or ovary. Then a question arises how sexual fate decision of germ cells is regulated. There were two possibilities to understand the mechanism. One was that, once the sexual clue is provided from the surrounding somatic cells, the sex determination and differentiation of germ cells proceeds to the end autonomously. The other way of understanding is that there is no intrinsic mechanism of sexual fate decision in germ cells. In this understanding, the oogenesis and spermatogenesis are completely controlled step by step by somatic cells as developmental events of gametogenesis. Recently we have identified *foxl3* as a switch gene for sexual fate decision of germ cells (1). This gene begins to express in germ cells right after germline stem cells commit to gametogenesis, and loses its expression before entry of meiotic prophase I. Disruption of *foxl3* results in production of fertile sperm in the ovary. Sperm production occurs in germinal cradle where niche structures for germline stem cells are formed (2-4). As a result, germinal epithelium expands and occupies stromal compartment where oocytes would otherwise grow and mature in the normal ovary. Very intriguingly, one or two months after the onset of spermatogenesis in the ovary of *foxl3* XX mutant, ovary resumes oogenesis although the number of oocytes is extremely small. The oocytes are ovulated and fertile. We suspected if the oocyte development might be due to a continuous effect of estrogen on germ cells in the XX mutant ovary. A block of estrogen production did not cause cessation of oogenesis, and administration of estrogen agonists did not induce oogenesis in the very young larva of *foxl3* XX mutant when only spermatogenesis occurs. These results suggest that the commitment of germ cells to oogenesis is estrogen-independent.

(1) Nishimura et al (2015) Science 349, 328-339. (2) Tanaka (2013) WIREs Dev.Biol. doi: 10.1002/wdev.131. (review). (3) Nakamura et al (2010) Science 328, 1561-1563. (4) Nishimura and Tanaka (2016) Sex.Dev. in press. (review).

OR-41

Regulation of the ovarian IGF system in the zebrafish ovary

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Recent studies provide evidence that the insulin-like growth factor (Igf) family plays important roles in the regulation of ovarian function during the periovulatory period in fish. In the zebrafish, the ovarian Igf system includes ligands (igf2a, igf2b and igf3), receptors (igf1ra and igf1rb) and multiple binding proteins. IGFs promote the development of maturational competence, oocyte maturation and steroid biosynthesis in the zebrafish. IGFs also function as survival factors and prevent caspase-3/7 induction in zebrafish ovarian follicles incubated in vitro. Endogenous expression of igf3 peaks in full grown (FG) ovarian follicles and then rapidly declines approaching ovulation. We undertook a series of studies to investigate the regulation of the igf3 mRNA and protein expression in zebrafish ovarian follicles. Treatment with human chorionic gonadotropin (hCG) an LH analog and adenylate cyclase activators increased igf3 expression and protein content in FG and mid-vitellogenic (MV) ovarian follicles from the zebrafish. In contrast, treatment with the maturation inducing steroid 17,20βP, activators of protein kinase C including phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 suppressed mRNA expression of igf3. In other studies, zebrafish that have ovulated exhibit low levels of igf3 expression relative fish treated with ethinylestradiol or progesterone both of which block ovulation. Collectively, these results suggest that there is a dynamic on and then off regulation of the IGF system during the periovulatory period which is regulated by luteinizing hormone/protein kinase A and protein kinase C dependent pathways, respectively. These findings support the contention that suppression of the Igf system may be essential for ovulation to proceed.

OR-42

Genetic evidence for the gatekeeping role of Ybx1 in controlling follicle activation and early folliculogenesis in the zebrafish

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Y box binding protein 1 (YB-1) is a multifunctional protein that is essential for embryonic development in the mouse, and knockout of YB-1 gene causes embryonic lethality. By comparison, YB-2, a homologue of YB-1, is specifically expressed in the germ cells, and its knockout in the mouse leads to infertility in both female and male. Interestingly, in the zebrafish, YB-1 (Ybx1/*ybx1*) is the only member of Y box-binding protein family. Although zebrafish Ybx1 is structurally close to YB-1, its expression is abundantly restricted to the germ cells, suggesting its functional resemblance to YB-2. In the ovary, Ybx1 is predominantly expressed in the primary growth (PG, stage I) oocytes, and its expression level decreased dramatically when the PG follicles are activated to enter the pre-vitellogenic (PV, stage II) stage, suggesting an important role for Ybx1 in controlling early follicle development. Using the genome-editing technique TALEN, we have successfully deleted *ybx1* gene from the zebrafish genome. Interestingly, the loss of Ybx1 in the zebrafish (Ybx1KO) resulted in partial mortality at early stage with about 8% mutant fish surviving to adult stage, lower than the expected ratio of 25%. The mortality occurred mainly during the period from 10 to 20 dpf, which might be caused by intestinal dysfunction, affecting food digestion and absorption. Interestingly, the mutant fish that survived this death window could grow normally to maturity, allowing for phenotype analysis of reproductive functions. The female mutant fish showed extremely low fecundity. Histological analysis showed that the ovarian follicles were mostly arrested at PV stage with only a few follicles entering vitellogenic growth. Further experiments suggest that the Ybx1KO-induced blockage at the PV stage might be due to reduced proliferation of follicle cells in the mutant ovary, which was likely caused by a significantly increased expression of *cdkn1a* (*p21*).

OR-43

Expression profiling identifies Sertoli but also Leydig cell genes as Fsh targets in adult zebrafish testis

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Spermatogonial stem cells (SSCs) are quiescent, undergo self-renewal or differentiate. Spermatogenesis is orchestrated by follicle-stimulating hormone (FSH), through the production of Sertoli cell-derived factors, and by Leydig cell-released androgens. Here, we investigate the transcriptional events induced by Fsh in a steroid-independent manner on the restart of zebrafish (*Danio rerio*) spermatogenesis *ex vivo*, using testis from adult males where type A spermatogonia were enriched by estrogen treatment *in vivo*. Under these conditions, RNA sequencing preferentially detected differentially expressed genes (DEGs) in somatic/Sertoli cells. Fsh-stimulated spermatogonial proliferation was accompanied by modulating several signaling systems (i.e. Tgf-β, Hedgehog, Wnt and Notch pathways). *In silico* protein-protein interaction analysis indicated a role for Hedgehog family members potentially integrating signals from different pathways during fish spermatogenesis. Moreover, Fsh had a marked impact on metabolic genes, such as lactate and fatty acid metabolism, or on Sertoli cell barrier components. Fish Leydig cells express the Fsh receptor and one of the most robust Fsh-responsive genes was *insl3*, a Leydig cell-derived growth factor. Follow-up work showed that recombinant zebrafish Insl3 mediated pro-differentiation effects of Fsh on spermatogonia in an androgen-independent manner. These results shed light on the transcriptional profile in the zebrafish testis associated with the Fsh-stimulated spermatogonial development. Our experimental approach allowed focusing on testicular somatic genes in zebrafish and showed that the activity of signaling systems known to be relevant in stem cell systems was modulated by Fsh, providing promising leads for future work, as exemplified by the studies on Insl3.

OR-44

Targeted gene disruption of secretogranin II in zebrafish reduces reproductive success

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Secretogranin II (SgII) is a member of the granin family of acidic secretory proteins whose main known function is as a precursor protein for bioactive peptides. There are two paralogs of teleost SgII that we have named SgIIa and SgIIb. These proteins are processed by prohormone convertases to produce the 31-34 amino acid peptides secretoneurin (SN) a and SNb, respectively. Our previous studies have shown that SN stimulates luteinizing hormone release *in vivo* and *in vitro*. In this study we examined the functional significance of SgIIa and SgIIb in zebrafish reproduction using TALEN (transcription activator-like effector nuclease)-mediated mutagenesis. Single and double knockouts for SgIIa and SgIIb were generated, reared until sexual maturity and assessed for reproductive output. While 62% of wild-type (WT) pairwise matings spawned, only 37% (P=0.0006), 44% (P=0.0169) and 6% (P<0.0001) of SgIIa, SgIIb and SgII(a+b) knockout (ko) animals spawned, respectively. Clutch sizes of mutant line pairwise crosses were not significantly different between WT and the three SgII-KO lines. Reciprocal breeding crosses were assessed to determine if these results were sex-specific. Spawning success of SgII(a+b) females crossed with WT males was significantly lower at 8% (P<0.0001) indicating a female specific effect. In contrast, all other reciprocal crosses were not different compared to WT pairwise crosses. Clutch sizes of reciprocal crosses were significantly different (p<0.0001) in that SgII(a+b) males crossed with WT females had significantly larger clutch sizes than WT pairwise crosses. To our knowledge these are the first data indicating that knockout of SgIIa and SgIIb significantly disrupts breeding. The percent fertilization in vitro fertilization is high (>87%) and is not significantly different between KO lines and WT. Therefore, we are currently testing the hypothesis that SgII-KO negatively affects sexual behaviour and this leads to impaired breeding success. Preliminary data shows reduced courtship behaviours in both SgIIa and SgIIb-KO lines. This is the first report of impaired reproduction following KO of SgII in any vertebrate.
Funded by NSERC and CAS.

OR-45

Effects of 11-ketotestosterone on the early ovarian development in sterlet, *Acipenser ruthenus*

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11-ketotestosterone (11-KT) has been identified as the most potent androgen in teleost while it has also been tested high level in female plasma from several chondrostean and teleost species including sturgeon. The plasma level of 11-KT increased sharply during the period of yolk deposition. Meanwhile, the ovary development of yolk deposition stage was always retarded in cultured sturgeon. Thus, whether 11-KT is one of the critical factors inducing the start of yolk deposition and how it works? The experiments were conducted using cultured sterlet *Acipenser ruthenus*. We examined the effects of exogenous 11-KT by implanting and incubated hepatic and ovarian explant with 11-KT in vitro during ovarian stage I-II testing 13 related gene expression, plasma hormone levels and histological changes. The results showed that 11-KT implantation promoted the ovarian development of sterlet obviously, and the role of high-dose treatment group was more significant than the low-dose treatment group, indicating that the effect was dose-dependent pattern.It had not induced ovarian masculinisation or sex reversal. The expression of foxl2 and cyp19a1 maintaining ovarian development also had no significant change.The expression of androgen receptor (ar), follicle stimulating hormone (fsh) and its receptor fshr significantly increased in brain. The expression of ar, vtg and lipoprotein lipase(lpl) were also significantly increased in liver. The expression of ar had not been changed obviously in ovary. The expression of fsh and vitellogenin receptor (vtgr) increased while the fshr reduced significantly. In serum, Vtg and T increased significantly while E2 decreased with no significant difference. These results indicated that 11-KT activated the expression and synthesis of Vtg. In ovarian explants incubating experiment with 11-KT,only the expression of gene era significantly increased, while the expression of other genes fshr, foxl2, cyp19a1, vtgr, ar and erb showed an upward trend with no significant difference. But in culture medium, T and E2 increased significantly, indicating that the ovaries of 11-KT just regulated the balance between steroid hormone. In hepatic explant incubating experiment with 11-KT, the expression of ar significantly increased, and vtg and lpl extremely significantly increased. The level of Vtg, T and E2 in the culture medium also increased significantly. The *in vitro* results further demonstrated that 11-KT could directly induce the expression and synthesis of Vtg in liver.

OR-46

Minutes and slight water temperature decrease triggers hormone mediated downstream migratory behavior in Pacific salmon

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We suggest slight decreases in water temperature, even for short periods of time, is an important triggers or perhaps regulates hormone mediated downstream swimming behavior, an initial step of seaward migration in juvenile Pacific salmonids. In hatchery tanks, we found that masu salmon (*Oncorhynchus masou*) and steelhead trout (*O. mykiss*) smolts, exposed to a minute decrease in water temperature (<2 C) exhibited high plasma cortisol levels within an hour, while non-migratory forms of masu salmon and *O. mykiss* (rainbow trout) did not increase their cortisol concentrations when exposed to similar decreases in temperature. Masu salmon, coho salmon and steelhead juveniles exhibited downstream swimming behavior when exposed to a 1 C drop in water temperature in laboratory circular troughs. Masu and coho salmon (*O. kisutch*) juveniles given cortisol implants via cholesterol pellets also exhibited downstream movements independent of water temperature changes. Similar elevations in temperature did not elicit this behaviour. We thus suggest that minute decreases in water temperature are one of the environmental cues resulting in and modification of downstream swimming behavior in Pacific salmonids. Moreover, this behavior is mediated partly by an increase in plasma cortisol, an important developmental hormone that also responds to stress. We thus also suggest that part of downstream migratory behaviour occurs as negative responses to some environmental factors.

OR-47

Change the expression profiles of digestive factors in red spotted grouper (*Epinephelus akaara*) by the water temperature

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The growth of fish is under the control of various environmental factors especially the water temperature (WT). It is suggested as one of the main factors in the eating behavior. Previously we explored the positive effects of high WT in the growth rate and feed efficiency in the red spotted grouper (110 DAH). The red spotted grouper (110 DAH) are faster growth and better feed efficiency in high WT (24℃ and 28℃) than natural WT. In this study, we studied the relationships between the water temperature and the expression profiles of digestive factors in red spotted groupers. Seventeen month-old juveniles were divided randomly into 3 groups and adjusted for 2 weeks with 3 different WT; natural control (15±1.0℃), 20±0.5℃ and 25±0.5℃. Commercial pellet diet was supplied at 11:00 (0 h) once a day to satiety. After 2 weeks, the fish were randomly sacrificed at 0 h, 3 h, 6 h and 21 h in all groups. They were applied to analyze the activity of goblet cell and the mRNA expression levels of cholecystokinin (CCK), leptin, trypsin and neuropeptide Y (NPY). The average lengths of intestinal villus were 220.92±14.80 ㎛, 247.08±23.09 ㎛ and 356.66±12.71 ㎛ (NC, 20 and 25℃ respectively). The numbers of intestinal goblet cells per tissue section were significantly many at 25℃; NC (462.3±67.8), 20℃ (461±91.2) and 25℃ (766±144.3). The mRNA levels of CCK, leptin and NPY were significantly high at 25℃ compared with other temperature but not trypsin. Based on them, it is suggested that the water temperature is a key factor for feeding and digestive activity. It is considered that the faster growth of the red spotted grouper at high water temperature have correlate with the digestive activity through the increased expression of digestive factors and the histological changes in intestine.

OR-48

Thermo-reception and smoltification in salmonids: a hot topic

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Rhythmic environmental inputs influence physiologic, metabolic and behavioral profiles of most organisms. Photoperiod and temperature are indicators of the daily and seasonal cycles that trigger many events such as development, migration and reproduction. The time-keeping hormone, melatonin (MEL) links environmental and physiological timing, targeting central and peripheral tissues, interacting with a wide variety of endocrine factors. Produced at night by the pineal and retinal photoreceptors in a Ca^{2+} dependent way, the duration of MEL secretion is determined by night length, while in ectotherms, ambient temperature controls the amplitude of the signal. The mechanisms of light/dark modulation of MEL production are well described, while temperature mechanisms are still unelucidated. In the present work we are interested in understanding thermo-sensation in teleosts and in deciphering how elevated temperatures, in the actual context of global climate change, affects the preparation for sea water tolerance and the downstream migration (smoltification), a crucial step on the life cycle of anadromous Atlantic salmon (*Salmo salar*). With this aim, photoperiod and/or temperature were manipulated, in order to assess their uncoupled relative effect, and also to mimic the predicted environmental changes to which animals will be confronted; *i.e.* a rapid temperature elevation but unaffected daily and seasonal photoperiod. We investigate the possible involvement of “Transient Receptor Potential” channels (TRP) from the vanilloid subfamily, known as thermo-receptors in different vertebrates, on thermo-sensation and temperature regulation of MEL production in fish. Gills Na^+/K^+-ATPase activity was measured as a marker of sea water tolerance acclimation. Circulating MEL was quantified, as well as the levels of TRPV1 and TRPV4 mRNA expression in the retina, pituitary and gills. *In vitro* pharmacology and temperature challenges were also carried out in salmon’s pineal gland. Our findings suggest a tissue-specific effect of photoperiod alone or combined with elevated temperature on TRPV channels expression. The results of MEL *in vivo* or *in vitro* are still not conclusive, but provide a first overview of time signaling mechanisms involving temperature cues, illustrating putative effects of a changing environment on the smoltification process.

OR-49

Stressor- and sex-specific effects of increased temperature and ocean acidification on the reproductive endocrine axis of the cinnamon anemonefish

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Although marine organisms face the dual climate change threats of increasing temperature and ocean acidification, the potential interactive effects of these stressors on the reproductive endocrine axis of marine fishes is not known. Previously, we reared adult breeding pairs of cinnamon anemonefish for 10 months at three temperatures (28.5, 30.0 and 31.5°C) cross-factored with three CO_2 levels (417, 644 and 1134 µatm) and quantified their reproductive performance (Miller et al. 2015, Ecol. Appl. 25:603-620). We found that temperature had a much greater impact than CO_2, with clear declines in reproduction occurring at 30.0°C, complete reproductive failure at 31.5°C, and CO_2 having a minimal effect. Elevated temperature also had a significant negative effect on plasma estradiol levels, suggesting that declines in reproduction with increasing temperature were due to a disruption of the endocrine reproductive axis. In this study, to further our understanding of the mechanisms by which elevated temperature and CO_2 levels affect reproduction, we assessed the effects of the above treatments on the expression of key reproductive genes in the brain-pituitary axis. In females, the effects of temperature and CO_2 on gene expression were target specific. Whereas LHβ mRNA levels decreased with increasing temperature and CO_2 levels, the expression of FSHβ and GPHα increased in the 30.0°C and 644 µatm treatment. Temperature and CO_2 significantly affected the expression of Kiss1, GnRH1, brain aromatase and tyrosine hydroxylase and there was an interaction between the two stressors. In contrast, the expression of the various target genes in males consistently increased with increasing temperature and CO_2 levels, and there was no interaction between the two stressors. Overall, elevated temperature and CO_2 had broad stressor- and sex-specific effects on the expression of reproductive genes in the brain-pituitary axis. These results provide new insight into the mechanisms by which future climate change may impact the reproductive performance of fish.

OR-50

Fish hormones and the ocean carbon cycle

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The impact of ocean acidification in the endocrine system of fish remains partially understood. The predicted ocean acidification generated by oceanic absorption of atmospheric CO_2 shifts the carbon equilibrium and reduces the availability of carbonates required for calcifying animals. Fish contribute to the carbon cycle by the production of intestinal mineralized carbonated aggregates generated as by-products of osmoregulation. The chemistry and solubility of the intestinal aggregates makes them relevant for the ocean carbon cycle, as they provide neutralizing alkaline buffer, preferentially in the uppermost, most productive section of the water column. This process of chemical ion trapping promotes continuous intestinal luminal fluid processing by decreasing osmolality, which impacts on calcium homeostasis and favors water absorption in the intestine. The production of intestinal carbonate aggregates is the end result of epithelial bicarbonate secretion in the intestine of marine fish, which is under a tight regulation by endocrine factors studied so far, including calcitropic hormones such as parathyroid hormone related peptides, stanniocalcin or the pituitary factor, prolactin. Bearing in mind this important involvement of the endocrine system in epithelial functions in fish we directed our efforts to the pituitary gland, the most important gland in the endocrine system. The pituitary incorporates the neuroendocrine information integrated by the hypothalamus to convert it into endocrine information to control all functions of physiology. Here we will show effects of changes of water CO_2 based in near future predictions in the expression of the main endocrine factors involved in body-wide physiology such as prolactin, growth hormone, somatolactin or proopiomelanocortin. In addition to the pituitary gland we have directed some effort to the response of the Stannius corpuscles, a fish specific gland, whose main endocrine product is stanniocalcin. In this initial approach, we analyzed expression and circulating plasma levels of Stanniocalcin in our model species, the sea bream. Taken together our results in the pituitary gland, and the corpuscles of Stannius show profound effects of water CO_2 in the endocrine system, that would be comparable to those evoked by exposure of fish to extreme salinities

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OR-51

Organochlorine pesticides disrupt fish immune responses

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Lou Guillette first brought attention to adverse endocrine and developmental effects of organochlorine pesticides (OCPs) in aquatic organisms inhabiting areas near Lake Apopka, Florida. In particular, muck farms around Lake Apopka were highly contaminated with OCPs for decades, and still present with high residue in the soils/sediments and the water. Largemouth bass (Micropterus salmoides) collected from clean sites and placed in ponds in the impacted areas rapidly uptake OCPs into their tissues. Transcriptomics analyses point to major alterations in the immune system, in addition to the expected endocrine endpoints. Follow up laboratory experiments with individual chemicals and binary combinations also point to both endocrine and immune alterations in the liver and gonad. For example, T-cell activation and regulation of macrophages are immune responses that are affected in LMB by OCPs at the transcriptome level. We have focused current studies on the hepatic lipidome and found alterations in phospholipid classes upon exposure. Enrichment was observed in the phosphatidic acids and phosphoinositols, while phosphatidylcholine and phophatidylethanolamine were suppressed, compared to controls. In addition, we observed specific alterations in sphingolipids and glycerophospholipids, which have been implicated with inflammation and other immune responses. We postulate that OCPs alter both the endocrine system and the immune system through adverse action on steroidogenesis and on soluble hormone receptors that control lipid biosynthesis.

Afternoon sessions Friday July 1

14:00-16:30	**Session 9: Aquaculture endocrinology** **Main Lecture Hall** Chairs: **Yonathan Zohar** (USA)**, Eva Andersson** (NOR)**, Oliana Carnevali** (ITA)	**Session 10: Steroid hormones: Receptors and synthesis** **Lecture Hall Pascal** Chairs: **Peter Thomas** (USA)**, Olivier Kah** (FRA)**, Deshou Wang** (CHN)
14:00-14:20	**OR-52** Production and uses of recombinant gonadotropins in fish. **Ana Gómez** (ESP)	**OR-58** Zinc- a novel second messenger regulated by steroid hormones through the membrane androgen receptor, ZIP9. **Peter Thomas** (USA)
14:20-14:40	**OR-53** Molecular mechanism of maturation-inducing hormone production in cultured ovaries of the Japanese eel, *Anguilla japonica*. **Shigeho Ijiri** (JPN)	**OR-59** Human and zebrafish nuclear progesterone receptors are differently activated by natural and synthetic progestins. **François Brion** (FRA)
14:40-15:00	**OR-54** Growth and reproductive measures in germ cell depleted Atlantic salmon (*Salmo salar* L.). **Anna Wargelius** (NOR)	**OR-60** Approaches for detailed analysis of membrane progestin receptor (mPR) protein and for physiological roles of mPR. **Toshinobu Tokumoto** (JPN)
15:00-15:30	**Coffee / Tea break**	**Coffee / Tea break**
15:30-15:50	**OR-55** Producing sterile fish by disrupting primordial germ cell development using a transient gene silencing bath-immersion approach. **Yonathan Zohar** (USA)	**OR-61** Haploinsufficiency of SF-1 causes female to male sex reversal in Nile tilapia, *Oreochromis niloticus*. **Deshou Wang** (CHN)
15:50-16:10	**OR-56** Endocrinology of temperature-induced masculinization and sterilization in the Nile tilapia. **Jean-François Baroiller** (FRA)	**OR-62** Comparisons of structure and co-regulator binding domain in teleost androgen receptors. **Shang Chien Lee** (TWN)
16:10-16:30	**OR-57** Endocrinological assessment of the fish immune system from a high through-put gene sequencing perspective. **Takashi Yada** (JPN)	**OR-63** Molecular characterization and quantification of the estrogen receptors ERα and ERβ from turbot (*Scophthalmus maximus*). **Yudong Jia** (CHN)
16:30-18:30	**Poster session II: Presenting authors requested to stand by even numbered posters**	

OR-52

Production and uses of recombinant gonadotropins in fish

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Understanding the differential roles of the pituitary gonadotropins Fsh and Lh in gonad maturation is crucial for a successful manipulation of the reproductive process in fish, and requires species-specific tools and appropriate active hormones. With the increasing availability of fish cDNAs coding for gonadotropin subunits, the production of recombinant hormones in heterologous systems has gradually substituted the approach of isolating native hormones. These recombinant hormones can be continually produced without depending on the fish as starting material and no cross-contamination with other pituitary glycoproteins is assured. Recombinant gonadotropins should be produced in eukaryotic cells, which have glycosylation capacity, but this post-translational modification varies greatly depending on the cell system, influencing hormone activity and stability. The production of recombinant gonadotropin beta-subunits to be used as antigens for antibody production has allowed the development of immunoassays for quantification of gonadotropins in European sea bass and Senegalese sole. Their use in combination with receptor-based bioassays to assess hormone bioactivity, have revealed as essential tools to study gonadotropin physiology. The administration *in vivo* of dimeric homologous recombinant gonadotropins has been used in basic studies and as a biotechnological approach to induce spermatogenesis and spermiation in immature European sea bass and European eel. In addition, gene-based therapies using somatic transfer of the gonadotropin genes have been tested as an alternative for hormone delivery *in vivo*. The hormones produced by the injected genes were functional and allowed different studies on gonadotropin action. In summary, the endocrine control of reproduction by *in vivo* administration of homologous recombinant gonadotropins or their coding genes opens new strategies in aquaculture, as homologous treatments can be designed to solve reproductive dysfunctions associated with low hormone levels or to develop out-of-season breeding programs in new or already domesticated species. Supported by AGL2015-67477-C2-1-R, PROMETEOII-214/051

OR-53

Molecular mechanism of maturation-inducing hormone production in cultured ovaries of the Japanese eel

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17α,20β-dihydroxy-4-pregnen-3-one (DHP) has been identified as a maturation-inducing hormone of the Japanese eel, *Anguilla japonica*. DHP production is regulated by production of its precursor, 17α-hydroxyprogesterone (17α-OHP), and an enzyme which converts 17α-OHP to DHP, termed 20β-hydroxysteroid dehydrogenase (20β-HSD). In a previous study, we suggested that 17β-hydroxysteroid dehydrogenase type12-like (hsd17b12l) is the 20β-HSD responsible for DHP production. Furthermore, our *in vivo* study demonstrated that DHP production can be induced by injection of high-dose (300 mg/kg) salmon pituitary extract (SPE) after completion of vitellogenesis and suggested that this DHP production is controlled mainly by 17α-OHP production, due to a rapid drop in cyp17a1 (17α-hydroxylase/C17–20 lyase) expression and not up-regulation of cyp17a2 (17α-hydroxylase lacking C17–20 lyase activity) and hsd17b12l mRNA expression. In this study, we aimed to understand molecular mechanism of DHP production in cultured ovaries of *A. japonica*. Feminized eels received weekly injections of SPE at 30 mg/kg body weight. After completion of vitellogenesis at the migratory nucleus stage, the eels were primed with a regular SPE injection, and 24 h later, primed eels received high-dose SPE injection to induce oocyte maturation and ovulation. Ovarian tissue collected at various developmental stages was incubated in L-15 medium in the presence or absence of 100 or 1000 μg/mL SPE, or 100 ng/mL 17α-OHP for 18 h at 20°C. After the incubation, DHP levels in the media were measured by TR-FIA, and cyp17a1, cyp17a2 and hsd17b12l mRNA levels in the ovarian tissue were measured by qPCR. Ovaries at all developmental stages were able to produce DHP from 17α-OHP. The DHP production by ovaries collected after high-dose SPE injection increased and maintained high at post-ovulation. SPE did not stimulate 20β-HSD activity in ovaries at all stages except for ovaries after high-dose SPE injection. However, hsd17b12l mRNA levels maintained stable level and were not stimulated by SPE in all incubated ovaries. In contrast, cyp17a1 was down-regulated and cyp17a2 was up-regulated by SPE in ovaries after high-dose SPE injection. These results suggest that 20β-HSD activity and hsd17b12l mRNA levels do not exhibit a linear relationship and the induction of DHP production in incubated ovary after high-dose SPE injection is controlled by 17α-OHP production which regulated by down-regulation of cyp17a1 and up-regulation of cyp17a2.

OR-54

Growth and reproductive measures in germ cell depleted Atlantic salmon (*Salmo salar* L.)

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Genetic introgression mediated into wild populations by farmed escapees is a concern, and currently limits growth of the Norwegian salmon industry. To address this problem, we investigate the possibility to induce sterility by vaccination, with the aim to abolish the germ cells in salmon. In this context we are exploring aspects of growth and reproductive physiology of fish lacking germ cells, to monitor potential effects of the loss of germ cells on traits relevant for aquaculture, such as growth and maturation. We have used the CRISPR-Cas9 technology to knockout the *dead end* (*dnd*) gene in salmon with the aim to produce germ cell-free salmon. This gene is essential for germ cell survival in both mammals and fish. Due to the long generation time of salmon we intended to generate complete (bi-allelic) loss-of-function mutants in the F0 generation. To avoid analysis of mosaic individuals, we simultaneously induced CRISPR-Cas9-mediated mutations in the *albino* (*alb*) and in the *dnd* gene. We observed that complete loss of pigmentation indicated bi-allelic disruption of *alb* but also of the *dnd* gene. This methodology allowed the production of germ cell-free salmon, in which we could show that in Atlantic salmon, like loach, goldfish and rainbow trout, sex determination is independent of the presence of germ cells. Germ cell-free fish (n=80) and wild-type (n=80) at ~100g body weight were exposed in a common garden setting to a precocious maturation regime; 16ºC and continuous light for 1 month. Subsequently the fish were transferred to ambient temperature, growth was measured and plasma samples were collected four times during one year when the fish had reached ~2-3 kg. Growth, 11-ketotestosterone and estradiol-17β were measured in these fish to determine the effect of lack of germ cells in the fish. Initial data analysis reveals that neither germ cell-free males nor females are recruited into puberty. Moreover, growth of germ cell-free fish was also affected in comparison to maturing controls.

OR-55

Producing sterile fish by disrupting primordial germ cell development using a transient gene silencing bath-immersion approach

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As global aquaculture is rapidly expanding, the need to farm reproductively sterile fish is gaining importance as the most effective strategy for genetic containment to enable environmentally-responsible aquaculture practices. Sterility also enhances muscle development and growth by minimizing energy invested in gonadal growth and preventing sexual maturation, which is known to cause deterioration of flesh quality and increase in susceptibility to stress and disease. We developed an embryo bath-immersion technology to produce infertile fish by disrupting the early migration of the primordial germ cells (PGCs) into the gonadal ridge. To enable the visualization of PGCs during early development in the zebrafish model, a transgenic line, *Tg(kop:DsRed-nanos3)*, that expresses fluorescent DsRed driven by the PGC-specific *kop* promoter and *nanos3* 3'UTR, was used for this study. We discovered that a molecular transporter, known as Vivo, can effectively carry silencing Morpholino oligomers (MO) across the chorion and reach the embryo. Immersing freshly fertilized zebrafish eggs in Vivo-conjugated MO against deadend (dnd-MO-Vivo), an essential gene for PGC development, effectively silenced Dnd expression and caused PGC mis-migration and differentiation into somatic cells, resulting in the production of reproductively sterile fish. Optimal conditions were achieved in zebrafish to induce 100% sterility with immersion durations as short as 5 hours post-fertilization. Under these conditions, 736 adult zebrafish from 8 independent experiments were all found to be infertile, possessing minimally-developed gonads that lacked any gametes but were otherwise phenotypically normal. We used the same approach to induce sterility in rainbow trout and Atlantic salmon, and current studies, in collaboration with commercial hatcheries, are aimed at optimizing conditions towards obtaining 100% sterility in salmonids. Our technology offers the aquaculture industry an efficient and practical non-GMO approach to generate sterile fish while maintaining fertile broodstock. It also provides an innovative strategy to eliminate germ cells that will enable us to dissect and fully explore, from very early development to adulthood, the roles of germ cells and gonads in regulating gene expression pathways and developmental organization of the reproductive endocrine network, without the need for gonadectomy.

OR-56

Endocrinology of temperature-induced masculinization and sterilization in the Nile tilapia

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In tilapia, male production mainly relies upon androgen treatments inducing masculinization, whereas sterility can be obtained through triploid induction. In the context of sustainable aquaculture, new sexing technologies and alternative approaches for controlling sex and/or sterilization of the gonads are currently major issues. As a first step, we have developed a precocious (2 weeks post-fertilization) sexing technology to define the sex-ratio (phenotype) of a progeny using the *amh* expression. Concerning alternative approaches, early high-temperature treatments (HT) can respectively masculinize (32 to 36 ° C = MHT) or sterilize (> 36 ° C = SHT) the gonads of the thermosensitive progenies. We have analysed the temperature effects of these 2 HT and the molecular pathways during the sex-differentiation process. MHT from 10dpf onwards can masculinize female monosex (all-XX) progenies. Temperature masculinisation is brought about by first rapidly (3-5 days following the beginning of the HT) stimulating testes differentiating genes such as *dmrt1* and *amh*. Two to three weeks later *dmrt1* and *amh* expressions were respectively 3-fold higher or similar in XX treated individuals *versus* XY genetic males. These high expression levels of *dmrt1* and *amh* down-regulate ovarian pathway genes, such as the aromatase *cyp19a1a* causing the decrease in gonad oestrogen levels. Furthermore, a much earlier up-regulation is seen for genes involved in testis development, such as *11βHSD* implicated in 11-ketotestosterone synthesis and *sox9*. In addition we have shown that following 60-day treatments of high-temperatures >36°C applied on 10 days fry caused permanent gonad sterility throught the processes of germ cell apoptosis and inhibition of proliferation. C*yp19a1a* and *amh* gene expressions were both reduced in HT fish, suggesting that Sertoli cells are directly or indirectly (lack of germ cells) affected by the temperature treatment. Masculinization of the gonads through germ cell depletion could be the first step of the temperature-induced sterility. On-going studies comparing thermosensitive and non-thermosensitive strains should be performed to analyse the involvement of germ-cell number and the roles of *amh* and *amhr2* in the temperature-induced masculinization.

OR-57

Endocrinological assessment of the fish immune system from a high through-put gene sequencing perspective

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Immune-endocrine interaction is important for pathogen resistance in fish. We review the immune-endocrine interaction in fish, with special reference to genome interpretation. An applied aspect of such information could be for selection of particular fish strains for aquaculture. By utilizing next generation sequencing, high levels of expression of genes related to endocrine and immune functions were detected in thermally selected strain of rainbow trout, Oncorhynchus mykiss that are candidates for aquaculture under climate change scenarios, including global warming. Such information is also relevant to the significant problem of decreased harvest of Japanese eel, Anguilla japonica, allowing for selection of appropriate disease resistant strains for breeding. We identified over thousands sequences responding to air-exposure in eel by comprehensive analysis of gene expression with next generation sequencing. Significant changes in expression were shown in numerous genes related to disease resistance after air-exposure even by the quantitative PCR. Another application of this information is in the development of therapeutants and/or the therapeutic application of hormones for disease-resistance in fish. For example, enhancement of fish immune function by short-term administration of purified growth hormone (GH) have been reported. However, long-term exposure to GH by transgene techniques resulted in contradictory results among species, some having enhancement, others depression or no effect. Oral administrations of GH to rainbow trout for several months resulted in significant modulation on the expression of immune-related genes independent of its growth-promoting effect. Other applications of the immune-endocrine interaction in fish will also be discussed.

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OR-58

Zinc- a novel second messenger regulated by steroid hormones through the membrane androgen receptor, ZIP9

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A membrane androgen receptor was recently discovered in Atlantic croaker granulosa cells in our laboratory and shown to be a member of the zinc transporter ZIP9 (SLC39A) subfamily, the first androgen receptor unrelated to nuclear steroid receptors identified in any vertebrate species (1). Testosterone (T) promotes serum starvation-induced cell death and apoptosis in croaker ZIP9-transfected cells and in croaker ovarian follicle cells that is associated with rapid increases in intracellular free zinc (Zn) concentrations, suggesting an involvement of Zn in this nonclassical androgen action to promote apoptosis. We demonstrated that human ZIP9 also functions as a membrane androgen receptor and Zn transporter in human prostate and breast cancer cells and mediates testosterone induction of apoptosis in these cancer cells through an intrinsic apoptotic pathway (2). Additional receptor and signalling characteristics of croaker and human ZIP9 were investigated in the present study. Ligand blot analysis of solubilized cell membranes showed [^{3}H]-T binding is associated with a 40kDa band corresponding to the position of ZIP9 on Western blots which suggests the ZIP9 protein can bind [^{3}H]-T alone, in the absence of other protein partners. Pretreatment with GTPγS and pertussis toxin decreased [^{3}H]-T binding to plasma membranes of ZIP9-transfected cells and blocked the testosterone-induced increase in ERK phosphorylation and influx of extracellular Zn, consistent with an association of ZIP9 with G proteins and an involvement of G proteins in both MAP kinase and Zn signalling. ZIP9 expression and [^{3}H]-T binding was also detected on mitochondrial and nuclear membranes of cells transfected with human ZIP9. T treatment caused deceases in the Zn contents of nuclei and mitochondria, suggesting that ZIP9 also regulates Zn signalling through releasing Zn from these intracellular organelles. The discovery that a single protein performs dual functions as a membrane androgen receptor and Zn transporter in croaker and human cells indicates that a previously unrecognized intimate relationship exists between steroid and Zn signaling pathways in vertebrate cells.

(1) Berg, A.H., et al., 2014. *Endocrinology* 155:4237-4249.
(2) Thomas, P., et al., 2014. *Endocrinology* 155:4250-4265.

OR-59

Human and zebrafish nuclear progesterone receptors are differently activated by natural and synthetic progestins

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As compared to (xeno-)estrogens, natural and synthetic ligands of the progesterone receptor (PR) have been scarcely studied on aquatic organisms while the potential risk posed by these compounds has been recently pointed out. However, there is still a lack of data to accurately characterize the hazards posed by these compounds. The capacity of synthetic progestins to interact with fish nuclear PR was poorly investigated up to now. Herein, we assessed the biological activity of a broad range of natural and synthetic progestins towards zebrafish (zf) PR and human (h) PR to identify possible interspecies differences. Two human cell lines co-expressing either hPR or zfPR and luciferase gene, namely HELN-hPRB and U2OS-zfPR cells, were developed and characterized using promegestone (R5020) as a reference agonist ligand. R5020 induced luciferase activity in both cell lines in a concentration-dependent manner. These effects were completely blocked by co-exposing the cells with mifepristone (RU486), a potent hPR antagonist. A large set of natural and synthetic progestins (25) was screened in the two cell lines. All tested chemicals, except the natural zebrafish progestin 17α,20β-dihydroxy-4-pregnen-3-one (DHP), activated hPR. Conversely, in U2OS-zfPR cells, only five of them induced luciferase activity, DHP being a potent and specific zfPR agonist. Coexposure of progestins with R5020 allowed us to further characterize progestins as partial or full agonists of h and zfPR. Lastly, several ubiquitous environmental endocrine disruptors (BPA, NP, BP,...) were screened on the two models and allowed us to identify one environmental ligand of the zfPR. Overall, two new luciferase reporter cell lines were developed and characterized, providing novel and relevant information regarding the activity of a large set of progestins on h and zfPR. We showed that h and Zf were differently activated by natural and synthetic progestins revealing major interspecies differences.

OR-60

Approaches for detailed analysis of membrane progestin receptor (mPR) protein and for physiological roles of mPR

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More than ten years have passed since the finding of membrane progestin receptors (mPRs). Although the identification of mPR genes in various organisms and expression patterns have been described since then, the precise physiological roles of mPRs are still unclear except the function as receptor for maturation-inducing steroid in fish. Wide distribution of mPRs predicts the variable actions of progestins through mPR in the tissues. Information about physiological roles of mPRs gradually accumulated recently such as progression of breast cancer and T cell proliferation etc. We established the cell line that make it possible to real time monitoring of the intracellular concentration of cAMP after stimulation by the ligands for mPR. We established the cell line that transformed with cDNAs for mPRα and recombinant luciferase gene, named Glosensor. The cells can be used for monitoring the effects of ligands for mPRα by intracellular cAMP levels. Study using these cell lines indicated that cAMP concentration was decreased by ligands for mPRα. The results provide the suportive evidence for previous results that suggested mPRα is coupled to Gi protein. Also we succeeded to express and purify recombinant mPR protein in yeast, *Pichia pastoris*. Relatively large amount of mPRα protein with hormonal binding activity can be purified by our method. The recombinant protein will be applicable to establish the molecular probe to detect mPR interacting agents and three demensional sturatural analysis of mPR protein. To get decisive evidence on the roles of mPRs, we are establishing strains of medaka fish that have deficient on mPRs. In Medaka, four subtypes of mPR genes (α, β, γ, and α2) were identified. By the reverse genetic screening system, we have selected three to four strains in which a point mutation was induced on the coding sequence of mPR subtypes. However homozygous mutants of each mPR genes showed no phenotype. The results suggested that mPR genes share redundancy. We are currently producing double and triple mutants of mPR subtypes. Physiological roles of mPRs will be proved by mutant medaka strains.

OR-61

Haploinsufficiency of SF-1 causes female to male sex reversal in Nile tilapia, *Oreochromis niloticus*

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Steroidogenic factor-1 (Sf-1, officially designated NR5A1) is a master regulator of steroidogenesis and reproduction in mammals. However, its function remains unclear in non-mammalian vertebrates. In the present study, we used immunohistochemistry to detect expression of Sf-1 in the steroidogenic cells, the interstitial, granulosa and theca cells of the ovary, and the Leydig cells of the testis, in Nile tilapia. CRISPR/Cas9 cleavage of sf-1 resulted in a high mutation rate in the F0 generation and a phenotype of gonadal dysgenesis and reduced steroidogenic cells in XX and XY fish. Sf-1 deficiency also resulted in decreased Cyp19a1a, Foxl2 expression and serum E2 (estradiol-17β) levels in XX fish. In XY fish, Sf-1 deficiency increased Cyp19a1a and Foxl2 expression but decreased Cyp11b2 expression and serum 11-KT (11-ketotestosterone) levels. MT (17α-Methyltestosterone) treatment successfully rescued the gonadal phenotype of Sf-1 deficient XY fish, as demonstrated by normal spermatogenesis and production of F1 mutants. In contrast, E2 treatment only partially rescued the gonadal phenotype of Sf-1 deficient XX fish, as demonstrated by the appearance of phase II oocytes. Furthermore, both sf-$1^{+/-}$ F1 XX and XY mutants developed as fertile males, though spermatogenesis was delayed and efferent duct formation was disordered. Our data suggest that Sf-1 is a major regulator of steroidogenesis and reproduction in fish, as it is in mammals. Sf-1 deficiency resulted in gonadal dysgenesis and feminization of XY gonads. However, unlike in mammals, Sf-1 deficiency also resulted in female to male sex reversal in 8.1% of F0 and 92.1% of sf-$1^{+/-}$ F1 in XX fish.

OR-62

Comparisons of structure and co-regulator binding domain in teleost androgen receptors

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In teleost, more and more subtypes of androgen receptors (ARs) were identified among different teleost species. Whole-genome duplication (WGD) was proposed to be one of the major events to construct different genome in vertebrates. In addition, studies of teleost genomes revealed that the third round (3R) WGD was occurred in teleost and these teleost duplicated genes were evidences to reveal more evolutionary functions. Distinct structural diversity of ARs can be observed among many teleosts. In this study, teleost ARs sequences were retrieved from gene database and were compared by sequence alignment software. Interestingly, the sequences of eels' ARs were separated into teleost ARα or ARβ clusters. However, both of them were diverged from other teleost. Furthermore, eel's ARα diverged from other teleost's ARα with a long distance. As results, the most conserved area was in DNA-binding domain (DBD) sequence, and followed by ligand-binding domain LBD. However, the most critical difference was short or no N-terminal domain (NTD) in many neo-teleosts. Following the alignment data, Hsp binding domain, P-box and D-box of DBD showed high similarity in teleost ARs. An important motif LxxLL was also very conserved in co-activator binding groove of LBD. In addition, NTD sequences with FxxLF-like motif were presented in several teleost and non-teleost fishes. In contrast, short and loss of NTD was obviously observed in many neo-teleosts. Moreo-ver, functional N/C terminal interaction of AR is reported and revealed that FxxLF peptide is play a crucial role in binding with LBD. This interaction significant affects co-activators like ARA70 and SRC2 binding during AR genomic actions. Furthermore, these N/C interaction modulated co-activa-tors have been cloned from zebra fish and rainbow trout, coincidentally, the AR of them are all NTD (FxxLF-like) conserved. Therefore, we hypothesized that, AR with or without NTD (FxxLF) sequences may result in various co-regulator binding patterns, and this may be the reason why highly diversify androgen or AR functions performs in teleost world.

OR-63

Molecular characterization and quantification of the estrogen receptors ERα and ERβ from turbot (*Scophthalmus maximus*)

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Estrogens play crucial roles in the regulation of reproductive activities in vertebrates via estrogen receptors (ERs)-mediated signaling pathway. In the present study, full-length sequences coding for the ERs (*erα* and *erβ*) were isolated from female turbot (*Scophthalmus maximus*) by homology cloning and a strategy based on rapid amplification of cDNA end-polymerase chain reaction (RACE-PCR). The nucleotide and amino acid sequences of turbot *erα* and *erβ* showed high homologies with the corresponding sequences of other fish species and significant homology with that of Japanese flounder (*Paralichthys olivaceus*). Both *erα* and *erβ* contained six typical nuclear receptor-characteristic domains and exhibited high evolutional conservation in the functional domains. Real time quantitative-PCR analysis revealed that the *erα* and *erβ* mRNAs were abundant in liver and ovary, respectively. Furthermore, the mRNA levels of *erα* in liver and *erβ* in ovary were found to increase gradually from pre-vitellogenesis to late-vitellogenesis stages, with the highest values observed during the late-vitellogenesis stage of the reproductive cycle. However, the mRNA levels of *erα* in liver and *erβ* in ovary were found to decrease dramatically from migratory nucleus to atresia stage. These results indicate that turbot *erα* is mainly involved in hepatic vitellogenesis and *erβ* may be related to the regulation of oocyte maturation as well as promotion of ovarian development. These findings give better insight into the understanding of the physiological functions of turbot ERs, which will be valuable for fish reproduction and broodstock management.

Morning sessions Saturday July 2

09:00-10:00	**Plenary Lecture III (Main Lecture Hall)** **Penny Swanson (USA) Perspectives on the physiology of Fsh in teleost fishes (OR-64)**	
10:00-12:30	**Session 11: Endocrine control of energy balance** **Main Lecture Hall** Chairs: **Mark Sheridan** (USA)**, Joaquim Gutierrez** (ESP)	**Session 12: Phenotypic plasticity** **Lecture Hall Pascal** Chairs: **Penny Swanson** (USA)**, Anna Wargelius** (NOR)**, Guan-Chung Wu** (TWN)
10:00-10:20	**OR-65** Functional plasticity of the intestine of Mediterranean farmed fish to cope with environmental and nutritional stressors. **Jaume Pérez-Sánches** (ESP)	**OR-71** The testis affects the ovary growth of digonic gonad in protandrous black porgy. **Guan-Chung Wu** (TWN)
10:20-10:40	**OR-66** Releasing stored lipids to fuel migration and reproduction in the eel, *Anguilla australis* – a role for 11-ketotestosterone? **Mark Lokman** (NZL)	**OR-72** Germ line stem cells and sexual plasticity in medaka, Oryzias latipes. **Tapas Chakraborty** (JPN)
10:40-11:00	**OR-67** Ceramides are involved in the modulatory actions of fatty acid and ghrelin in the central metabolic control of food intake in rainbow trout. **José L Soengas** (ESP)	**OR-73** Adaptive plasticity in Atlantic salmon- genetic predisposition of time at maturity and environmental triggers. **Rolf B Edvardsen** (NOR)
11:00-11:30	**Coffee / Tea break**	**Coffee / Tea break**
11:30-11:50	**OR-68** Central leptin signalling and effects on appetite regulation in rainbow trout. **Ningping Gong** (SWE)	**OR-74** The effects of migratory stage and 11-ketotestosterone on the expression of rod opsin genes in the shortfinned eel (*Anguilla australis*). **Georgia Thomson-Laing** (NZL)
11:50-12:10	**OR-69** Leptin is a carbohydrate catabolic stress hormone that stimulates glycolysis: Insights from transcriptomic and physiological studies in the tilapia. **Russell J Borski** (USA)	**OR-75** Brain and adipose tissue global gene network responses to photoperiod in pre-pubertal European sea bass. **Rute Martins** (PRT)
12:10-12:30	**OR-70** The effect of long-term feed deprivation followed by re-feeding and feed-flavour stimulation on gene expression of putative hypothalamic appetite regulators in Arctic charr. **Anja Striberny** (NOR)	**OR-76** Personality and the biological clock – consistent inter-individual patterns of clock gene expression, diurnal activity and endocrine expression correlate in adult zebrafish. **Christian Tudorache** (NLD)
12:30-13:30	**Lunch**	

OR-64

Perspectives on the physiology of Fsh in teleost fishes

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Nearly thirty years ago, follicle-stimulating hormone (Fsh) was discovered in Pacific salmon by Kawauchi and colleagues. This discovery overturned the dogma that reproduction in all fishes was regulated by a single luteinizing hormone (Lh)-type gonadotropin. Since that time, Fsh and receptors for both Fsh and Lh have been identified in numerous and diverse fish species, largely through molecular genetic techniques. Detailed studies of the physiological functions of Fsh in many species were limited by challenges with purifying native Fsh protein due to low pituitary content and overlapping chemical properties with Lh. However, with new methods to produce biologically active recombinant glycoprotein hormones, large-scale transcriptomic approaches, and gene knock out techniques, we now have information on Fsh function in diverse species, including fish with group synchronous and asynchronous spawning. Results from our work in salmonids and that of other groups in diverse species demonstrate that Fsh has actions that are both distinct from and overlapping with those of Lh. In this talk I will give a short history of the discovery of Fsh and an overview on what is known about the physiology of Fsh, focusing on the importance of Fsh in puberty onset and early stages of gametogenesis.

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OR-65

Functional plasticity of the intestine of Mediterranean farmed fish to cope with environmental and nutritional stressors

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Fish intestine is a key organ for the regulation of energy balance and metabolism, and other important processes like immune response and water and electrolyte balance. Thus, intestine constitutes an important target tissue for the assessment of cultured fish health and performance. We review in the present work data on intestine gene expression of two important species for Mediterranean aquaculture, the gilthead sea bream and the European sea bass. These outcomes are based on targeted and untargeted transcriptomic approaches (focused PCR-arrays, microarrays, RNA-seq), in order to assess the plasticity of fish intestinal transcriptome and the effects of new diet formulations and additives. In sea bass, microarray analysis highlighted a constant gene expression profile of middle (MI) and anterior (AI) intestine regions. Conversely, more than 1,900 genes were differently expressed between posterior intestine (PI) and AI-MI. PI emerged as a highly immune-regulated tissue, with also relevance on vitamin B12 and bile acid metabolism. A differential expression of chemosensors (G-protein coupled receptors) along the intestine sections was evidenced. In gilthead sea bream, microarray analysis showed minor gene expression changes in the intestine of fish fed balanced diets with a high replacement of fish meal and fish oil. By contrast, a large (>5,000 genes) different spatial expression pattern (AI *vs* PI) was found, which was especially evident in active feeding periods (summer). RNA-seq analysis also highlighted major gene expression differences due to the intestine section rather than to dietary challenges. However, pathway-focused PCR-arrays revealed a pro-inflammatory status of the AI in fish fed extreme plant vegetal diets, but importantly dietary butyrate supplementation was able to reverse this effect. These findings highlight the refractoriness of the intestinal transcriptome to dietary changes when nutrient requirements are met by diet. By contrast, the large differences found in temporal and spatial molecular signatures offer the possibility to identify and validate reliable markers of diagnostic and/or prognostic value for intestinal health and integrity. Acknowledgments: this work was funded by EU (ARRAINA, FP7-KBBE-2011-5-288925) and Spanish (Mi2-Fish, AGL2013-48560; PrometeoII/2014/85) projects.

OR-66

Releasing stored lipids to fuel migration and reproduction in the eel, *Anguilla australis* – a role for 11-ketotestosterone?

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The long-distance migration of freshwater eels to remote spawning locations occurs in the absence of any food intake – the fish thus are dependent on stored fats to fuel both migration and reproductive investment. The endocrine signals responsible for mobilising stored fats have not, to date, been identified. Given the key role of 11-ketotestosterone (11KT) in inducing the migratory eel phenotype, we hypothesised that this steroid would also affect muscle lipid physiology. To test this hypothesis, we employed Nanostring analysis to measure the expression of lipolytic and lipogenic enzyme genes in white muscle of wild-caught feeding non-migratory (yellow) eels and fasting migratory (silver) eels. From among ~ 20 genes, 11 were differentially expressed between both life history stages (based on FDR), six of which were identified as generically lipogenic, five as lipolytic. Subsequently, we experimentally manipulated yellow eels with or without 11KT, resulting in a dose-dependent increase (control + 3 doses of 11KT) in gonadosomatic index (GSI). The increase in GSI was accompanied by increased expression of four lipogenic enzyme genes and one lipolytic enzyme gene in white muscle, in the same fashion as seen when comparing silver with yellow eels. Furthermore, there was a significant decrease in expression of androgen receptor (AR)-β in a pseudo dose-dependent manner. A follow-up experiment on silver eels saw GSIs decrease with prolonged captivity, but they recovered in part by 11KT treatment. The expression of only one (lipolytic) enzyme gene in muscle was significantly up-regulated by 11KT, here too matching the trend seen in wild-caught silver females. Unlike yellow eels, expression of ARβ in muscle from 11KT-treated silver eels showed an *increasing* trend, whereas ARα expression was significantly decreased by 11KT. We contend that only a part of the change in gene expression profiles between yellow and silver eels appears to be dependent on regulation by 11KT. Furthermore, we provide compelling evidence for expressional regulation of eel ARs by 11KT.in muscle of eel.

OR-67

Ceramides are involved in the modulatory actions of fatty acid and ghrelin in the central metabolic control of food intake in rainbow trout

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Ceramides is a family of lipid composed of a sphingosine linked to a fatty acid, and are especially abundant in brain. In brain areas of mammals like hypothalamus the synthesis of ceramides (modulated by hormonal signals like ghrelin and leptin) is involved in the control of feeding and body weight through effects on fatty acid sensing systems and expression of neuropeptides. We have previously demonstrated in fish the existence in hypothalamus of rainbow trout of fatty acid sensing systems modulated by ghrelin and involved in the control of food intake. We hypothesize that ceramides in rainbow trout are involved in the control of food intake modulated by central fatty acid sensing systems and peripheral hormones like ghrelin. In a first study, we assessed changes in ceramide levels in brain areas after ICV treatment with oleate, ghrelin or oleate+ghrelin. Once demonstrated a relationship between ceramides and action of fatty acids and ghrelin, in a second study we administered ICV to 100g rainbow trout 1µl of DMSO-Saline alone (control) or containing 2.5 µg of C6:0 ceramide to assess changes in fatty acid sensing systems and the expression of neuropeptides involved in the regulation of food intake. In a third study, we evaluated the effects of ceramide treatment on food intake. Ceramide treatment activated fatty acid sensing systems in hypothalamus and hindbrain and increased anorexigenic potential in the same brain areas. Particularly, we observed decreased expression of the orexigenic factors AgRP and NPY, and increased expression of the anorexigenic factors POMC and CART, whose overall balance would be an increased anorexigenic potential in agreement with the decreased food intake observed after treatment with ceramide. These responses, in some cases different to those known in mammals, are exclusive of hypothalamus and hindbrain, since midbrain was unaffected. We therefore provide, for the first time in fish, evidence for a specific role of ceramides in hypothalamus and hindbrain of rainbow trout connecting the effects of changes in levels of fatty acids and ghrelin with the control of food intake. Acknowledgements: Funded by Ministerio de Economía y Competitividad and European Fund for Regional Development (AGL2013-46448-3-1-R and FEDER).

OR-68

Central leptin signalling and effects on appetite regulation in rainbow trout

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Leptin is an anorexigenic hormone and a key regulator of appetite and energy balance in human and rodents. Lep is present in all vertebrate groups, but with remarkably low conservation of protein sequences and expression patterns, which raises fundamental questions on conservation/ divergence of endocrine function. In rainbow trout, leptin action is mediated and modulated by five leptin receptor (LepR) isoforms, including a full-length LepR ($LepR_L$), a truncated LepR ($LepR_T$), and three binding proteins (LepBPs). Leptin stimulation of rainbow trout hypothalamus-derived cells RTHy activates Jak2-Stat3, Pi3k-Akt, and Erk1/2 pathways. Coexpression of $LepR_T$ and $LepR_L$ in rainbow trout hepatoma cells RTH149 attenuates leptin activation of the Jak2-Stats pathway, suggesting $LepR_T$ as a negative modulator in leptin signalling. The LepBP1, which contains the complete extracellular domain, can diminish leptin-induced phosphorylation of Jak2, Stat3 and Akt in a concentration-dependent manner in the RTHy cells, indicating that LepBP can also be a negative modulator. In the rainbow trout hypothalamus, the full-functional $LepR_L$ has the highest gene expression, and $LepR_T$ has higher expression than the LepBPs. LepR-immunoreactive (LepR-ir) cells are widely distributed in the lateral tuberal hypothalamus, particularly within the nucleus lateralis tuberis, the periventricular zone along the infundibulum, and the nucleus anterior tuberis. Intracerebroventricular (ICV) administration of homologous leptin activates and phosphorylates Akt in the LepR-ir hypothalamic region, while coinjection with the Pi3k inhibitor LY294002 reverses this effect, suggesting involvement of Pi3k-Akt pathway in hypothalamic leptin action. ICV leptin administration strongly suppresses rainbow trout food intake. A low dose stimulates hypothalamic transcription of anorexigenic cocaine- and amphetamine-regulated transcript (Cart) and orexigenic neuropeptide Y, while a high dose stimulates hypothalamic transcription of proopiomelanocortin (Pomc). LY294002 reverses this upregulation. The data suggest that leptin suppresses food intake through stimulating anorexigenic neuropeptides Cart and Pomc, and the Pi3k-Akt pathway is activated in the regulation of Pomc. Altogether, these results demonstrate that the anorexigenic leptin effect is at least partially conserved through vertebrate evolution, located to the mediobasal hypothalamus.

OR-69

Leptin is a carbohydrate catabolic stress hormone that stimulates glycolysis: Insights from transcriptomic and physiological studies in the tilapia

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Leptin is a cytokine that is thought to work primarily as an adipostat in mammals, whereby the hormone circulates in proportion to fat deposition and inhibits appetite and stimulates lipolysis and fatty acid oxidation to prevent excessive lipid accumulation. Its function on energy homeostasis in fish is poorly understood despite leptin's well-conserved anorexigenic actions. We have shown that hepatic expression of leptin increases in response to seawater challenge and other catabolic stressors (fasting) and induces hyperglycemia and glycogenolysis in tilapia, *Oreochromis mossambicus*. A transcriptomic analysis of the tilapia rostral pars distalis (RPD), containing a nearly pure population of prolactin (PRL) cells, was performed in order to identify novel cellular actions of leptin, as well as to characterize leptin effects on central genes involved in glucose metabolic pathways. Advanced clustering of the RNAseq data revealed that leptin stimulates the expression of the glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), in a covariable manner to hypoxic stress response gene networks. Additionally, orthogonal tests show that recombinant tilapia leptin A (LepA), the dominant leptin paralog in fishes, increases the mRNA level of GAPDH and phosphofructokinase (PFK), the rate-limiting enzyme of glycolysis, after 6 h incubation of RPD. Likewise, leptin stimulates total glycolytic activity (lactate secretion) and PFK activity within 6 h. Glycolytic activity correlated significantly to both PFK mRNA levels and enzymatic activity. The potential signaling mechanisms for leptin action were also assessed. LepA stimulates STAT3 and ERK1 phosphorylation (activation) in the RPD. The stimulatory effect of leptin on glycolysis and PFK activity was suppressed by a STAT3 but not an ERK blocker, indicating the hormone stimulates glycolysis through a STAT3 mediated increase in glycolytic enzyme gene expression. Leptin stimulation of ERK signaling is most likely linked to leptin action in stimulating acute PRL release. To assess whether LepA might broadly regulate glycolysis, we tested its effects in another cell type, namely hepatocytes, where LepA is predominantly produced and may exert autocrine/paracrine effects. In hepatocyte incubations, LepA stimulates total glycolytic activity and PFK mRNA levels, but had little effect on GAPDH expression. Overall the results identify a novel action of leptin as a direct stimulator of glycolysis through a STAT3-mediated mechanism.

OR-70

The effect of long-term feed deprivation followed by re-feeding and feed-flavour stimulation on gene expression of putative hypothalamic appetite regulators in Arctic charr

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Research on appetite regulation in fish is based on knowledge derived from mammals, assuming similar roles of central appetite stimulators such as Agouti Related Peptide (AgRP) and Neuropeptide Y (NPY) and inhibitors such as Proopiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART), melanocortin 4 receptor (MC4-R), corticotropin-releasing factor (CRF) and leptin receptor (LepR) in fish as in mammals. After two decades of research, the knowledge of the appetite regulatory function of these regulators in fish is still contradictory. The anadromous Arctic charr (*Salvelinus alpinus*) has a seasonal feeding cycle characterized by a short, intense feeding period during summer and months of voluntary fasting during winter. The charr is therefore an interesting model to study appetite regulation. The aim of this experiment was to induce hunger in the charr, anticipating a stimulation and inhibition of orexigenic and anorexigenic signalling, respectively. Therefore, we feed-deprived immature two-year old charr for 4 weeks during summer. A control group was fed ad libitum. On the last day of the experiment, feed-deprived fish were divided in sub-groups and were either re-fed, exposed to fish-feed flavour, or left unfed. The control group was fed in the same manner as the re-fed group. At 1 and 5 hours, 12 fish per group were euthanized. Weight, length and stomach contents were measured and hypothalami were sampled for gene expression analysis of the above listed genes. In addition, expression of insulin-like growth factor 1 (IGF-1) and IGF-binding protein 5 (IGFBP5) were measured as potential markers of energy status. The feed-deprived fish had a significantly lower condition factor than the fed fish, and this negative energy status was associated with a lower expression of IGF-1 and IGFBP-5, pointing towards a role of hypothalamic IGF-1 in the regulation of long-term energy homeostasis. Surprisingly, re-fed charr had significantly lower stomach content than the control group and only a trend for an increased expression of POMC A2 at 5 h in the feed-deprived fish compared to the control. The exposure to fish-feed flavour and re-feeding increased AgRP, POMCA 2 and MC4-R gene expression. In conclusion, 4 weeks of feed deprivation did neither lead to a strong feeding response during re-feeding, nor to changes in expression of hypothalamic appetite regulators in the Arctic charr, while exposure to flavour and re-feeding lead to a stimulation of both putative orexigenic and anorexigenic signalling pathways.

OR-71

The testis affects the ovary growth of digonic gonad in protandrous black porgy

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In sequential sex change fish, the timing of sex changes is regulated by age, body size, and social cues. High levels of plasma estrogen are important for ovary development. However, chemical-stimulated sex change is transient, and a reversible sex change occurs after chemical treatment is withdrawn. Thus, the sexual phase is tightly regulated by the endogenous cues. Black porgy has a stably male in first two reproductive cycles, and chemical-stimulated female is a transient status in < 2-yr-old fish. Conversely, a precocious female with vitellogenic oocytes developed after the testes were removed from < 2-yr-old fish. In present study, our results reveal that sexual phase alternation is regulated by gonadotropins in the testis but not in the ovary. Lower levels of methylation at the *cyp19a1a* promoter in the ovary were correlated with natural sex change. In addition, decreased methylation levels were observed in the ovaries of fish whose testes were removed. Taken together, our data demonstrated that in addition to a bidirectional signal from the brain-pituitary-testis axis which regulates temporal effects, there is a reciprocal cross-talk between both sex tissues that regulates spatial effects, from the testis to the ovary. Finally we show that the modifications to the DNA methylation of the ovarian *cyp19a1a* promoter play an important role during sexual phase alternation.

OR-72

Germ line stem cells and sexual plasticity in medaka, *Oryzias latipes*

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Compounding effects of global warming and pollution increase the risks of reproductive failure and intersexuality, thus posing a threat to population existence. Hence, it is high time to understand the molecular mechanism of sexual trans-differentiation and plasticity in vertebrates. Stem cells and their potency can be crucial to understand the sexual re-orientations in adulthood. Our recent studies have shown that female fish can trans-differentiate into fertile males at any stages of life by exogenous steroid treatment or genetic mutation. The present study was conducted to explore the mechanism of bidirectional gonadal sexual plasticity in adult medaka. We found that immature males are significantly more plastic, and prone to complete sex reversal upon estradiol-17β (E2) administration than breeding ones. Apart from more permanent changes in sex-biased markers (*Gsdf, Sox9, Fig1a*, etc.), *DNMT1* (methylation marker) expression was significantly higher in immature fish (early gonia's > somatic cells). However, no such stage-specific differences were observed in females. This highlights that methylation might be a key factor for maintaining adult sexual plasticity. We found that E2 could also induce germ cell proliferation (positive for GFR1α and Oct4) in the testicular periphery, even at 3 days after treatment (dat), which later subsides at around 40dat. The undifferentiated stem cell marker genes were initially reduced and then re-induced from 45dat, while the differentiated stem cell marker genes showed totally opposite pattern. We isolated these germ cell clusters (hereafter named as germ line stem cells, GSCs) from both stages of each sex, and validated their stemness *in vitro* and *in vivo*. Surprisingly, surrogate fish produced donor-specific-fertile gametes depending on host's genetic makeup, irrespective of donor cell sex. When transplanted with PKH-26 treated-GSCs, fractions of both somatic and germ cells were PKH-26 positive. While, similar SSCs (somatic stem cells) only produced a population of somatic cells, which later developed as phenotypic males, probably due to non-existence of germ cells in the gonad. Our study suggests that bi-directional sexual plasticity mostly depends on GSCs and their rejuvenating potential and methylation patterns. However, the maturation-related inhibition of sex change needs further investigation.

OR-73

Adaptive plasticity in Atlantic salmon- genetic predisposition of time at maturity and environmental triggers

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Genetic diversity in wild populations can be utilized to identify the genetic basis of specific traits present in part of the population. By means of a whole genome scan in Atlantic salmon, we have recently revealed that sea age at maturity is largely controlled by the *vgll3* locus. Time of maturation is also strongly influenced by environmental parameters such as light and temperature. We have in this context explored how the environmental and genetic predisposition interacts in an environment which triggers maturation in males. In our experiments pit-tagged fish were exposed to a post-smolt maturation regime in common garden with fish genetically predisposed to mature after either at 1, 2 and 3 seawinters. Twenty weeks later, the gonado-somatic index was determined and used to classify the males as maturing or immature. Individuals were also genotyped for the genetic variants associated with age at maturity. Our results show that the genetic predisposition reduces the chance of maturing also in environments where maturation is triggered. Which suggest that time of maturation genotyping can be used to decrease environmentally induced maturation in salmon aquaculture. We have also investigated populations from Northern and Southern Norway displaying significant, but thus far, uncharacterized genetic differences. These differences may result from separate postglacial colonization patterns, diversifying natural selection and adaptation, or from a combination of these factors. In order to elucidate the genetic differences between Northern and Southern salmon populations, we conducted a genome wide association study, generating whole genome resequencing data from eight populations. The study identified ten selective sweeps, comprising more than 50 genes with significantly different allele frequencies between these geographically separated salmon. Our analysis also suggests an evolutionary mechanism where the whole genome duplication in salmonids has provided raw material for evolutionary adaptation.

OR-74

The effects of migratory stage and 11-ketotestosterone on the expression of rod opsin genes in the shortfinned eel (*Anguilla australis*)

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The spawning migration of freshwater eels is preceded by changes that prepare eels for a future oceanic environment. For example, in preparation for migration, some eel species have demonstrated plasticity in their spectral sensitivity through the modification of visual pigments in the retina. Notably, a novel rod opsin is expressed in the retina to increase sensitivity to blue-light wavelengths in the deep-sea environment. Given its recognized role as a physiological driver of changes related to migration in freshwater eels, 11KT was hypothesized to affect the production of both the novel seawater-type opsin and the existing freshwater-type opsin. We investigated the relationship between rod opsin expression and the ontogeny of the New Zealand shortfinned eel (*Anguilla australis*). Freshwater opsin (*fwo*) and seawater opsin (*swo*) cDNAs were isolated and the expression of opsins and androgen receptors were analysed in wild-caught non-migrating (yellow) and migrating (silver) female eels. Thereafter, the effects of 11KT on opsin expression were examined by *in vivo* and *in vitro* experiments. Yellow eels were treated with sustained-release implants containing 11KT and the androgen receptor blocker flutamide. Additionally, isolated retina pieces from yellow eels were exposed to *in vitro* treatment with 11KT (0 to 300 nM) and flutamide (0 to 300nM), both separately and in combination. Following experimentation, treated eye tissue was subjected to expressional analysis of opsins (*fwo* and *swo*) and androgen receptors (ARα and ARβ). This study demonstrated an increase in *swo* expression and a decrease in *fwo* expression in silver eels in comparison to yellow eels. This supported the existence of the same 'switch' in opsins previously established in other freshwater eel species. Additionally, *in vivo* and *in vitro* treatment with 11KT increased *swo* expression in the eel retina. Preliminary results also suggest a possible synergistic effect of 11KT and flutamide on *swo* expression. The effects of 11KT on *fwo* and androgen receptor expression in the retina were more elusive and this aspect of the study is still ongoing. Overall, this study supports the hypothesis that 11KT affects opsin expression related to changes in spectral sensitivity in the eyes of migrating freshwater eels. This evidence further strengthens the idea that 11KT is a major driver of migration-related changes in catadromous freshwater eels.

OR-75

Brain and adipose tissue global gene network responses to photoperiod in pre-pubertal European sea bass

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Puberty is a developmental process in which the hypothalamus-pituitary-gonadal axis is activated and animals become reproductively active. In European sea bass, *Dicentrarchus labrax*, precocious puberty among males results in smaller size at the time of marketing. Photoperiod manipulations are able to inhibit (continuous light - CC) or advance (shift from long to short days - AP) this process, although the underlying mechanisms are largely unknown. In this study, we explored the effects of CC and AP compared to natural photoperiod (NP) in the brain and adipose/pancreatic transcriptomes of pre-pubertal sea bass applied before the summer solstice (from April). We hypothesized that larger fish, who become reproductive during the first year, would have a different response to AP compared to non-reproductive smaller fish and other photoperiods. We constructed SuperSAGE libraries from brains and adipose/pancreatic tissue of immature fish of two sizes (L-large fish and S-small fish) reared in NP, CC or AP, and sampled at different times after photoperiod change. Comparison of the brain transcriptome under NP showed the larger fish with higher levels of circadian clock, glycolysis-related and fatty acid sensing genes, as well as differential expression of growth regulating neuropeptides - higher PACAP/PAC1 and lower somatostatin (SST). In the adipose/pancreatic tissue, downregulation of pancreatic hormones – insulin (INS), STT, and glucagon (GCG) - and up-regulation of lipolysis-related genes in L compared to S. When L were exposed to AP, the opposite expression was seen, as well as the activation of SST in the brain and SST and INS/GCG genes in the pancreatic tissue, indicating inhibition of lipolysis and growth stimulating signals. Exposure to CC downregulated growth promoting signals in L but had no effect on SST in the brain nor on SST or INS/GCG levels in pancreatic cells. This may explain the stimulation of lipolysis related genes and previously reported reduced growth/weight seen in fish under CC. Overall these results suggest that precocious puberty is associated to growth/metabolic networks which respond differently according to fish size and photoperiod – activation of puberty seems to be associated with lipid storage and activation of SST and INS/GCG axis. Funded by the European Community's Seventh Framework Programme (FP7/ 2007- 2013) under grant agreement n° 222719 – LIFECYCLE.

OR-76

Personality and the biological clock – consistent inter-individual patterns of clock gene expression, diurnal activity and endocrine expression correlate in adult zebrafish

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In zebrafish and other animal species, inter-individual differences exist in behavioural and physiological responses to environmental challenges. These differences, when consistent over time and across context, are termed (animal) personality, and can vary between two extremes, proactive and reactive. Proactive individuals are generally more bold, aggressive and explorative than reactive individuals. A generally recognised test for the differentiation of boldness is the emergence test, where a group of individuals has to emerge from a familiar, sheltered compartment into a novel, potentially dangerous environment, with early emerging fish (EE) being bolder and more explorative than late emerging fish (LE). When applying RNA sequencing (RNAseq) to the brains of EE and LE fish in order to evaluate the relative genetic expression across the entire genome, the largest differences can be found in genes related to the biological clock. This means that personality related differences are found especially in the genetic regulation of time-related traits. Further investigation into the inter-individual differences over time on other levels of biological organisation revealed that EE differ from LE in diurnal activity and regulation of melatonin and cortisol, suggesting that personality and genetic, behavioural and endocrine regulation over time are interlinked. These findings have great value for the further development of zebrafish as a model for human neuroendocrinology, psychopathology and personalised medicine. Additionally, it will add greatly to the understanding of the origins and the regulation of consistent inter-individual differences in behaviour, physiology and genetics.

Afternoon sessions Saturday July 2

13:30-16:00	**Session 13: Endocrine disruption** **Main Lecture Hall** Chairs: **Charles Tyler** (GBR), **Nancy Denslow** (USA), **François Brion** (FRA)	**Session 14: Endocrine control of development** **Lecture Hall Pascal** Chairs: **Deborah Power** (PRT), **Hamid Habibi** (CAN), **Luiz Renato de Franca** (BRA)
13:30-13:50	**OR-77** Estrogens regulate heart rate via the G protein-coupled estrogen receptor. **Daniel A Gorelick** (USA)	**OR-83** Changes in zebrafish gut microbiota modulates IGF system and metabolic hormones during larval development. **Oliana Carnevali** (ITA)
13:50-14:10	**OR-78** Estrogenic effects of several bisphenol A substitutes in the developing zebrafish brain. **Pascal Coumailleau** (FRA)	**OR-84** Thyroid hormones key integrating factors of embryonic and post-natal development of teleosts. **Marco Campinho** (PRT)
14:10-14:30	**OR-79** Development of quantitative adverse outcome pathways for aromatase inhibition in fish. **Natàlia Vinas** (USA)	**OR-85** Multifactorial regulation of reproduction and gonadal development in fish. **Hamid Habibi** (CAN)
14:30-15:00	**Coffee / Tea break**	**Coffee / Tea break**
15:00-15:20	**OR-80** Extensive regulation of diurnal transcription and metabolism by glucocorticoids in the zebrafish (*Danio rerio*). **Thomas Dickmeis** (GER)	**OR-86** Endocrine control of the sexual fate development in the gonad and the activation of the early brain in the protandrous black porgy. **Ching-Fong Chang** (TWN)
15:20-15:40	**OR-81** Using larval zebrafish behaviour to assess endocrine disruption. **Thomas Fraser** (NOR)	**OR-87** Dynamic and differential expression of the gonadal aromatase during the process of sexual differentiation in novel transgenic cyp19a1a-GFP zebrafish line. **Nathalie Hinfray** (FRA)
15:40-16:00	**OR-82** Addressing the impact of exposure to oestrogenic effluents on fish populations. **Patrick Hamilton** (GBR)	**OR-88** ASIP1 knockout perturbs pigment cell development and color pattern formation in fish. **Laura Cal** (ESP)
16:00-16:50	**The Yoshitaka Nagahama Lecture (Main Lecture Hall)** **Suraj Unniappan (CAN) Current trends in the neuroendocrine regulation of feeding and metabolism in fish: an overview** (**OR-89**)	
16:50-17:30	**CLOSING CEREMONY**	
19:00	**BANQUET**	

OR-77

Estrogens regulate heart rate via the G protein-coupled estrogen receptor

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Exposure to environmental endocrine disruptors can significantly disrupt fish physiology. Previous studies demonstrate how endocrine disruptors affect gonadal development and function, however less is known about how endocrine disruptors affect the development and function of non-gonadal tissues. Here we show that estrogens influence heart rate in zebrafish embryos. Using genetic and pharmacologic approaches, we find that the G protein-coupled estrogen receptor (GPER) is required for estradiol-dependent increase in heart rate, whereas canonical nuclear estrogen receptor signaling is not required. Acute exposure to estrogens increased heart rate in wildtype and in estrogen receptor alpha and beta mutant embryos but not in GPER mutants. Nuclear estrogen receptor signaling remained normal in GPER mutant embryos, however GPER mutant embryos exhibited reduced basal heart rate, while heart rate was normal in nuclear estrogen receptor mutants. Our results demonstrate that estradiol plays a previously unappreciated role in the acute modulation of heart rate during zebrafish development and that GPER functions as an autonomous estrogen receptor to regulate basal heart rate. Our results support the need to evaluate the impact on cardiac function when considering the effects of estrogenic environmental endocrine disruptors on fish physiology.

OR-78

Estrogenic effects of several bisphenol A substitutes in the developing zebrafish brain

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Many studies have demonstrated the endocrine disrupting activity of bisphenol A (BPA) and provide increasing support that environmental BPA exposure can be harmful to humans. For these reasons several regulatory agencies in the world have banned the use of BPA in infant formula bottles. Such restrictions along with increased public concerns have led to increasing use of alternative bisphenols such as BPS and BPF among many. However, little is known about the toxicity and/or endocrine disrupting effects of alternative bisphenols. The present work aimed at defining estrogenic-like activity of several BPA structural analogs, including BPS, BPF, BPAF, and BPAP, on the developing brain of zebrafish larva as an *in vivo* model. We measured the induction level of the estrogen-sensitive marker *cyp19a1b* gene (Aromatase B), expressed in the brain, using different *in situ/in vivo* strategies: RT-qPCR and *in situ* hybridization in wild type larvae, and fluorescence detection in *cyp19a1b*-GFP transgenic larvae. These different experimental approaches demonstrated that BPS, BPF, or BPAF exposure, similarly to BPA, significantly activates the expression of the estrogenic marker in the brain of developing zebrafish. In addition, *in vitro* experiments using both reporter gene assay in a glial cell context and competitive ligand binding assays strongly suggested that up-regulation of *cyp19a1b* is largely mediated by the zebrafish estrogen nuclear receptor alpha (zfERα). Importantly, and in contrast to other tested bisphenol A analogs, the bisphenol AP (BPAP) did not show estrogenic activity in our study.

OR-79

Development of quantitative adverse outcome pathways for aromatase inhibition in fish

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Adverse Outcome Pathways (AOPs) offer a biological pathway-based toxicological framework to support hazard assessment and regulatory decision-making. However, it remains unclear how AOPs, and toxicology data developed in that context, can be used to quantitatively facilitate decision-making. Here, we present several areas for quantitative application and development of the AOP concept as a hypothesis-driven framework including a scoring-based weight of evidence approach, a probabilistic approach, and a mechanistic approach. Mechanistic quantitative AOPs approaches have the highest biological fidelity and incorporate sufficient mechanistic details of the events in an AOP such that compensatory mechanism are accounted for and biological behaviors can be predicted in the absence of training data. The development of increasingly quantitative models from AOPs also enables end users to shape development of increasingly sophisticated and quantitative models depending on the needs of the hazard or risk application. As an example, the AOP for inhibition of aromatase leading to reproductive dysfunction in fish provides a well-characterized pathway with which to highlight essential features of a mechanistic quantitative AOP (qAOP). Here, we show how to build a mechanistic qAOP for endocrine disruption by aromatase inhibition using mathematical and probabilistic models representing different key events within the aromatase inhibition AOP.

OR-80

Extensive regulation of diurnal transcription and metabolism by glucocorticoids in the zebrafish (*Danio rerio*)

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Altered daily patterns of hormone action are suspected to contribute to metabolic disease. We examined diurnal metabolite and transcriptome patterns in a zebrafish glucocorticoid deficiency model. Several metabolic pathways were deregulated, particularly those linked to amino acid metabolism and central carbon metabolism. Constant glucocorticoid treatment normalized changes in most of these pathways, with some notable exceptions. Surprisingly, the constant glucocorticoid treatment rescued a large portion of the deregulated diurnal transcriptome. A simple combination of E-box and glucocorticoid response elements, regulatory elements which are enriched in rescued genes, is sufficient to drive glucocorticoid-dependent circadian reporter gene expression. Our work highlights metabolic pathways which may contribute to disease symptoms in patients with glucocorticoid deficiency and mediate effects of endocrine disruption in the glucocorticoid system. Moreover, we provide mechanistic insight into cooperation of the circadian clock and glucocorticoids in the transcriptional regulation of metabolism.

OR-81

Using larval zebrafish behaviour to assess endocrine disruption

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Zebrafish larval behaviour is an increasingly popular tool used to identify neurotoxic compounds. We have begun to assess larval behaviour for use in the field of endocrine disruption. Using a standard light/dark behavioural assay five days post fertilisation, we have found behavioural responses to two endocrine disruptors, bisphenol A and tetrabromobisphenol A, are dependent on the age of the larvae, and the photo-regime prior to testing. For example, larvae exposed to bisphenol A were hyperactive when maintained under constant darkness, but hypoactive when reared on a day/night cycle. Similarly, the lowest effect concentration was found to be dependent on the age of larvae within a 24-hour period. For TBBPA, we found a 1000 fold difference in the lowest effect concentration depending on methodology. Further experiments using the same assay, demonstrate bisphenol A exposure results in a similar behavioural phenotype to both 17β-estradiol and testosterone exposure. However, the behavioural phenotypes observed following TBBPA exposure are unlikely to be related to thyroid disruption.

OR-82

Addressing the impact of exposure to oestrogenic effluents on fish populations

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Wastewater treatment work (WwTW) effluents comprise a large proportion of the flow of many lowland rivers across the world. Many WwTW effluents are oestrogenic and induce a range of feminised phenotypes in wild male fish. These include the presence of vitellogenin, an egg yolk precursor, in the blood of males and the intersex condition – the presence of developing eggs in the testes of otherwise male fish. While the effects of oestrogen exposure on individuals are relatively well understood, less is known of the impacts of exposure at a population level. We have conducted a series of experiments to address the impact of WwTW effluents on populations of roach (*Rutilus rutilus*), a common cyprinid fish. In a breeding study using wild-caught roach from contaminated UK rivers and using DNA microsatellites to assign parentage, we found most intersex fish were capable of reproduction; however, the reproductive success of the most feminised male roach was reduced by up to 76%. Using DNA microsatellites, we investigated the population genetic structure of roach in southern England. No evidence was found for a reduction in effective population sizes, which relates to the number of breeding individuals. We also identified populations that had been largely restricted to stretches of river with a high proportion of oestrogenic effluent over several multiple generations, i.e. they appear to be self-sustaining despite the oestrogen exposure. This raised the question of whether roach can, and have, adapted to the harmful effects of exposure to oestrogens. Genetic selection results from the poor survival or reproduction in genetically susceptible individuals, so analysing the genetic footprints of selection provides insights on the impact of chemical exposure on fish populations. We have investigated this in wild roach populations using a combination of analysis of genetic variation in populations using single nucleotide polymorphisms (SNPs) and an experiment to investigate evidence for adaptation of roach to oestrogen. We have found evidence of strong selection for variants of the androgen receptor gene and for Cyp1A1, suggesting that roach have adapted to exposure to human derived chemicals in some English rivers. However, as yet, we have found no strong evidence of adaptation specifically to exposure to steroid oestrogens.

OR-83

Changes in zebrafish gut microbiota modulates IGF system and metabolic hormones during larval development

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The gut microbiota has been identified as an environmental factor that may play an important role in host's energy balance and development. In the present research, we evaluated the effects of probiotic *Lactobacillus rhamnosus* administration on gut microbial community composition during early stage of larval development, and its ability to modulate the IGF system and metabolism during different stages of development. Metagenomic results revealed that *L. rhamnosus* administration shifted the overall gut microbiota composition of zebrafish gut larvae and such changes modulated the expression of genes whose products encode for hormones involved in lipid and glucose metabolism, in appetite control and growth. During following stages of larval development, from 8 dpf until 90 dpf, the changed microbiota, significantly up-regulated *igf1*, *igf1ra* and *igf1rb* gene expression, concomitantly with a down regulation of *mstn*, particularly from 8 dpf until 30 dpf and higher body weight. In addition, changed microbiota up-regulated gene expression igf2 and igf2r at all stage analysed. In addition, during zebrafish early larval development from the hatching until 8 dpf, the changes in gut microbiota down-regulated *hnf4α* gene trasncript, whose hormonal product regulate lipid metabolism. Concomitantly, the probiotic treated larvae showed lower content of total body cholesterol and triglyceride. The changes in gut microbiota during early zebrafish larval development, also up-regulated *nucb2a, glp-1* and *insulin* gene transcription, whose hormonal products regulate glucose metabolism. The IHC analysis showed NUCB2a presence in the enterocytes cytoplasm in the first and medium tract of intestine. Such data corroborate with the lower content of glucose found in the probiotic treated larvae. Moreover, the changed microbiota composition up-regulated *lept,* whose hormonal product reduce food intake. These results clearly underline the capability of microbiota to positively affect zebrafish larval development by regulating genes encoding hormones that are involved in lipid and glucose metabolism, appetite control and IGFs system providing higher growth rate during early stage of development.

OR-84

Thyroid hormones key integrating factors of embryonic and post natal development of teleosts

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Thyroid hormones (THs), thyroxine and its active form tri-iodothyronine, constitute a key endocrine axis in vertebrate homeostasis. THs have a well established role in growth and metabolism of adult vertebrates but recently accumulating evidence supports the notion that they constitute essential factors in embryonic and post-natal development of vertebrates. During early development THs are fundamental for correct neural development and failures lead to serious brain and central nervous tissue disorders from teleosts to humans. The THs also play a central role as integrating factors of the diverse signals that underpin appropriate development of the blood-hindbrain barrier (BHB) in zebrafish. The role of THs in development does not stop here and they are obligatory for metamorphosis and the larval to juvenile transition in teleosts and lower vertebrates. The action of THs during metamorphosis is most dramatic in the pleuronectiforms that change from a pelagic symmetric larva to an asymmetric flat benthic juvenile. The most notable feature of this transformation is the migration of one of the eyes to the opposite side of the head generating a flat morphology with both eyes on the same side of the head (ocular). Recent evidence suggests that the hypothalamic regulation of metamorphosis in teleosts might have species-specific characteristics. Taken together the evidence shows that THs have a variety of actions during teleost development and that alterations in its biology and regulation is a key factor in the diversity of teleosts species and their success in colonizing different aquatic environments.

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OR-85

Multifactorial regulation of reproduction and gonadal development in fish

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Fishes are among the most diverse species with respect to sex determination and reproduction. In gonochoristic species such as goldfish, primordial germ cells in the developing gonads differentiate into either ovary or testis and the phenotype remains as male or female for life. Hermaphrodite fishes, however, can change sex from female to male (protogynous), or male to female (protandrous). We observed significant variations in hormonal control of gonadal development and reproduction, depending on sex, species, and environmental conditions. Here we focus on the role of gonadotropin-releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH), and thyroid hormones (T3/T4) in the control of reproduction and gonadal development in different species of fish. GnRH is a key regulator of reproduction, not only as a stimulator of gonadotropin production, but also as a paracrine regulator of gonadal function which interact with GnIH both at the level of pituitary and gonads. We provide evidence that T3/T4 are also involved in the control of reproduction and gonadal development by interacting with hormones of HPG axis in addition to influencing metabolic function and energy balance in order to sustain gonadal development. Reproductive process in fish require significant energy investment, and T4/T3 work in concert with GnIH to regulate shift from somatotropic to gonadotropic phase leading to gonadal recrudescence. Our findings demonstrate that T4/T3 and GnIH in mature fish influence estrogen and estrogen receptor levels in the liver and gonads, leading to changes in the synthesis of vitellogenin which is an important component of ovarian follicular development. Furthermore, GnRH and GnIH, in addition to controlling pituitary gonadotropin production, exert direct actions at the level of ovary and testis to modulate steroidogenesis and apoptosis which is critical to synchronous development of ovary and testis in fish. Our findings provide a support for the hypothesis that GnRH may regulate testicular regression in protandrous hermaphrodite fish via an apoptotic mechanism prior to ovarian development. Overall, GnRH, GnIH and T4/T3 are important components of multifactorial regulation of reproduction and gonadal development in fish, and their action are influenced by gonadal steroids and environmental factors.Funded by grants from NSERC to HRH.

OR-86

Endocrine control of the sexual fate development in the gonad and the activation of the early brain in the protandrous black porgy

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Sex change occurs in a variety of fish, invertebrates and plants. This ability is lost in amphibians, reptiles, birds and mammals. In fishes, hermaphroditism has been documented in many species representing more than 20 taxonomic families in 9 orders. Black porgy, *Acanthopagrus schlegelii*, a marine protandrous hermaphrodite fish, is differentiated at 3-4 mo of age (primary sex determination) and remains functional male for the first 2 yrs of life (primary sex determination), but sexually change to female after third year (secondary sex determination). Testicular tissue and ovarian tissue are separated by connective tissue in the digonic gonad. Black porgy provide a unique model to examine the endocrine control of the gonadal sexual fate and brain development in fish. The annual profiles of plasma estradiol, vitellogenin and 11-ketotestosterone in males were significantly different from those in the 3-yr-old sex changing females. Plasma LH levels and *dmrt1* transcripts in the testis were higher in male fish than sex-changing female. Oral administration with aromatase inhibitors for one year blocked the natural sex change in 3-yr-old black porgy and all fish became functional males. The male-phase maintenance was mediated by the Gnrh-Gth-Dmrt1 axis and knockdown of *dmrt1* in the testes resulted in the testis regression and germ cells loss, and then caused a sex change to female ovary. Removal of testicular tissue induced the activation and development of ovarian tissue and produced a vitellogenic ovary in 1-2-yr-old fish. The testicular portion regulated the ovarian *cyp19a1a* promoter methylation and repressed the activation of ovarian development in 1-2-yr-old black porgy. During the gonadal sex differentiation, the aromatase activity, steroidogenesis, estradiol production, cell proliferation, and neurogenesis were increased in the early brain. Aromatase inhibitor further blocked the cell proliferation in the early brain. The function of these peak activity patterns in the early brain occurred at the time of gonadal sex differentiation remains unclear. Our studies suggest that the process of the primary sex determination is related to the brain *cyp19a1b*-associated signaling, and then the secondary sex determination is switched by the fate of testis through brain-pituitary-gonad axis in black porgy.

OR-87

Dynamic and differential expression of the gonadal aromatase during the process of sexual differentiation in novel transgenic cyp19a1a-GFP zebrafish line

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In most gonochoristic fish species, aromatase, the enzyme responsible for the synthesis of estrogens, has been shown to play a critical role in the process of sexual differentiation (SD). In zebrafish, that undergoes a juvenile intersex stage, there is still a lack of knowledge regarding the precise localization and pattern of expression of gonadal aromatase (Cyp19a1a) impeding the formal characterization of the role of cyp19a1a in this process. To fulfil this gap, the expression of cyp19a1a was analyzed in a novel transgenic zebrafish model expressing GFP under the control of the zebrafish cyp19a1a gene promoter. First, we showed a perfect co-expression of GFP and endogenous Cyp19a1a protein in adult gonads that was localized in the cytoplasm of oogonia, young oocytes and peri-follicular cells of the ovary and in the cytoplasm of Leydig and germ cells in the testis. Then, the spatio-temporal expression pattern of cyp19a1a was studied during SD using GFP fluorescence imaging in gonads between 20 and 40 dpf. GFP was expressed in all undifferentiated gonads of 20 dpf-old zebrafish. Then, GFP expression increased in early differentiated female at 30 and 35 dpf to reach high GFP intensity in well-differentiated ovaries at 40 dpf. On the contrary, males consistently displayed low GFP expression as compared to female whatever their stage of development, resulting in a clear dimorphic expression between both sexes. Interestingly, fish that undergoes ovary-to-testis transition (35 and 40 dpf) presented an intermediary GFP level. Our results suggest that (1) cyp19a1a is expressed early during gonadal development before the histological process of SD, (2) the down-regulation of cyp19a1a expression is critical for the testicular differentiation, (3) although cyp19a1a expression exhibit a clear dimorphic expression in gonads during SD, its expression persists whatever the sex suggesting that estradiol synthesis is important for gonadal development in both sexes. Monitoring the expression of the GFP in control and exposed-fish will help to identify the interest of this model to study the mechanisms of action of endocrine disruptors on SD.

OR-88

ASIP1 knockout perturbs pigment cell development and color pattern formation in fish

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Vertebrate pigment patterns frequently show a clear distinction between a pale ventral area and a darker dorsal area. We have known for some time that in mammals this pattern results from spatially regulated expression of Agouti-signaling protein (ASIP). In fish, little is known about the nature and interaction of the genes controlling this process. So far only Asip1 have been identified that, when over-expressed, can disrupt the dorso-ventral countershading pattern in adult zebrafish. However, 'loss of function' analysis has not yet been reported. Using zebrafish, we show that Asip1 is essential for pigment cell development and dorso-ventral color pattern formation in adult fish. Inactivation of Asip1 function by targeted mutagenesis using the CRISPR-Cas9 system results in the disruption of dorso-ventral pigmentation phenotype in zebrafish. Therefore, we show that this dorso-ventral patterning in zebrafish is also Agouti-signalling protein-dependent and combined with the data from mammals, our data strongly suggests that an Agouti-signalling protein-dependent mechanism regulating dorso-ventral pigment pattern establishment during development is a conserved feature of vertebrates.

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OR-89

Current trends in the neuroendocrine regulation of feeding and metabolism in fish: An overview

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The humble contributions of this speaker to fish endocrinology were initiated during a very fortunate graduate (PhD) studentship under Dr. Richard (Dick) E. Peter, a doyen in fish endocrinology. In the first ever R.E. Peter lecture at the fifth ISFE held at Castellon (Spain) in 2004, Dr. Peter summarized the neuroendocrine regulation of feeding in fish. Since then, especially over the past decade, dedicated excellence of numerous fish endocrinologists led to notable advances in understanding how hormones regulate feeding and metabolism in fish. The focus of this inaugural lecture named after Dr. Yoshitaka Nagahama, yet another doyen in fish endocrinology will be to highlight the recent advances in neuroendocrine regulation of energy balance. The contributions of Unniappan's research lab during the past 8 years will be the core of this plenary lecture. Neuroendocrine regulation of energy homeostasis is achieved by the coordinated actions of multiple, redundant endocrine factors derived from many tissues. The past decade has witnessed the identification of appetite regulatory actions of a large number of already known peptides. In addition, several novel peptides with metabolic functions were also identified. The speaker's laboratory made seminal contributions to better our understanding on ghrelin, ghrelin-*O*-acyl transferase, peptide PYY, nesfatin-1, nesfatin-1 like peptide, xenin and irisin as metabolic peptides in fish. This lecture will summarize advances in discovery research, mechanism of actions, role of processing enzymes, and the species-specific similarities and/or differences in regulatory peptides with endocrine like actions in fish. We will compare how species and tissue specific functions of recently identified metabolic factors are evolutionarily conserved across fishes.

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Posters

PO-01

Dopamine modulates aggressive, submissive and affiliative behaviour of cooperative breeders

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Dopamine is part of the reward system of animals and is involved in the modulation of the social decision network. It is hence important for the regulation of social behaviour. The effect of dopamine on the social behaviour of highly social animals is currently obscure. We studied the role of the dopaminergic system in the cooperatively breeding cichlid *Neolamprologus pulcher* by blocking or stimulating the activity of the dopaminergic receptors D1 and D2. Our results show that the D2 receptors in these fish modulate aggressive, submissive and affiliative behaviour. Interestingly, social context is of primary importance for the particular role of D2 receptors in behavioural regulation. Furthermore, subordinates show a higher dopaminergic activity in the diencephalon and higher dopamine concentrations in the forebrain than control animals, suggesting that family living is perceived as rewarding. These results provide insight into the role of dopamine for the social behaviour of cooperative breeders.

PO-02

Enriched environment affects aggressive behaviour and sexual maturity in Siamese fighting fish

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Enriched environment increases numerous neurochemicals in the animal brain, and may facilitate behavioural change. A technique called “Environmental Enrichment” has been gradually applied in zoos and aquariums as a new aspect of animal welfare. It is known that teleost fish can respond to environmental manipulation as well. Siamese fighting fish *Betta splendens* is solitary and highly territorial, and display fierce stereotyped aggressive behaviour toward other fish. Thus, adult fish should be kept isolated in captivity. We found that complexed and enriched rearing environment reduced their aggression and allowed adults to be kept in a group. *B. splendens* which hatched in our laboratory had been raised in a group under enriched environment. At the age of four months, some individuals were moved to poor environment and kept isolated. Then, all the fish was kept until at the age of six months, either in enriched environment (EE) or in poor environment (PE). To evaluate their aggressiveness, mirror image test was conducted at two, four, and six months of their age. After the mirror image test, the fish were deeply anesthetized, and the measurements of body parameters and the collection of blood samples were conducted. Regardless of sex, PE fish showed longer duration of aggressive display to self-image and higher plasma cortisol concentration after mirror image test than EE fish. However, plasma concentrations of 11-ketotestosterone in EE males were significantly lower than PE males, showing the same level as four months old males. The gonadosomatic index of EE females was lower than PE females, showing the same level as four months old females. These results suggested that inhibition of the development of aggressiveness also inhibit the normal sexual maturation in *B. splendens*.

PO-03

Photoreceptor gene expression during the juvenile development in red spotted grouper *Epinephelus akaara*

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Photoreceptors in fish are the membrane receptors that receive the light and transmit the light-induced signals to the brain regions to make an image of the outside world. They include rods and cones. Rod photorecetpors recognize the light and darkness, while cone photoreceptors respond to different wavelengths of light depending on their subtypes. To help understand the juvenile behaviors of red spotted grouper (*Epinephelus akaara*), we cloned their photoreceptors here and examined their expression during the juvenile development. Using TA cloning method, we successfully cloned SWS2 (blue opsin), MWS (green opsin), LWL (red opsin) and Rhodopsin in this fish species. Then, their expression levels were determined by RT-PCR in various adult tissues including forebrain, midbrain, hindbrain, retina, kidney, heart, intestine, muscle, gonad and gill. We found that all photoreceptor genes were strongly expressed in the retina. To examine their expression during the eye development, we isolated tissue samples at different developmental stages (lens and ear vesicle formation stage, 1, 2, 3, 5, 6, 10, 21, 50 dah) and determined their expression level by real-time RT-PCR. SWS2, a cone photoreceptor, began to be expressed from lens and ear vesicle formation stage and its expression gradually increased until 10 dah. The expression of LWS was first observed from 3 dah and their expression decreased thereafter. In case of MWS, its expression was detected 3 dah and reached the highest level at 21 dah. Rhodopsin, a rod photoreceptor, was found to be expressed from 2 dah and its expression reached the highest level at 50 dah. The outer (ONL) and inner nuclear layer (INL) began to differentiate at 2 dah, while choroid first appeared at 4 dah so that the eyes became black. In addition, ganglion cell layer was observed only at 50 dah. Taken together, these results indicate that the development of retina mostly completes around 50 dah.

PO-04

Egg cannibalism vs loving parent: which hormones initiate the behaviour shift?

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The parental care strategy of a species is highly dependent on its mating system. Teleost fish exhibit an enormous and diverse set of mating systems. Cichlid fish have a wide range of parental care types, going from no parental care to mouthbrooding until cooperative breeding. The two congeners *Neolamprologus caudopunctatus* and *Neolamprologus pulcher* are very similar in their ecological requirements, reproductive behaviour and morphology but show different parental care types: in *N. caudopunctatus* both parents share the investment in parental care, while *N. pulcher* is a cooperative breeder where both kin and non-kin helpers engage in alloparental care. Nevertheless egg cannibalism is ubiquitous in both species, and therefore the inhibition of egg cannibalism is essential to become a successful parent. Oxytocin and prolactin initiate and regulate parental care behaviours in mammals. In fish, Isotocin, non-mammalian homolog of oxytocin, enhances parental care behaviour. In contrast, prolactin in fish has a highly species-sex-specific effect, hence a very pleiotropic influence. Furthermore, in the case of egg cannibalism suppression, ghrelin, a hypothalamic hormone that enhances food intake, is known to exhibit low levels during spawning and increases after spawning. The aims of this study are: 1) Identify the point of switching from egg cannibalism to loving parent and, 2) Which hormones are important mediators of this behavioural turnaround. We will sample at three different points during reproduction: i) at pair formation, ii) egg laying and iii) during parental care, and compare it to non-reproducing fish. We will sample the brain, pituitary gland and blood, which will be used for hormonal quantification. Additionally we will collect behavioural information on parental care and pair relationship. We predict that spawning is the turning point between egg cannibalism and caring parent. Further we hypothesize that both isotocin and prolactin will increase from pair formation onwards until clutch maintenance and that we will find behavioural and hormonal differences between the sexes.

PO-05

Involvement of hormones in olfactory imprinting and homing in chum salmon

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The olfactory hypothesis for salmon homing to their natal stream was proposed in the 1950s, and the mechanisms of olfactory imprinting and homing in salmon have been intensively researched. However, until now, it has been impossible to link hormonal control mechanisms to imprinting and homing because we lacked molecular markers that would permit the evaluation of olfactory memory formation and retrieval in the salmon brain. In brains of hatchery-reared underyearling juvenile chum salmon (*Oncorhynchus keta*), thyrotropin-releasing hormone gene expression increased immediately after release from a hatchery into the natal stream, and the expression of the essential NR1 subunit of the N-methyl-D-aspartate receptor increased during downstream migration. The olfactory discrimination ability of stream odors, measured by electro-olfactogram (EOG), showed significantly greater discrimination ability for the natal stream water in juveniles when they encounter different streams during downstream migration. Thyroid hormone treatment in juveniles enhanced NR1 gene activation. These data suggest that during the initiation of the downstream migration of juvenile chum salmon, the environmental changes involved in the release into the river may induce the expression of the brain-pituitary-thyroid hormones, which then stimulate the upregulation of NR1, enhancing the olfactory memory formation capability. Gene expression of salmon gonadotropin-releasing hormone (sGnRH) and NR1 increased in the adult chum salmon brain during homing from the Bering Sea to the natal hatchery. Adult chum salmon collected near the natal hatchery showed significantly higher EOG responses to their natal stream water than to non-natal stream water. GnRHa treatment in adults improved stream odor discrimination. These data on adult homing migration suggest that the activation of sGnRH may cause both the onset of homing migration and upregulation of NR1, facilitating olfactory memory retrieval as well as natal stream odor discrimination during upstream migration. The gene expression levels of NR1 in the brain are a useful molecular marker to clarify the olfactory imprinting and homing abilities of both juvenile and adult chum salmon. Further biochemical and molecular biological studies investigating the protein levels and the gene expression profiles of NR1 and NR2A-D with treatments of NMDAR antagonist will be able to reveal olfactory long-term potentiation in salmon brain.

PO-06

An oxytocin receptor antagonist inhibits social preference in zebrafish

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The zebrafish is a fairly novel model system that is particularly suitable for psychiatric research due to the developed behavioural tests, ease of pharmacological treatment, and the available mutated lines of genes involved in human syndromes and disorders. Due to their strong tendency to socialise in shoals, zebrafish serves as a good model to study social behaviour. Although the neuropeptide oxytocin is well-known to be crucial for social behaviours in mammals the role of isotocin, the fish homolog of oxytocin, for shoaling behaviour in zebrafish is as yet not clarified. Aim: to investigate if an oxytocin receptor antagonist inhibits sociability in adult zebrafish. Methods: adult male and female zebrafish were intraperitoneally injected with either the non-peptidergic oxytocin receptor antagonist L-368,899 or vehicle, and subsequently tested in a social preference paradigm. Results: zebrafish treated with the oxytocin receptor antagonist displayed a tendency to decreased social preference compared to fish injected with vehicle. Conclusions: these results indicate that endogenous isotocin may be involved in social preference in zebrafish, and show promise for future explorations of the network underlying social behaviour in the zebrafish.

PO-07

Effect of ICV administration of sulfated cholecystokinin octapeptide on psychomotor activity in goldfish

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Sulfated cholecystokinin octapeptide (CCK-8s) regulates appetite and satiety as an anorexigenic factor in vertebrates. In rodents, intracerebroventricular (ICV) administration of CCK-8s has also been shown to affect locomotor activity. However, as there is still no information regarding the psychophysiological effects of CCK-8s in goldfish, we investigated the effect of CCK-8s on psychomotor activity in this species. ICV administration of synthetic CCK-8s at 1, 2.5 and 5 pmol/g body weight (BW) enhanced locomotor activity. Since intact goldfish prefer a black to a white background area, or the lower to the upper area of a tank, we used two types of preference test (black/white and upper/lower preference tests) for measuring anxiety-like behavior in goldfish. ICV administration of CCK-8s at 5 pmol/g BW shortened the time spent in the white background area, and prolonged the time taken to move from the lower to the upper area. This action of CCK-8s mimicked that of the central-type benzodiazepine receptor inverse agonist, FG-7142 (an anxiogenic agent), at 4 pmol/g BW. The anxiogenic-like effect of CCK-8s was abolished by treatment with the CCK receptor antagonist, proglumide, at 50 pmol/g BW. These results indicate that CCK-8s potently affects psychomotor activity, and that CCK-8s exerts an anxiogenic-like effect via the CCK receptor-signaling pathway in goldfish.

PO-08

Endocrine and olfactory regulation of sexual behavior in goldfish: sexual bipotentiality of the brain of goldfish

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Sexual behavior of goldfish, Carassius auratus, is regulated by hormones and pheromones. Sexually mature male goldfish producing testicular androgen perform sexual behavior stimulated by sex pheromones released from females. Sexual behavior in females is triggered by ovarian prostaglandin (PG) and ovarian estrogen is not required for the female behavior. Olfaction is also an important factor for the occurrence of sexual behavior in goldfish. Male goldfish possess a stimulatory olfactory regulation for sexual behavior, and female have an inhibitory regulation. In males, sexual behavior is not elicited by olfactory blockage. Nasal occlusion (NO) blocks the reception of pheromone, and olfactory tract section (OTX) blocks the transmission of pheromonal information to the telencephalon. In females, NO suppresses the sexual behavior even under the stimulation by PG. However, unlike males, PG-injected females perform sexual behavior after OTX, which indicates that NO causes inhibition of the behavior and OTX removes this inhibition. It is known that brain sex differentiation occurs in mammals, and they do not normally perform heterotypical sexual behavior. However, in goldfish heterotypical sexual behavior can be induced by hormonal treatments in sexually mature fish. Androgen treatment induces male-typical behavior in females and PG treatment triggers female-typical behavior in males. These facts led us to propose a hypothesis that unlike mammals, brain sex differentiation does not occur in goldfish, and the brain is sexually bipotential which can regulate both male- and female-typical sexual behavior. Recently, we found out that both male and female goldfish have the stimulatory and the inhibitory olfactory regulation systems. Male-typical behavior of androgen-treated females was suppressed by nasal occlusion or OTX. Female-typical behavior in PG-injected males was suppressed by nasal occlusion but resumed by OTX. These results neuroanatomically indicate that both male and female possess the bisexual brain system for olfactory processing for the regulation of sexual behavior.

PO-09

How do individuals cope with stress? A neuroendocrine substrate for proactive and reactive coping styles in fish

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Despite the use of fish models to study human mental disorders and dysfunctions, knowledge of regional telencephalic responses in non-mammalian vertebrates expressing alternate stress coping styles is poor. Since perception of salient stimuli associated with stress coping in mammals is mainly under forebrain limbic control, we tested region-specific forebrain neural (*i.e.* mRNA abundance and monoamine neurochemistry) and endocrine responses at basal and acute stress conditions for previously characterized proactive and reactive Atlantic salmon. Reactive fish show a higher degree of neural plasticity under basal conditions in Dl (proposed hippocampus homologue) and higher post-stress plasma cortisol levels. Proactive fish displayed post-stress higher serotonergic signalling in Dm (proposed amygdala homologue) and increased neural plasticity in both Dl and Vv (lateral septum homologue), in line with active coping neuro-profiles reported in the mammalian literature. We present novel evidence of proposed functional equivalences in the fish forebrain with mammalian limbic structures.

PO-10

New contributions on distribution and localization of the neurons of arginine-vasotocin in *Steindachneridion parahybae* (Siluriformes: Pimelodidae).

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The key component regulating fish reproduction is the endocrine system, primarily through the brain-pituitary-gonad axis. This axis synthesizes and releases gonadotropin-releasing hormones, follicle-stimulating hormone, luteinizing hormone and gonadal steroid hormones, besides neurohormones that modulators of the reproductive process. Attempting to understand the endocrine regulation of fish reproduction, several groups of neuropeptides, via interactions with other neurotransmitter or neurohormones system have been investigated. Arginine-vasotocin (AVT) is an important hypothalamic neurohormone with functions in various aspects of social behaviour and physiology of teleosts, especially in events related to modulate several processes in reproductive physiology, including both aggressive and reproductive behaviours. *Steindachneridion parahybae* is a freshwater catfish endemic to the Paraiba do Sul River Basin, Brazil, classified as an endangered Neotropical species. Therefore, our studies focused on identify the hypothalamic nuclei responsible for the production and release of AVT in *S. parahybae*, highlighting to the reproduction behaviour in captivity. AVT neurons were visualized by immunohistochemistry. Three main hypothalamic nuclei responsible for the production of AVT were identified in the preoptic area in the *S. parahybae* brain: parvocellular (pPOA), magnocellular (mPOA) and gigantocellular (gPOA) nucleus. AVT cell bodies located in the pPOA area were the most rostral, ventral and numerous cells, round or oval in shape and presented small cellular and eccentric nuclear area than others cell subpopulations. The mPOA neurons extended more dorsal and posteriorly from the pPOA, and they were also numerous and with round or pyriform in shape, with larger cellular and eccentric nuclear area than pPOA cells. Finally, gPOA cell subpopulation were identified at more dorsal and posterior positions. These cells were characterized with irregular in shape, largest cellular area within POA area, and presented spherical and eccentric nuclear than pPOA and mPOA cells. Additionally, AVT fibers were observed in the pituitary gland, especially at the proximal pars distalis and pars intermedia. Our results contribute for a better reproductive performance in captivity, since their endangered status deserves special attention and urgent action for contribution to the knowledge of reproductive physiology, which is the basic premise for the program of fish restocking in the Paraíba do Sul River Basin.

PO-11

The pineal gland regains its role as a central circadian clock organ

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The pineal gland has been considered the master circadian clock organ in fish. However, this dogma has been challenged by the finding that most zebrafish tissues contain molecular clocks that are directly reset by light, pointing to a de-centralized circadian system in this species, thereby questioning the role of the pineal gland as a master clock organ.
To further examine the role of the pineal gland oscillator in the zebrafish circadian system, we generated a transgenic line in which the molecular clock in the melatonin-producing cells of the pineal gland is selectively blocked, by over expression of a dominant-negative form of the CLOCK protein. As a result, clock-controlled rhythms of melatonin production and gene expression in the pineal gland are disrupted. Importantly, circadian rhythms of locomotor activity under constant darkness are also disrupted, whereas peripheral molecular oscillations are sustained. These findings provide evidence that the organization of the zebrafish circadian system is composed of both independent peripheral components and a hierarchical organization in which the pineal gland serves as a master circadian clock that regulates rhythmic behavior, partially mediated by the rhythmic secretion of melatonin. The genetic approach used here helped to define the organization of the circadian system in zebrafish and to re-establish the pineal gland as a central circadian clock organ.

PO-12

Elevated plasma cortisol levels and decreased intestinal barrier function in Atlantic cod (*Gadus morhua*) reared in brackish water – result of acclimation to low salinity or chronic stress?

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The intestinal barrier consists of a single epithelial cell layer, lining the gut lumen and serves two essential and critical functions. It is responsible for absorption of nutrients, electrolytes, and water from the intestinal lumen. But it also serves as a barrier to harmful substances in the intestinal lumen such as foreign antigens, pathogens, and other toxins. Thus, the intestine performs partly opposing tasks that demand epithelial transfer selectivity. This is achieved through both trans- and paracellular pathways. Paracellular permeability is controlled by the cell–to-cell junctions, of which the tight junctions (TJ) are most important. TJs can be affected by a range of factors including stress and environmental salinity. Seawater (SW) epithelia are considered to have higher paracellular permeability than fresh water (FW) epithelia. However, in several studies on the salmonid intestine (both rainbow trout and Atlantic salmon), the revers have been demonstrated, so that the paracellular pathway becomes tighter after acclimation to seawater (SW). The increased intestinal integrity in SW indicates a strengthening of the intestinal barrier. This may be a protection towards the increased exposure of the intestinal epithelium to environmental pathogens and other harmful agents when the fish start drinking after SW entry. It is currently not known if this phenomenon is unique to the anadromous salmonids and/or if the permeability of the intestinal barrier is related to environmental salinity also in more stenohaline fish. The present study aimed at elucidating the effect of environmental salinity on the intestinal barrier function of Atlantic cod (*Gadus morhua*). Atlantic cod were maintained in duplicate tanks at 12, 22 and 34 ppt salinity for 3 months. Thereafter, intestinal barrier function was assessed *in vitro* using an Ussing chamber set up and blood plasma was analyzed for cortisol content. The transepithelial electrical resistance (TER) was significantly lower in brackish water (12 ppt) compared to 34 ppt, suggesting tighter epithelium at the highest salinity. The diffusion rate of ^{14}C-mannitol tended to support this result. Plasma cortisol levels showed high individual variation especially in the 12 ppt group, indicating that individual fish were stressed when reared in brackish water. However, no significant differences between groups could be observed. The current results underpin the hypothesis that a tighter intestinal barrier is correlated to higher environmental salinity indicating a stronger barrier against ingested water.

PO-13

Expression and localization of relaxin related genes in the brain of threespine stickleback (*Gasterosteus aculeatus*)

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Relaxins are hormones that are widely recognized as regulators of pregnancy and childbirth in mammals. Recent mammalian studies demonstrated that relaxins also possess potent osmoregulatory actions. Genome database searches in mammals and non-mammalian vertebrates including fishes have revealed the presence of multiple members of the relaxin family. However, the functions of relaxins in non-mammalian vertebrates are yet unclear. In order to gain insight into teleost relaxins, the localization and expression of relaxin related genes were examined. Tissue distribution analysis showed that relaxin (*rln*) and its receptor (*rxfp*) transcripts were especially abundant in the brain. Since the stickleback brain atlas is currently under construction, relaxin gene localization in stickleback was estimated using the Japanese eel model. *In situ* hybridization analysis showed that *rln3a* and *rln3b* transcripts were localized in the corresponding regions of the nucleus of the lateral lemniscus of midbrain and the griseum centrale of the hindbrain in this species. Localizations of stickleback *rln3* transcripts were similar to those in eel *rln3*. This also seems to be the cese for rainbow trout. These data suggest that the sites of expression of *rln3* genes in the brain are conserved among teleosts. Microarray analysis to compare gene expression profiles in the brain between marine and stream stickleback ecotypes revealed that the transcript levels of *rln*, *rln3b* and *rxfp1-4* were significantly higher in the marine ecotype than in the stream ecotype. Genome scan analysis showed that there are slight differences in F_{ST} between marine and stream ecotypes at the genomic regions where relaxin and receptor genes are located. However, they are not located in genomic islands of high differentiation. Thus, it is likely that there is no recent divergent selection on relaxin related genes between ecotypes. These results suggest that the differences of relaxin gene expression between marine and stream ecotypes are unlikely due to coding mutations at these genes.

PO-14

The integrative role of serotonin and melatonin in ion transport in fish

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The role of serotonin and melatonin in ion transport has not yet adequately addressed in fishes besides the extensive studies on the role of classic hormones such as cortisol and thyroid hormones in ionoregulation. On the other hand, as transporters that provide the driving force for many other transport systems, Na^+/K^+-ATPase and H^+ ATPase are vital for Na^+/K^+ and H^+ homeostasis. It is hypothesized that serotonin and melatonin can drive the functions of these transporters so as to modify the cellular and extracellular Na^+/K^+ and H^+ gradients. Serotonin (5-hydroxytryptamine; 5-HT), a monoamine neurotransmitter synthesized in serotonergic neurons of CNS and enterochromaffin cells of gastrointestinal tract and melatonin, a pineal hormone have been implicated in the ionoregulation in fish. The responses of 5-HT and melatonin to hypoxia and net confinement and its effects on ionoregulation were examined in an air-breathing fish. Analyses of pattern of ionoregulation in these experimental fish indicate that 5-HT and melatonin have direct control on ion transporter functions. In addition, 5-HT and melatonin can interact with cortisol and thyroid hormones and this further suggest the interactions of these hormones in our fish. Overall, our data provide evidence that serotonin and melatonin can modify the cortisol and thyroid hormone release while integrating the ion transport capacity of our fish model.

PO-15

Neurohypophysial homologs convergent mediate homeostasis in cuttlefish embryos

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Cephalopods were proved to process epithelial acid-base regulation. The identification and characterization of those key acid-base transporters demonstrated that the molluscan pH regulatory machinery shows many evolutionary conserved features to those found in vertebrate and mammalian systems. However, its functional basis of how intracellular pH modulation correlate with hormone signalling is poorly understood in cephalopods so far. In this study, we used cuttlefish *Sepia pharaonis* embryos to examine expressions of neurohypophysial hormones (pro-sepiatocin and sepiatpcin) and respective receptor (sepiatocin receptor, str) under CO_2-induced acidic perturbation. RNA *in situ* hybridization images appeared that, on one hand, pro-sepiatocin and sepiatocin were both expressed in optic lobe neurons. On the other hands, str was found to be expressed in embryonic epithelium, the dominant sites for acid-base regulation. Moreover in CO_2-acidified condition, pro-sepiatocin and str were up-regulated accompanied with those acid-base regulation genes in epithelium (e.g. *vha*, *nbc*, *nhe3*, *rhp* and *nka*). The present work inferred that the activated features of neurohypophysial hormone signalling would be beneficial to operate epidermal ion fluxes in cuttlefish; accordingly, in order to cope with acid-base disturbances during their oviparous development, cephalopod embryos have evolved convergent endocrinal pathway regulating intact homeostasis. Comparative studies using a range of marine invertebrates will create a novel and exciting research direction addressing the evolution of pH regulatory and excretory systems.

PO-16

Hormonal changes over the spawning cycle in the female three-spined stickleback, *Gasterosteus aculeatus*

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Female three-spined sticklebacks are batch spawners laying eggs in a nest built by the male. While seasonal endocrine changes in the brain-pituitary-gonad axis have been extensively studied in this species, hormonal changes over the spawning cycle *per se* have not. We sampled female sticklebacks over different time points before (ready to spawn) and 0.25, 1, 2 and 3 days after spawning with a male. Almost all females had ovulated eggs again at Day 3 after spawning. At sampling, plasma, brain and pituitaries were collected, and the ovary weight measured for gonadosomatic index (GSI) determination. Circulating sex steroid hormones [testosterone (T), estradiol (E2), 17,20β-dihydroxypregn-4-en-3-one (17,20β-P) and 17,20β,21-trihydroxypregn-4-en-3-one (17,20β,21-P)] were measured by RIA. Moreover, the mRNA levels of follicle-stimulating hormone (*fsh-β*) and luteinizing hormone (*lh-β*) in the pituitary, and of the gonadotropin-releasing hormones (GnRHs: *gnrh2*, *gnrh3*) and kisspeptin (*kiss2*) and its G protein-coupled receptor (*gpr54*) in the brain were measured by real-time qPCR. GSI peaked in ready to spawn females, dropped to a minimum immediately after spawning and then again increased progressively from Day 0.25 to Day 3. Plasma T levels showed peaks at Day 1 and 2 while significantly decreased at Day 3. E2 levels increased a little earlier than T (at Day 0.25) and remained at high levels up to Day 2. There was a strong positive correlation between T and E2 levels over the spawning cycle. Both progestogens were non-detectable in most samples, though the detectable 17,20β-P values were found at Day 2. Pituitary *lh-β* mRNA levels showed a peak at Day 2, while *fsh-β* did not change. The neuropeptides and *gpr54* in the brains did not show any changes over the spawning cycle.

PO-17

Hormones and environmental involvement in growth and sex differentiation in eels, *Anguilla anguilla*

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Based on the results of hormone and gene transcriptions, the secretion and treatment controlling both the somatic axis and gonadotropic axis were affected by the environment parameter, directly or indirectly, by hormones that were studied in my laboratory. A model was proposed for sex differentiation and gonadal development correlating to the growth of European eel (*Anguilla Anguilla*). A high growth variation is affected by the environment. At a low density of eels, the gonadotropin-releasing hormone (GnRH) affected the secretion follicle stimulation hormone (FSH) in the pituitary, steroidogenesis and aromatase (CYP19) synthesis, and the 17β – estradiol (E2) from 11-ketotestosterone (Kt-11) causing ovary development. The ovary secretion E2 affecting the adenylate cyclase-activating polypeptide (PACAP), growth hormone (GH) and the insulin-like growth factor (IGF) stimulated rapid growth in females. On the other hand, a high density of eels caused the pituitary gland to secrete FSH at a lower level and CYP19 was not synthesized in the gonads. The secretion of Kt-11 affected differentiation to testis, which inhibits the somatic axis in reducing growth rate.

PO-18

Study of the regulation of follicle-stimulating hormone (Fsh) expression and it's role during puberty in Salmo salar

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The Brain-Pituitary-Gonad axis regulates reproduction integrating hormonal messaging from internal and environmental stimuli. Luteinizing hormone (Lh) and follicle-stimulating hormone (Fsh) produced by the pituitary gland are main hormones involved in reproduction. Available knowledge suggests that in mammals, both Lh and Fsh, produced by the same cell type, are required to trigger the entry into puberty. In fish, where Lh and Fsh are produced by two distinct cell types, evidence suggests a major role of Fsh in the regulation of this developmental process. However, knowledge about the regulation of Fsh synthesis and secretion is still fragmentary. The aim of this project is to identify possible regulators of pubertal initiation studying and comparing the expression of candidate receptors in Fsh-producing cells before and after puberty. We used Atlantic salmon (*Salmo salar)* as a model. An anadromous fish with a huge economical interest, where environmental factors play important roles in regulating reproduction. However, among the male population, some called dwarf or precocious males, mature during the first years in river without migrating to the sea. First, immature and mature dwarf males were identified and separated based on gonado-somatic index (GSI) and histology of gonadal tissue. Then, we performed qPCR analysis on whole pituitaries for genes encoding candidate receptors such as those of melatonin, dopamine, Gnrh and Kiss. The results revealed presence of several isoforms from each receptor group in the pituitary. When comparing the expression of those receptors between the developmental stages, we observed differential levels of expression for some of them, suggesting an important role in puberty regulation. The preliminary study give us useful information about possible candidates of puberty regulation, the following step of this project is to study in detail the regulation of gonadotrope cells with *in situ* hybridization to identify the receptors expressed in Fsh- vs Lh-producing cells at different stages of pubertal development.

PO-19

Phosphatidylinositol 3,4,5-trisphosphate signal transduction differentially modulates stimulated and basal LH and GH secretion in goldfish.

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Receptor-mediated activation of phosphoinositide 3-kinases (PI3Ks) generates phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) which interacts with pleckstrin homology (PH) binding domains to facilitate the recruitment of downstream signalling molecules. We have demonstrated that class I PI3Ks mediate the LH and GH secretion responses to the endogenous gonadotropin-releasing hormones, GnRH2 and GnRH3, in goldfish. In this study, we investigated the involvement of pleckstrin homology (PH) domain-dependent signalling downstream of PI3K activation during hormone release responses in primary cultures of dispersed goldfish pituitary cells. Results from Western blotting of dispersed goldfish pituitary cells extracts using antibodies against mammalian pan- and phosphorylated Akt (protein kinase B), as well as pan- and phosphorlated phosphoinositide-dependent protein kinase 1 (PDK1), indicated the presence of these canonical PtdIns(3,4,5)P_3–sensitive downstream signalling proteins in the goldfish pituitary. Imaging flow cytometry further verified the expression of Akt-immunoreactivity in identified goldfish gonadotropes and somatotropes. In cell column perifusion experiments, antagonizing PH domain interactions using a small molecule mimetic of PtdIns(3,4,5)P_3 inhibited GnRH2- and GnRH3-induced LH and GH responses, and elevated basal LH and GH secretion. Application of an inhibitor of Akt elevated basal LH and GH release, as well as GnRH3-induced GH secretion, while inhibition of PDK1 only enhanced basal GH secretion. In contrast, blockade of Bruton's tyrosine kinase (BTK), another known PtdIns(3,4,5)P_3- interacting target, reduced GnRH2-stimulated GH release, as well as basal LH and GH levels. When taken together, these observations indicate that PtdIns(3,4,5)P_3-sensitive transduction components differentially participate in the integrated control of basal and GnRH-stimulated hormone release in a cell- and agonist-specific manner.

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PO-20

Identification of three glycoprotein hormones from the pituitary gland of cloudy catshark, *Scyliorhinus torazame*

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Cartilaginous fishes are ancient groups of jawed-vertebrates, and they have diversified reproductive patterns such as oviparity, ovoviviparity and viviparity. Nevertheless, our knowledge on the endocrine controls of their reproductive strategies has been poorly understood. To clarify the reproductive endocrine systems of this animal group, we have identified three glycoprotein hormones (GPHs) from the pituitary of cloudy catshark (*Scyliorhinus torazame*). We also have examined the cellular localization and possible function of shark GPHs in this oviparous species. Based on the transcriptome analysis of the hypothalamus-pituitary organs, we identified one common GPH α subunit and three GPH β subunits, luteinizing hormone (LH) β, follicle-stimulating hormone (FSH) β and thyroid-stimulating hormone (TSH) β subunits. The shark LHβ/FSHβ/TSHβ had possible N-glycosylation sites and 12 cysteine residues that were exactly conserved at homologous positions to three kinds of other gnathostome GPHβ subunits. The three GPHβs showed 58-82% sequence identity with those of sturgeon and lungfish. The histological observations revealed that shark adenohypophysis was divided into three major parts, rostral and proximal pars distalis and pars intermedia. In addition, there was an additional part, the ventral lobe, beneath the proximal pars distalis and pars intermedia. The ventral lobe was attached to the cartilaginous palate that divides their brain from oral cavity. Our *in situ* hybridization and immunohistochemical analysis revealed that the three GPHs were synthesized specifically in the ventral lobe of the pituitary gland. In early vitellogenic stages, the mRNA levels of FSHβ were significantly higher than those of LHβ and TSHβ. It is suggesting that shark FSH has crucial functions in the formation of egg vitellogenin through sex steroids, as other gnathostome FSH has. Furthermore, in mature cloudy catshark which spawn periodically every 30 days, the mRNA levels of two GTHβs (LHβ/FSHβ) increased with the formation of eggshell, whereas those levels were quite low before eggshell formation. These results suggest that shark GTHs have significant actions on the eggshell gland to induce periodical eggshell formation.

PO-21

Cortisol directly affects Atlantic cod (*Gadus morhua*) pituitary reproductive function in a seasonally dependent manner

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Stress, a state in which there is a perceived threat to an organism's homeostasis, can affect reproduction in vertebrates, including teleost fish. Part of the stress response involves increasing concentrations of circulating cortisol (F), which has been demonstrated to affect all levels of the brain-pituitary-gonad axis. The effects, however, can be contradictory between studies, indicating that factors such as dose, duration, species, gender and maturational stage influence the F effect. We have previously demonstrated that Atlantic cod gonadotropes express two versions of Gnrh receptors (*gnrhr1b* and *gnrhr2a*), with Gnrhr2a likely being the main regulator of gonadotrope reproductive function. Gnrhr1b may also have a role in regulation of growth hormone synthesis. It is not known if F affects pituitary gonadotropin regulation in cod, and the aim of the current study was therefore to investigate direct effects of F exposure on cod pituitary. To explore potential effects of maturational status, experiments were conducted at different times of the reproductive cycle. Pituitary primary cultures were treated with either base line (10 ng/ml) or stress level (100 ng/ml) F for 72 hours, and cell viability (metabolic activity and membrane integrity) and gene expression levels (*fshb*, *lhb*, *gnrhr1b* and *gnrhr2a*) recorded. The data show that F stimulated cell metabolic activity, except in cultures prepared from spent and early recrudescent fish. Membrane integrity was stimulated by F just prior to the spawning period, but was not affected in the rest of reproductive cycle. The effect on gene expression levels depended both on target gene and on maturational status; *fshb* expression was stimulated in early spawning fish, but inhibited in spent fish, while *lhb* transcript levels were stimulated just prior to spawning, but unaffected for the rest of the reproductive cycle. *gnrhr2a* levels were generally increased after F treatment, whereas *gnrhr1b* expression was down-regulated in spent and early recrudescent fish, but remained at control levels in cultures prepared at other maturational stages. In conclusion, F has the potential to directly affect both cell viability and reproductive gene transcription in Atlantic cod pituitary, suggesting stress as a potent regulator of reproductive status also in this species.

PO-22

Development of ELISAs for FSH and LH and their application to a study of reproduction in greater amberjack, *Seriola dumerili*

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Gonadotropins (GtHs; follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) play central roles in the regulation of vertebrate reproduction. However, little is known about the physiological mechanisms by which GtH regulates gonadal development in fish, because a suitable method for measuring FSH and LH has been established in only a few species. In this study, we developed competitive enzyme-linked immunosorbent assays (ELISAs) for measuring FSH and LH in greater amberjack, *Seriola dumerili*, which is a major aquaculture species worldwide, and characterized seasonal changes in plasma FSH and LH in both sexes. We also studied the secretion of FSH and LH by gonadotropin-releasing hormone analogue (GnRHa) implant in female fish. Recombinant GtHs consisting of greater amberjack FSHβ (or LHβ) and glycoprotein α (GPα), were used as standards for the ELISAs, while recombinant chimeric GtHs, consisting of greater amberjack FSHβ (or LHβ) with rabbit GPα, were used to produce the antisera. The validation study showed that the ELISAs were precise (intra- and inter-assay coefficient of variation, <10%) and sensitive (detection limit, <0.8 ng/ml) with mutual low cross-reactivity. In females, plasma FSH significantly increased during the post-spawning period, while plasma LH were high during the spawning period. In males, levels of plasma FSH were high at the onset and completion of testicular development and decreased during the post-spawning period, while plasma LH showed no significant changes. Plasma FSH levels were not affected by GnRHa implantation, while plasma LH increased significantly. The present results suggest the differential roles of the two GtHs in gametogenesis of greater amberjack. In addition, the gonadal development may be regulated differently by GtHs between females and males. In females, secretion of LH but not FSH may be controlled by the GnRH stimulus.

PO-23

Molecular characterization and quantification of the gonadotropin receptors FSH-R and LH-R from turbot (*Scophthalmus maximus*)

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In order to elucidate regulatory mechanisms during final oocyte maturation and spawning, full-length sequences coding for the receptors for follicle-stimulating hormone (*fshr*) and luteinizing hormone (*lhr*) were isolated from female turbot (*Scophthalmus maximus*) by homology cloning and a strategy based on rapid amplification of cDNA end-polymerase chain reaction. The nucleotide and amino acid sequences showed high homologies with the corresponding genes of other teleosts and significant homology with that of *Hippoglossus hippoglossus*. Both the *fshr* and *lhr* are composed of a typical structural architecture of glycoprotein hormone receptors consisting of the large N-terminal extracellular domain, seven transmembrane domains and a short C-terminal intracellular domain. The mRNA levels of *fshr* and *lhr* were found to be abundant in the ovary, but deficient in extra-ovarian tissues. Meanwhile, *fshr* and *lhr* mRNA were found to increase gradually from pre-vitellogenesis to migratory nucleus stages, with the highest values observed in late vitellogenesis and migratory nucleus stage during reproductive cycle, respectively. However, *fshr* and *lhr* mRNA were found to decrease dramatically during the atresia stage. Meanwhile, functional analysis with HEK293T cells continual expressing *fshr* demonstrated that *fshr* was specifically stimulated by ovine FSH, but not ovine LH. These results suggested that *fshr* is mainly involved in the stimulation of vitellogenesis and regulation of oocyte maturation via specific ligand binding, whereas *lhr* may be related to the final maturation and ovulation of oocyte. These findings open doors to further investigation of physiological functions of gonadotropin receptors FSHR and LHR, which will be valuable for fish reproduction and broodstock management.

PO-24

Melatonin implants affect the kiss/gnrh systems during spermatogenesis in male sea bass, *Dicentrarchus labrax* L.

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Melatonin may drive the seasonal changes in kisspeptin-expressing cells and GnRH/gonadotropin secretion in mammals, thus controlling reproduction. In this work, we investigated the brain expression changes of the elements of the kisspeptin/gnrh systems including the two kisspeptin genes (*kiss1*, *kiss2*) and their receptors (*gpr54-1b*, *gpr54-2b*) as well as *gnrh-1*, *gnrh-2*, *gnrh-3* and *gnrh-II-1a* and *gnrh-II-2b* and gonadotropin genes (*fsh*β, *lh*β) in response to long-term melatonin administration. Its potential effects on the activity of the pituitary-gonad axis and certain biometric parameters and gonadal maturity in male sea bass were also examined. Melatonin reduced the fish weight and condition factor as well as affected gonadogenesis, showing lower gonadosomatic index values after 150 days of treatment in comparison to controls. Melatonin-treated fish also exhibited a lower percentage of running males during the spermatogenesis and full spermiation stages. Furthermore, exogenous melatonin resulted in lower plasma androgen levels during the reproductive period, and showed a significant decrease in serum Lh and Fsh concentration after 30 and 60 days of treatment, respectively. The hypothalamic expression of *kiss1* was significantly higher in melatonin-treated fish than in controls after 30 days of treatment, while a significant increase in *kiss2* expression was detected on day 90 of treatment. By contrast, melatonin showed a significant decrease in kisspeptin expression in the dorsal brain on day 150 of treatment and also affected the expression of *gnrh-1* and *gnrh-3* and *gnrhr-II-1a and 2b* and the *fshβ* gene in the pituitary. Thus, these findings indicate that melatonin presumably has an anorexic action and induces changes that affect gonadogenesis in adult sea bass if administered over an extended period of time throughout their entire reproductive cycle. In addition, although the mechanisms of action of melatonin are still unclear in this species, our experimental evidence raises the possibility that melatonin might presumably induce the downregulation of kisspeptin-gnrh members on the dorsal brain, which might affect $\phi\eta\sigma\beta$ □ transcription at the pituitary level during early gametogenesis, thus evoking disturbances in the further testicular development of sea bass.

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PO-25

Changes in expression of sex differentiation-related genes during sex change in the protogynous wrasse, *Halichoeres trimaculatus*

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The three-spot wrasse, Halichoeres trimaculatus, which inhabits the coral reefs of Okinawa may undergo sex change from female to male (i.e., is protogynous). During the process of this female-to-male sex change, ovaries physically degenerate and transform completely into functional testes. It is reported that rapid depletion of estrogen plays crucial role in the onset of the gonadal transformation. However, little is known about the subsequent molecular mechanism during the sex change. To address this question, we focused on the expression profile of sex differentiation-related genes, which have been investigated in gonochoristic vertebrates, during the sex change induced by aromatase inhibitor treatment in the three-spot wrasse. The genes examined include R-spondin 1 (rspo1), factor in the germline alpha (figla), gonadal soma derived factor (gsdf), doublesex and mab-3 related transcription factor 1 (dmrt1), Anti-Müllerian hormone (amh) and sex determining region Y box 9 b (sox9b). Changes in the expression of each of these genes during the sex change were observed. Expression levels of rspo1 and figla, which are involved in ovarian differentiation and maintenance in vertebrates, declined from middle to late stages of the sex change. At that timing, almost oocytes degenerated and disappeared in the gonads. These results suggested that degeneration of ovarian tissues resulted in the decrease of two genes expression levels. On the other hand, the expression levels of gsdf, dmrt1, amh, and sox9b, which play important roles in the differentiation and maintenance of testis, rise before and after the beginning of spermatogenesis during the gonadal transformation. These findings indicated that these genes contribute to testes formation during the sex change. In particular, the expression levels of gsdf rise early among these genes. This result implied that gsdf might be involved in proliferation of spermatogonia-like cells and subsequent spermatogenesis. In the future, it is important to investigate the relationship between these genes and estrogen, which is key factor of the sex change.

PO-26

Sexual characteristics of sterilized tilapias

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We recently succeeded to induce complete germ cells loss in gonads in tilapias by high temperature. Heat treatment at 37℃ for 45–60 days caused a complete sterilization in female Nile tilapia, *Oreochromis niloticus*, and that sterility was achieved in fish at all stages of their life cycle. Unlike previous observations, germ cells did not repopulate even after returning them to the water at control conditions suggesting permanent depletion of germ cells. Ovarian somatic cells immunopositive for 3β-hydroxysteroid dehydrogenase (3β-HSD) were clustered at one end of the germ cell depleted ovaries close to the blood vessel. Serum level of testosterone, 11-ketotestosterone, and 17β-estradiol was significantly decreased in sterile female fish compared to control. Body weight of sterile female fish was higher than control female fish at the end of experiment. Juveniles of the Mozambique tilapia, *Oreochromis mossambicus*, at 3 dah were reared at a high temperature (37 ± 0.5 °C) for 50 days. The heat-treated fish were then cultivated at a normal water temperature for over six months. The testes of all individuals heat-treated for 50 days were sterile. Histological analysis revealed the complete absence of all stages of spermatogenic germ cells in the testes of the heat-treated males; however, structures within a layer of epithelial cells lining the efferent ducts were observed to actively secrete sperm fluid into the ducts, as in the mature testes of normal males. Clusters of cells immunopositive against P450scc and 3β-HSD were observed in the sterilized testes. Leydig cells had developed smooth endoplasmic reticulum and several mitochondria with tubular cristae indicating active steroidogenesis. The sterilized males displayed male nuptial coloration, actively dug spawning nests, and mated with normal mature females. However, normal females mated with these males initially brooded their eggs normally but released them prematurely at 4–5 days. All the released eggs were unfertilized and dead. Heat-sterilized male tilapia matures endocrinologically but completely lacks spermatogenic germ cells. Our observations of permanent sterilization in the high temperature-treated fish suggest that this method could be an appropriate and eco-friendly tool for inducing sterility in fish with a higher thermal tolerance.

PO-27

Effects of sex steroids on leptin and the leptin receptor in immature male Atlantic salmon, *Salmo salar* L., parr

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In mammals, leptin plays an important role in reproductive function and is a major player in the interaction between nutritional status and the regulation of the brain-pituitary-gonad axis (BPG-axis). Leptin modulates all levels of the reproductive endocrine axis and, in turn, both leptin and the leptin receptor are regulated by sex steroids. We have recently found that sex steroids are positive regulators of two leptin genes in a teleost *in vitro.* The aim of this study was to investigate the role of androgens in the control of components of the leptin system in the liver and at different levels of the reproductive axis in fish. One-year old immature salmon were implanted either with empty capsules or capsules containing T or 11-ketoandrostenedione (11-KA). The effects of these two main androgens on leptin gene expression in the liver and pituitary, leptin receptor expression in pituitary and testis and circulating leptin plasma levels were assessed after a 35-days treatment period. Both 11-KA and T stimulated the reproductive axis by increasing testes weight and up-regulating pituitary *lh-β* mRNA levels, and for T also *fsh-β*. Transcription levels of *lepa1* and *lepr* were significantly up-regulated by T in the pituitary, while 11-KA had no effect. *lepr* expression in the testis was unaltered by either androgen. Hepatic *lepa1* but not *lepa2* mRNA levels were significantly up-regulated by T, while 11-KA had no effect. Plasma leptin levels did not differ significantly between treatments. These results indicate that androgens regulate gene expression of leptin and the leptin receptor in different tissues in fish, suggesting a role for leptin in fish reproduction.

PO-28

Development of specific ELISAs for Medaka (*Oryzias latipes*) gonadotropins Fsh (follicle-stimulating hormone) and Lh (luteinizing hormone) using recombinant proteins

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Vertebrate puberty and sexual maturation is regulated primarily through the endocrine system known as the hypothalamo-pituitary-gonad (HPG) axis, of which Fsh (follicle-stimulating hormone) and Lh (luteinizing hormone) play integral parts. There are indications that these hormones play important roles also during early development, although detailed functions and underlying mechanisms have not been described. The focus of the present study is to generate key tools and methodologies to study the functional roles of Fsh and Lh during early development of medaka. As a tool to investigate the function of early pituitary gonadotropin expression, we are developing specific and homologous competitive enzyme-linked immunosorbent assays (ELISA) to quantify Fsh and Lh protein levels in medaka pituitary and plasma, including development of antisera against the two hormones. Plasmids containing Fshβ, Lhβ, or single-chain Fshβα or Lhβα were expressed using the methylotrophic yeast *Pichia pastoris*. Hormone production in *P. pastoris* in 1 l cultures over 3 days (large production of recombinant protein) resulted in 0,813 mg highly purified medaka Fshβ and 0,201 mg of highly purified medaka Lhβ, both based on one-step nickel batch purification. Western blot analysis using his-hrp antibody showed bands with expected sizes of 12.5 kDa for Fshβ and 15 kDa for Lhβ. Specific antisera were raised in rabbits, using three injections of purified protein in 0,9 % NaCl and emulsified with complete Freund adjuvant at 3-wk intervals. By conducting immunohistochemistry using the Lhβ antibody on medaka pituitary sections from an established *lhb*:*hr*Gfp-II transgenic line a complete overlap of labeling was shown. This result validates clearly the antibody specificity raised against Lhβ. The same testing needs to be done for the antibody specificity against Fshβ. Gpα was joined with medaka Fshβ or Lhβ mature protein-coding sequences to form a fusion gene that encodes a “tethered" polypeptide, in which the gonadotropin β-subunit forms the N-terminal part and the α-subunit forms the C-terminal part. We produced Fshβα and Lhβα single-chain peptides in *P. pastoris* (resulting in 0,367 mg Fshβα and 0,053 mg Lhβα) to be used as standards in the specific ELISAs and for characterization in ligand-Lhr/Fshr receptor-binding studies that are under preparation. A further validation of the recombinant proteins Fshβ, Lhβ, Fshβα, and Lhβα by an *in vitro* bioassay is under preparation as well. The *in vitro* bioassay is planned to investigate the biological activity by secretion of the steroids estradiol (E_2), testosterone (T), and 17α,20β-dihydroxy-4-pregnen-3-one (DHP) from ovaries from female medaka in response to recombinant Fshβα and Lhβα. The validated assays for medaka Fsh and Lh will be important tools to reveal the functional roles of these hormones during different stages of medaka reproductive development.

PO-29

Roles of male-released pheromone associated with gonadotropin action on final oocyte maturation in the honeycomb grouper, *Epinephelus merra*

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The honeycomb grouper *Epinephelus merra* is lunar related spawner that spawns for 3 days, a few days after full moon. It is known that the final oocyte maturation (FOM) is induced by some kind of substance found in mature male rearing water. In other words, the process of FOM is regulated by the male-released pheromone. It is also reported that the male pheromone induces maturation of the female in the Mozambique tilapia and the African catfish. However, the roles of maturation inducing pheromone are unknown. Therefore, in this study, we examined the effects of pheromone on FOM associated with the action of gonadotropin, which is key hormone for regulating FOM. Experimental fish were collected from coastal area around Sesoko Island, Okinawa, Japan. Females and males were separately reared until the start of the following two experiments. Exp. 1) Just prior to the spawning phase, females which have not yet started FOM were divided into two experimental groups with or without human chorionic gonadotropin (HCG) treatment. HCG was injection at 100 IU/kg BW at two days after full moon. At 40 hr after injection, ovaries were collected from all female for histological observation of oocyte development. There was only tertiary yolk stage oocyte in the ovary before HCG treatment. Progression of FOM was observed only in HCG injected females. However, not every oocyte initiated FOM at same time by HCG treatment. The oocyte of three different stages (tertiary yolk, migratory nucleus, and ripe) appeared in the ovary after HCG treatment. Exp. 2) Females were divided into three experimental groups, which were the HCG (100 IU/kgBW) injection group, rearing with male group (Male+ group), and an only female group (control). Blood, ovary, and pituitary in female were sampled at 0, 24, 48, and 72 hr after the start of experiment. Ovulated eggs were observed in HCG group and Male+ group at 48 and 72 hr after treatment. Luteinizing hormone (LH) beta gene expression increased only in female pituitary of Male+ groups, and plasma E_2 and T levels increased in both HCG and Male+ groups. This study confirmed that male pheromone induced FOM via LH synthesis in the pituitary. Moreover, LH-induced steroids may enhance physiological activity for FOM.

PO-30

The corticotropin-releasing factor system has anti-steroidogenic actions in the zebrafish ovary

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Beyond its central role in the coordination of the stress response, recent studies have implicated the corticotropin-releasing factor (CRF) system in the regulation of physiological functions in peripheral tissues. In this study, we sought to characterize the ovarian CRF system in fish and its role in the regulation of steroidogenesis. We established that all the components of the CRF system are expressed in zebrafish ovary (*crfr1* >> *crfr2* > *crf* > *unc3* = *uts1* = *crfbp*), that whole ovary *crf* and *crfr1* gene expression are highest at 7:00 when ovulation is expected to occur, and that *crfr1* expression levels are higher in full grown follicles than in earlier stages. *In vitro*, incubating follicles with CRF suppressed human chorionic gonadotropin (hCG)-stimulated production of 17-β estradiol and testosterone, effects that were abolished by the CRFR1 receptor antagonist antalarmin. Incubating follicles with CRF also suppressed hCG-stimulated increases in steroidogenic acute regulatory protein (StAR) and P450 aromatase gene expression. We show that the CRF system is expressed in fish ovary and that CRF acting through CRFR1 inhibits steroidogenesis by suppressing StAR and P450 aromatase. Collectively, these results suggest that the CRF system may modulate steroidogenesis via autocrine or paracrine actions in the fish ovary.

PO-31

Photoperiodic control of reproduction in the three-spined stickleback, *Gasterosteus aculeatus*

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The three-spined stickleback has a marked seasonal reproductive cycle and spawns in spring and early summer. The reproductive cycle is to a large extent controlled by photoperiod but also by temperature. Sticklebacks exposed to a combination of long photoperiod and high temperature in winter start to reproduce, whereas this does not take place under short photoperiod. Under intermediate photoperiods, some fish mature and others not. The proportion of fish that mature changes gradually with the photoperiod, but each individual either matures fully or not at all. The breeding season is followed by a refractory period, even under a stimulatory long photoperiod, and under constant long photoperiod reproduction displays a circannual cycle. Extra retinal photoreceptors can control breeding and appear to be more important than the retina. Melatonin, on the other hand, appears not to be involved in the control of seasonal reproduction. There are marked differences in feedback on the brain-pituitary-gonad axis under different photoperiods. Under non-stimulatory short photoperiod, feedbacks are generally negative, especially on FSH, but also on LH, suppressing reproduction. Under stimulatory long photoperiod, on the other hand, feedbacks are positive, stimulating reproduction. There is also a dose effect; low doses of testosterone suppress especially LH, but also FSH, whereas high doses are more positive. The all-or-nothing effect on reproduction in individual fish could be due to a positive feedback accelerating maturation if a certain threshold in androgen level is reached. The negative effect of low androgen doses is dependent on aromatisation to estrogens and aromatase inhibitors can stimulate increased maturation also under short photoperiod.

PO-32

Characterization of temporal changes of Lh producing cells in medaka

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Final oocyte maturation is dependent on accurate and sufficient release of luteinizing hormone (Lh). In order to get a better understanding of the novel components behind the well coordinated events of Lh synthesis and release, we have investigated different temporal characteristics of the Lh producing gonadotrope cells in a transgenic line (tg(*lhb*:hr-GfpII)) of medaka (*Oryzias latipes*), where hrGFP-II expression is controlled by the endogenous medaka *lhb* promoter. Gfp labeling specificity in these cells was first confirmed in juveniles and adults by immunofluorescence, utilizing a recently developed medaka Lhb specific polyclonal antiserum. The Lh producing cells were isolated by fluorescence activated cell sorting (FACS) and revealed a remarkable increase in the proportion of these cells from juvenile to adult fish. These data were supported by observation of the distribution of the *lhb* expressing cells by 3D imaging. The FACS sorted Lh gonadotropes were further analyzed by whole transcriptome analysis using RNA-seq, and the cells displayed a high correlation between Gfp and *lhb* expression. In addition to offering a global overview of the transcriptome in the Lh producing cells, the RNA-seq data provide good indications to genes that could be of importance during sexual maturation. For instance, we found important differences in the ion channel repertoire expressed in these cells before and after puberty. Electrophysiology experiments indicate that the Lh producing cells could be placed into different groups depending on their ion channel composition. BrdU incorporation experiments performed in adults demonstrated a small number of newborn *lhb*-Gfp cells that could partly explain the cellular heterogeneity between cells at the adult stage in these cells. Studies are ongoing to elucidate if the higher abundance of the Lh producing cells in adult fish is caused by active cell division during the transition from juvenile to adult fish, or alternatively, if it is caused by other mechanisms. These results demonstrate time-dependent changes in the characteristics of Lh producing cells and give indications of heterogeneity with different sub-populations cells.

PO-33

Kisspeptins and their receptors are differentially expressed in the brain-pituitary-gonadal axis during gametogenesis in *Odontesthes bonariensis*.

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The brain-pituitary-gonadal (BPG) axis is implicated in the regulation of vertebrate reproduction, and kisspeptin has emerged as a key player of this axis. Two ligands, *kiss1* and *kiss2* and receptors, *kissr2* and *kissr3*, were characterized in *Odontesthes bonariensis*, and changes in the expression profile of these transcripts were analyzed at the BPG axis during female and male gametogenesis. Adult fish were collected every month from the wild, and the relative abundance of these transcripts was measured by real-time PCR. In females, brain *kiss1* levels were higher at final maturation (FM); meanwhile *kiss2* levels were high at primary growth stage, with no differences in receptors levels. At the pituitary, *kiss1* and *kiss2* peaked at the cortical alveoli (Ca) stage, and *kissr3* at initial vitellogenesis. Finally, there was an increase of *kiss1*, *kissr2* and *kissr3* in the ovary during the Ca stage and *kissr2* and *kissr3* at FM. These results showed that these genes were differentially expressed at the three levels of the BPG axis throughout oogenesis. Changes of brain *kiss1* expression levels suggested that it could be related to FM, in relation with the rise of *gnrh1* observed at the same stage. Meanwhile *kiss2* seemed to be associated with initial stages of gametogenesis. In males, the four genes were highly expressed in the brain at the arrested stage. In the pituitary, *kiss2* peaked at spermatogonial (SG) and spermatocytary (SC) stages; while *kissr3* reached a peak at the spermiogenic stage (SP). In testes, *kiss1* and *kiss2* significantly increased during the SG and SC stages; meanwhile, *kissr2* increases at SG and SC, whereas *kissr3* levels were significantly high at SC and SP stages. Taken together, these results suggested that kisspeptin ligands and receptors are involved in the regulation of pejerrey gonadal development at the three levels of the BPG axis, showing differential expression profiles in both sexes, suggesting different roles in female and male gametogenesis.

PO-34

Modelling and functional prediction of two paralogous kisspeptin receptors, kissr2 and kissr3, in pejerrey fish

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The kisspeptin system has a main role in the control of puberty in mammals and it is composed by the neuropeptide, KISS, and its associated receptor, KISSR. However, in teleost fish species two *kissr* paralog genes have been identified. Here, we report the gene organization and the *in silico* structural analysis of two receptors, *kissr2* and *kissr3*, in the pejerrey, *Odontesthes bonariensis*. The *kissr2* gene in pejerrey comprises five exons and four introns and *kissr3* presented six exons and five introns. The organ/tissue distribution expression analysis evidenced two distinct transcripts for both receptors. The *kissr2* contained an insert of 98 nucleotides caused by the retention of the whole intron III and *kissr3* an insertion of 79 nucleotides product of the whole intron IV retention. In *kissr2* the predicted amino-acidic sequence comprises one premature termination codons in transmembrane helix 4; in the case of *kissr3*, there is a shift in the reading frame at the intratracellular domain 3, leading to the loss of TM6 and TM7, which may result in a non-functional truncated receptor. Molecular modelling of the Kissr2 and Kissr3 3D structure were performed by the on-line platform GOMoDo. Both receptors were then analyzed by a virtual docking protocol included in the GOMoDo server. Indeed, the docking procedure was performed by the Haddock program version accessible through the server. The predicted binding cavity in both, Kissr2 and Kissr3 is formed by residues of TM3, TM5, TM6 and TM7. Remarkably, almost all the identified residues are conserved in vertebrates. The lack of TMs 4 and 5 upon the insertion of premature stop codons will cause not only the lost of the binding cavity but also fundamental regions for the stability of the protein structure. In conclusion, the reported alternative splicing events might contribute to regulative mechanism of the Kiss/Kissr-signalling pathways.

PO-35

Saccus vasculosus is a photoperiodic sensory and endocrine organ for reproductive regulation in Japanese eel

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Recent studies in the masu salmon (*Oncorhynchus masou*) have shown that the saccus vasculosus functions as a photoperiodic sensor, and thus, plays an important role in regulating seasonal reproductive changes (Nakane et al., 2013). To date, however, no studies have shown the function of this organ in other fish species. Here, we investigate the physiological function of the saccus vasculosus in Japanese eel (*Anguilla japonica*) through histological characterisation as well as gene and protein expression. Histological analysis showed the saccus vasculosus to be a specialised circumventricular organ located within the caudal hypothalamus, consisting of a neural epithelium with coronet cells, supporting cells, and an extensive vascular plexus. Neurons of the anterior tuberal nucleus (NAT) of the hypothalamus extended axonally into the saccus vasculosus, while a band of nerve fibres protruded from the saccus vasculosus to the hypothalamus. Immunoreactivity was observed for thyroid stimulating hormone beta subunit (TSHβ) and glycoprotein alpha subunit (GPα) in the coronet cells of the saccus vasculosus. Furthermore, photoreceptor genes (such as rhodopsin 2 and fresh water opsin of the rhodopsin family), as well as key genes associated with seasonal reproductive changes (such as TSHβ, GPα, and type 2 iodothyronine deiodinase), were found to be expressed in the saccus vasculosus, in addition to the specific expression of kisspeptin receptor genes (Japanese eel *kissr2 and kissr3*). These results suggest an important role of the saccus vasculosus in seasonal reproductive regulation in the Japanese eel. However, further studies are necessary to obtain direct evidence for such a role.

Nakane et al., (2013). The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nature Communications, DOI: 10.1038/ncomms3108

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PO-36

Gnrh-induced Ca^{2+} responses in varicosity-like structures on membranous tubular extensions from Lh-producing gonadotropes in medaka (*Oryzias latipes*)

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Luteinizing hormone (Lh) is a key regulator in the reproductive axis in vertebrates. During our studies of Lh-producing gonadotropes in medaka, using a transgenic line in which Lh-producing gonadotropes express a reporter gene (GFP), we have consistently observed membranous tubular extensions radiating from the cell soma. These extensions have not been described in any detail, and their function is unknown. In the present study Ca^{2+} imaging (Fura-2) was used to investigate the effect of 10^{-6} M gonadotropin-releasing hormone (Gnrh) on the cytosolic Ca^{2+}-level ($[Ca^{2+}]_i$) in these extensions, focusing on the Ca^{2+} responses in varicosity-like structures on the extensions. The results from our experiments show that the Ca^{2+} response in the varicosity-like structures occurs at the same time as the response in the cell soma. In fact, the Ca^{2+} response in the varicosity-like structures started 330 ± 847 ms prior to the response in the cell soma (n=28). These results indicate that the response in the varicosity-like structures is not due to propagation of a signal from the cell soma, but a parallel event. The elevation of $[Ca^{2+}]_i$ may depend on both intracellular and extracellular sources of Ca^{2+}. In order to eliminate the extracellular contribution we replaced the normal extracellular solution (ECS) with Ca^{2+}-free ECS. This significantly reduced the duration of the responses in the varicosity-like structures from 189 ± 14.83 s (n=13) in the control to 138.86 ± 27.92 s (n=7). As in the cell soma tThere was a partial lossreduction of the second phase, but the change to Ca^{2+}-free ECS did not result in elimination of the response altogether. This implies that at least part of the Gnrh-response is due to Ca^{2+} from internal stores within the varicosity-like structures.

PO-37

Testicular steroidogenesis and locomotor activity are regulated by gonadotropin-inhibitory hormone in the male European sea bass.

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Gonadotropin-inhibitory hormone (GnIH) suppresses reproduction in several vertebrate species by acting at both the brain and pituitary levels. New evidence obtained in birds and mammals suggests that, in addition to the brain, GnIH may also be produced in gonads and can regulate gonadal steroidogenesis and gametogenesis. Sex steroids are essential for gonadal function and cyclicity and play a key role in referring the sexual status to the brain and pituitary. However, the function of GnIH in gonadal physiology has received little attention in fish. One of the main objectives of this study was to evaluate the effects of peripheral sbGnIH-1 and sbGnIH-2 implants on gonadal development and steroidogenesis along the reproductive cycle of male sea bass. Implants of sbGnIH-1 and sbGnIH-2 did not affect plasma levels of the maturation-inducing steroid 17,20 □dihydroxy-4-pregnen-3-one. Although similar profiles of plasma testosterone (T) and 11-kestostestosterone (11-KT) were observed in controls and GnIH-implanted animals, with a peak in January, both GnIHs decreased T and 11-KT plasma levels in November and December. In February, control fish exhibited only late meiotic and full spermiogenic testicles containing mature sperm. In contrast, both sbGnIH-1 and sbGnIH-2 treated groups exhibited a significant percentage of fish with testicles containing abundant type A spermatogonia and partial spermatogenesis. In addition, we determined the effects of peripheral GnIH implants on plasma FSH and LH levels, as well as on brain and pituitary expression of reproductive hormone genes (*gnrh1, gnrh2, gnrh3, kiss1, kiss2, gnih, lhbeta, fshbeta*) and their receptors (*gnrhrII-1a, gnrhrII-2b, kiss1r, kiss2r and gnihr*) during the spawning period (February). Treatment with sbGnIH-2 increased brain *gnrh2, gnih, kiss1r* and *gnihr* transcripts. Moreover, both sbGnIH-1 and sbGnIH-2 treatments decreased *lhbeta* expression and plasma LH levels, and sbGnIH-1 reduced plasmatic FSH. Finally, through locomotor activity recording we showed that both GnIHs prevented the characteristic increase in nocturnalism observed in sea bass during the reproductive season. Taken together, our results indicate that GnIH may regulate the reproductive axis of sea bass acting not only on brain and pituitary hormones but also on gonadal physiology and locomotor activity.
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PO-38

Molecular identification of the Dyn/Kor system and its potential role in the reproductive axis of goldfish

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Dynorphin（Dyn）inhibits gonadotropin releasing hormone (GnRH) pulsatile secretion by acting upon kappa opioid receptors (Kor) in mammals. However, the action of Dyn/Kor system on fish reproductive axis is unclear. In this study, full-length cDNAs encoding for prodynorphin (*pdyn*) and *kor* were isolated from the goldfish. Sequence analysis showed that *pdyn* encodes two mature peptides (Dyn A, Dyn B). Quantitative real-time PCR and *in situ* hybridization analyses demonstrated that *pdyn* and *kor* mRNAs are highly expressed in the brain regions. The expression patterns of *pdyn* and *kor* at different developmental stages of goldfish were also studied. The mRNA expression of *pdyn* and *kor* in the brain are lower in the early vitellogenic stage. No significant changes of these genes in the brain were found during the testis development. The effects of Dyn on the expression of GnRH and gonadotrophin were further investigated *in vivo*. Intraperitoneal administration of synthetic goldfish Dyn A and Dyn B peptides significantly reduced the hypothalamic salmon *gnrh* and pituitary *fshβ* and *lhβ* mRNA levels at 6 h. Our findings suggest that Dyn/Kor system may play a negative role in the regulation of the reproductive axis in goldfish.

PO-39

Molecular mechanisms of feedback regulation of 17β-estradiol on two kiss genes in protogynous orange-spotted grouper, *Epinephelus coioides*

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Kisspeptins stimulates the secretion of GnRH in vertebrates. On the other hand, Kiss1 mediates the feedback regulation of sex steroids on hypothalamus-pituitary-gonadotropic axis. Distinct from mammals, multiple kiss1 paralogous genes (kiss1, kiss2) have been identified in teleosts, raising the complex signal pathways in the feedback regulation. In the present study, molecular pathways by which 17β-estradiol (E2) exerted feedback regulation on two kiss genes via three types of ERs were investigated in orange-spotted grouper (*Epinephelus coioides*), a protogynous species. Moreover, E2 treatment decreased two kiss promoter activities in the presence of erβ1 and erβ2 rather than erα in HEK 293T cells. Further deletion analysis and site-directed mutation on two kiss promoters indicated that kiss1 was regulated by E2 via ERE-dependent classical pathway with erβ1 and Ap1-dependent non-classical pathway with erβ2. The kiss2 was differently regulated by E2 through unknown transcription factors with erβ1 and 1/2ERE-dependent classical pathway with erβ2.

PO-40

Kiss2 acts at various levels of the reproductive axis in a teleost fish species

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Kisspeptins are key players in the neuroendocrine control of puberty and other reproductive processes in mammals. Contrary to placental mammals, where only one KISS1 and one KISS1R gene are present, a second ancient paralog of Kiss1, referred to as Kiss2, has been described in different vertebrate lineages and species, including European sea bass (*Dicentrarchus labrax*). Both genes are expressed in the brain and pituitary of this teleost species, but their differential roles in the central control of fish reproduction are only beginning to be elucidated.In this study, we investigated whether the brain represents the only site of kisspeptin action on reproductive function, and also the potential actions of kisspeptin at the pituitary level could be considered. First, we examined the effects of intracerebroventricular injections of the highly active sea bass peptides Kiss1-15 and Kiss2-12 on spermiating male sea bass. Physiological saline, Kiss1-15, or Kiss2-12 were injected into the third ventricle. Blood samples were collected at different times after injection to analyze the effects of kisspeptins on the release of gonadotropins (Lh and Fsh) and androgens (testosterone and 11-ketotestosterone). Sperm samples were also collected to study the effects on the sperm quality. Our results showed that Kiss2-12 provoked a marked effect on plasma luteinizing hormone (Lh) levels, which in turn had a strong stimulatory effect on the release of androgens and on sperm quality parameters.On the other hand, both synthetic peptides, Kiss1-15 and Kiss2-12, were used to stimulate dispersed sea bass pituitary cells obtained from mature males. Our results showed that Kiss2-12 induced Lh and follicle-stimulating hormone (Fsh) release, whereas Kiss1-15 had no effect on gonadotropin secretion. Furthermore, the distribution and origin of Kiss2 and its potential interactions with the gonadotropins in the pituitary were analyzed using dual fluorescence immunohistochemistry. Kiss2 cells were found in the proximal pars distalis and colocalized with gonadotropin immunoreactive cells. In summary, our results provide, for the first time in a teleost species, functional and neuroanatomical evidence that Kiss2 may act through different routes to directly modulate the activity of gonadotrophs, either as a brain neuropeptide or as an autocrine/paracrine factor in the pituitary. Funded by grants: LIFECYCLE FP7-22719-1; AGL2011-28890; PROMETEOII-214/051.

PO-41

Electrophysiological Characterization of Genetically Labeled *Ihb* Producing Cells

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Sexual maturation and puberty is regulated through the Brain-Pituitary-Gonadal (BPG) axis. At the pituitary level, activation of gonadotropin releasing hormone (Gnrh) receptors on gonadotrope cells leads to biosynthesis and release of the two gonadotropins; follicle stimulating hormone (Fsh) and luteinizing hormone (Lh). These two hormones are subjected to differential regulation. However, the different mechanisms behind the control of Fsh and Lh synthesis and release are not fully understood. To elucidate key mechanisms behind gonadotropin release our group utilizes a transgenic medaka (*Ihb*:hrGFP-II) where GFP is controlled by the *Ihb* promoter. In earlier studies we have shown that *Ihb* producing cells are electrically excitable with ability to fire action potentials (AP) spontaneously and that Gnrh can increase AP firing frequency and subsequently increase cytosolic Ca^{2+}. To further characterize the *Ihb* producing cells we first sequenced the mRNA from FACS sorted GFP positive *Ihb* producing cells to identify all ion channels expressed within the *Ihb* producing cell population. The data show a large selection of ion channels responsible for the inward and outward currents. We therefore aimed at elucidating the ion channel composition from single isolated *Ihb* cells employing the perforated patch clamp technique. Interestingly, we found a large heterogeneity among the cells. Based on the current-voltage relation we clustered the *Ihb* producing cells into three different groups (Group 1-3). Group 1 lacks the transient inward Na^+ current necessary for driving action potentials. The outward current of group 1 cells displayed a slow time dependent activation persisting over the 200 ms voltage step used. Groups 2 and 3 had a clear transient inward Na^+ current. In addition, two different cellular characteristics were observed, in particular with regards to the outward current. Group 2 cells had a typical N shaped current-voltage relation indicative for Ca^{2+} activated K^+ channels $K_{(Ca)}$ known to regulate action potential frequency. These findings were confirmed with a tail current protocol comprising a prepulse to elevate cytosolic Ca^{2+}. The group 2 cells also displayed an inactivating component in the outward current at potentials more positive than 50 to 60 mV, similar to the I_A current and known to regulate interspike activity. The last group of *Ihb* producing cells, group 3, shared similar characteristics in the outward current as group 1 and lacked the $K_{(Ca)}$ currents and the I_A type inactivation component of the outward current found in group 2. Taken together, medaka *Ihb* producing cells show great heterogeneity in their electrophysiological properties. The functional role of this cellular heterogeneity is still being investigated. However, earlier findings have shown that *Ihb* producing cells are clustered in the pituitary and that communication between cells in the pituitary also involves direct electrical connection through gap junction. Thus, having different electrophysiological properties may reflect cell specific tasks like receiving and passing information within a particular cell cluster.

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Genes at the brain level associated with photoperiod-induced sexual maturation in the three-spined stickleback (*Gasterosteus aculeatus*).

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Sexual maturation of three-spined stickleback (*Gasterosteus aculeatus*) is controlled by the day length, in winter it is suppressed under short-day (L8D16), but stimulated by long-day (L16D8) photoperiod. To understand the mechanisms underlying light-induced sexual maturation at the brain level, the gene expressions in brains of males kept under L8D16 or L16D8 for 3, 10, 19 and 29 days were analyzed using RNA-seq transcriptome and qPCR. Changes in transcripts in the brain following the day lengths that may be involved in sexual maturation were found in: (1) Sex steroid biosynthesis: expressions of transcripts related to steroid conversions (aromatase, 17alpha-hydroxylase and 17, 20-lyase) and StAR (steroidogenic acute regulatory protein) were significantly induced by L16D8 from the 3rd day and lasted to the end of experiment. Accompanied with steroid biosynthesis, an increase of estrogen receptor (*er2*) expression were found under L16D8 at day 10 and 19. (2) Monoamine neurotransmitters: 3 days of L16D8 exposure suppressed transcripts of enzymes involved in dopamine metabolism (catechol-O-methyltransferase and dopa decarboxylase) and 5-HT/5-HIAA and 5HT/melatonin conversions (MAO and ANNT1). The alteration of the genes transcript involved in 5-HT conversions lasted to the 19th day, but changes of the genes expression for dopamine metabolism were not pronounced at other time points. (3) GnRH/Kisspeptin system: L16D8 up-regulated the expressions of GnRH2 (*gnrh2*) and GnRH3 (*gnrh3*) from the 3rd and the 19th day respectively. L16D8 also increased transcripts of Kisspeptin receptor (*gpr54*) at 3rd day, but no such effect was found on Kisspeptin gene (*kiss2*) itself until 19th day. Furthermore, the males under L16D8 were found with higher expression of *lpr* (leptin receptor) at early time points (3rd and 10th day). These results suggest that both the sex steroid conversions and monoamine neurotransmitters in the brain could be important for mediating photoperiodic effects. Moreover, leptin may also be associated with this photoperiod-induced maturation.

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Evidence for a possible interplay between IL-1β and autophagy in the fine flounder skeletal muscle

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In higher vertebrates, it is demonstrated that autophagy modulates transcription, processing and secretion of the pro-inflammatory cytokine IL-1β, acting as a modulator of the immune response, and as an effector of the stress response. However, the relationship between autophagy and IL-1β in fish has not been yet described. According to this, the aim of this work was to study the effect of rearing stressful conditions on the immune response and its relationship with autophagy in a fish model. Two experimental setups were used. In the first model (confinement), juvenile fine flounder were maintained on high stock density, for 4 and 7 weeks. Skeletal muscle samples were collected from control and stressed fish groups. In the second model (pathogen infection), fish were subjected to a bath infection challenge with a sub-lethal dose of *Vibrio ordalii* (Vo-LM-18 strain). Fish were removed from each tank after 2, 4 and 10 days post-infection and skeletal muscle samples were collected. Growth- and stress-related hormones, e.g. GH, IGF-I and cortisol; IL-1β mRNA and protein content; autophagy activation; stress- and immune-related gene expression were analyzed by western blot and real-time PCR. Autophagy and synthesis of IL-1β were increased under confinement-induced stress; however, mRNA levels of most immune-related genes were decreased. On the other hand, no changes were detected on the stress-related genes from fish challenged with *V. ordalii*; nevertheless, the mRNA levels of immune-related genes, the autophagy and IL-1β were up-regulated. These results suggest a molecular interplay between IL-1β production and autophagy activation in fish skeletal muscle under stress condition. Further functional analyses are required to determinate the specific molecular pathways connecting autophagy and IL-1β synthesis in fish.

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Acute stress of high density modulates mRNA levels of marker genes for reproductive and detoxifying pathways in liver of *Salmo salar*

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High density stress in aquaculture procedures such as handling or during transfer of juvenile salmon to farming sites in the sea, might be related to decreased growth, higher incidence of disease or mortality. Chilean salmon contribute 27% of global production, ranking second on national exportations involving about 60.000 jobs especially in the southern regions where sustainable development of aquaculture industry is of pivotal importance. We found biologically relevant effects on marker gene expression via RT-qPCR analyses in liver of juvenile male *Salmo salar* maintained for three days at high density (70 kg/m3) respect to controls (10 kg/m3). Salmon were acclimated in tanks at natural temperature and luminosity before the experimental period. Primers were designed on salmon gene sequences extracted from GenBank and *gpx*3 sequences were cloned from salmon liver cDNA. Primers were optimized for parallel quantitative real time PCR, all amplicons were cloned and specificity was confirmed by sequencing. EF1α served as normalizer. Although after one day of overcrowding no difference was found at the transcriptional level in all genes tested, after three days changes occurred. mRNA levels of glutathione peroxidase *gpx3* were significantly decreased in comparison to control indicative of repressed antioxidant response, concomitantly cytochrome P450 *cyp1A* was increased as well as metallothionein *mt*. *Cyp1A* plays an important role in detoxifying pathways, whereas *mt* is related to heavy metal homeostasis, both systems seemed to be involved in the physiological stress response to three day overcrowded situation *in vivo*. In addition from vitellogenin *vg*, a egg precursor protein usually only present in female, transcripts were revealed in liver of fish after exposure to three days of high density. Therefore these results suggest that determination of gene expression of a panel of marker genes could serve as early indicators of biologically relevant changes thus helping to avoid potential detrimental effects of acute high density stress in juvenile salmon.

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Blocking the adrenocorticotropin receptor – novel tool to understanding the stress response in the eel, *Anguilla australis*

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The function and effects of glucocorticoids are vital in the stress response of vertebrates. Given that elevated levels of glucocorticoids have been associated with interrupted growth and development, reproduction and immune system functions, the ability to manipulate glucocorticoid levels can be a beneficial tool for breeding and maintaining healthy fish stocks in aquaculture and for basic science aimed at understanding stress responses. Glucocorticoid synthesis is upregulated through endocrine signalling by adrenocorticotropic hormone (ACTH) in the hypothalamus-pituitary-interrenal pathway. We aimed to examine the effects of chronic blockage of the ACTH-receptor in *Anguilla australis in vivo* using a novel ACTH antagonist (ACTH-X: Justia Patent 8524664). Its effects were measured through changes in glucocorticoid (cortisol) levels in the blood plasma. We hypothesised that increasing the concentration of ACTH-X would decrease the stress response through a reduction of plasma cortisol. To test this hypothesis, sustained-release cholesterol-cellulose implants containing 0, 5, 50 or 500µg of ACTH-X were administered to 48 eels. On each of Day 1 and 3, an acute stressor was imposed on 24 eels by holding them out of water at short intervals prior to being placed in a bath with anaesthesia. Twenty minutes after onset of the disturbances, eels were euthanized and bled. Blood samples from a further 6 eels that were not implanted and had not received the stressor were collected on each of day 0, 1 and 3. Radioimmunoassay analyses indicated that treatment with 50 and 500µg of ACTH-X reduced plasma cortisol concentrations in comparison to the control on both days, with a greater reduction observed on day 1. In addition, eels that received 0µg had higher cortisol levels than eels that were not manipulated. This study demonstrates the potential value of ACTH-X as a new tool to suppress cortisol levels so as to further understand the regulation of the stress response and develop means to limit the negative effects of stress on animal well-being and production.

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Transcriptional response to confinement stress in skeletal muscle of fine flounder (*Paralichthys adpersus)* determined by RNA-seq.

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Fish under intensive rearing conditions are subjected to diverse stressful factors, which negatively affect animal welfare and growth. Confinement stress is an inherent aquaculture condition that induces low growth rate and increased vulnerability to pathogens in fish, among other undesirable effects. Until now, little is known about the effects of confinement-stress conditions on transcriptional response in fish and the impact on its physiological adaptation. Recently, we sequenced the transcriptome of the fine flounder *(Paralichthys adpersus)*, an endemic fish species with farming potential. In order to identify gene expression reprograming induced by stress, we evaluated the transcriptional response on skeletal muscle of juvenile fine flounders submitted to 4 and 7 weeks of confinement, using an RNA-seq approach. In addition, growth- and stress-related hormones, e.g. GH, IGF-I and cortisol were measured. We found 116 and 63 differentially expressed genes on 4 and 7 weeks, respectively. Particularly, we identify genes encoding to ubiquitin proteasome system, transport channels, metabolic enzymes and structural proteins. We also detected 14 differentially expressed genes with no functional annotation. Moreover, enrichment ontology analysis reveals that biological process associated to myofibril assembly and muscle cell differentiation are the most impacted in fine flounder skeletal muscle under confinement stress. Finally, expression data obtaining *in silico* of candidates genes were validated using RT-qPCR. This work represents an important contribution to identify genes and skeletal muscle cellular process involved in fish stress response adaptation.

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Changes of brain monoaminergic activities and interrenal cortisol synthesis of rainbow trout (*Oncorhynchus mykiss*) during recovery from stress.

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In fish, serotonergic and dopaminergic systems appear to play a main role in initiating and maintaining the response to stress. Such response involves the activation of the hypothalamus-sympathetic-cromaffin (HPC), and the hypothalamus-pituitary-interrenal cells (HPI) axes, leading to increased plasma catecholamines and cortisol levels, but also brain monoaminergic activity. As a consequence, cortisol synthesis and release increase. After stress exposure, the physiological response tends to dissipate, with cortisol levels, among other parameters, turning back to normal. However, little is known regarding the role of brain monoamine neurotransmitters during chronic stress and indeed no studies focused after stress, when cortisol decays to basal non-stress levels. To address that question, a cohort of rainbow trout (*Oncorhynchus mykiss*) was subjected to stress by high stocking density for up to 7 and 10 days and then sacrificed, whereas another cohort was stressed for 7 days and afterwards un-stressed and sacrificed for the following 2-h, 6-h, 24-h and 72-h. Individual samples of blood (for cortisol, and metabolites assessments), different brain regions (for HPLC assessment of monoaminergic activities and qPCR assessment of *THP1* and *TH* mRNA abundance), head kidney (for the assessment of parameters related to cortisol synthesis: *StAR*, *3βHSD*, *P450ssc* and *11βH*) were collected. Our results show enhanced plasma cortisol levels and dopaminergic and serotonergic activities in telencephalon, hypothalamus, optic tectum and hindbrain of stressed fish. Increased mRNA abundance for *StAR*, *3βHSD*, *P450ssc* and *11βH* (head kidney) and *TPH1*, *TH* (brain) were also observed. After stress, monoaminergic activities decreased to basal values from 2 to 6 hours in parallel to plasma cortisol. Thus, the above described physiological response to chronic stress in rainbow trout dissipates in a relatively short time period (6-h) after exposure to stress, which supports the role played by brain monoamines in mediating the upstream initiated physiological response to stress in trout. Funded by Ministerio de Economía y Competitividad and European Fund for Regional Development (AGL2013-46448-3-1-R and FEDER).

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The regulation of leptin by glucose and the stress axis in the tilapia

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Leptin is a cytokine critical for regulating energy expenditure in vertebrates, yet little is known about how the hormone interacts with the endocrine stress axis, particularly in fishes and other ectotherms. Previous studies in tilapia have shown that leptin A (LepA) is the dominant form of leptin and that liver *lepa* mRNA acutely rises along with systemic glucose during seawater challenge, and under hypoxic conditions in other fishes. Leptin increases plasma glucose and decreases liver glycogen *in vivo* in tilapia, suggesting it promotes glycogenolysis. These data suggest that LepA may be involved in the adaptive stress response by mobilizing energy reserves, namely carbohydrates. Currently the regulatory interactions between leptin and the classical stress hormones and glucose are unclear. Here we evaluated the actions of epinephrine, cortisol, and glucose in directly regulating LepA in the liver, the major site of hormone production in the tilapia (*Oreochromis mossambicus*). Using hepatocyte incubations and a homologous LepA ELISA, we show that LepA decreases intracellular glycogen and increases glucose release. LepA synthesis and secretion declines as ambient glucose levels increase. These data suggest a negative feedback inhibition whereby leptin stimulates glucose release during the initial stress response and glucose subsequently acts to inhibit leptin synthesis and secretion. Cortisol at physiological concentrations, comparable to stressed levels, stimulates hepatic *lepa* mRNA and LepA secretion. Epinephrine stimulates LepA secretion in a dose-dependent fashion within 15 minutes, but had little effect on *lepa* mRNA expression. The response was accompanied by increases in glucose release likely indicating a classical glycogenolytic effect of the adrenergic hormone. These data suggest hepatic LepA promotes glycogen breakdown at the hepatocyte, is sensitive to ambient glucose, and is stimulated by both catecholamines and glucocorticoids. The results indicate that leptin plays an integral role in the vertebrate stress response to promote energy mobilization in conjunction with the classical stress hormones.

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The role of NO in brain ion transport during stress/ease response in fish

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In teleost fishes, nitric oxide (NO) has a vital role in the physiological processes. As transporters that provide the driving force for many other transport systems, Na^+/K^+-ATPase and H^+ ATPase are vital for Na^+/K^+ and H^+ homeostasis. Induction of stress by hypoxia altered the functions of these transporters during stress response and later these transporters regain its basal activity level during recovery or ease response by way of modifying the disturbed cellular and extracellular Na^+/K^+ and H^+ gradients. Dose-responsive effect of NO agonist SNP and antagonist NAME were tested in the brain ion transporters of air-breathing fish *Anabas testudineus* Bloch. The data indicate that both SNP and NAME modified the tested ion transporter's function in the fish brain. The response of fish to hypoxia with or without NAME was examined to delineate the role of NO in the adaptive processes. Analyses of the response pattern of ion transporters indicate that NO can promote ease response as it mitigates the magnitude of stress-induced variations in the transporter activities. Collectively, the data provide evidence that NO has a role in stress and ease response in fish.

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Cortisol induces gonadal masculinization in common carp

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Gonadal sex differentiation depends on estrogenic activity under the primary control of genetic factors in teleost fishes. It is also affected by environmental factors such as temperature in some gonochoristic fishes. It has been demonstrated that environmental stress induces cortisol secretion and masculinization by suppressing the estrogenic activity. Common carp (*Cyprinus carpio*) is known to have relatively stable sexuality scarcely modified by environmental factors. In this study, we investigated whether cortisol affect gonadal sex differentiation in carp to verify the generality of the interaction between gonadal and interrenal endocrine systems. Genetically controlled female (all-XX) siblings were fed with cortisol (1, 0.1mg/g diet) or corticosterone (1mg/g diet) for 8 months. Six months after the treatment, gonadal tissues were subjected to the histological evaluation of gonadal status. Gonadal masculinization was highly induced by high dose of cortisol (1mg/g diet), whereas low dose (0.1mg/g diet) resulted high percentage of gonads difficult for sexing (undeveloped or sterile), and this masculinizing effect was reversed by cotreatment with estrogen. Corticosterone (1mg/g diet) showed lower effect than cortisol, resulting in ovotestis, while genetically female (all-XX) inbred strains with extremely elevated plasma corticosterone was reported. The results show that cortisol can induce defeminization and/or masculinization by suppressing the estrogenic potentials at least in pharmacological dose, and that high level of suppression of estrogenic effects can induce masculinization while low level only suppress ovarian development resulting in unsexable (undeveloped) gonad.

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The endocrinal responses to long-term acoustical stress in milkfish (*Chanos chanos)*

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Strong underwater acoustic noise from shipping, seismic experiments or sonar has been know that may cause hearing loss and actual stress in teleost. However, the long-term physiological effects of relatively weak but continuous ambient sound on fish were less understood. In present study, milkfish, *Chanos chanos*, were exposed to the wind farm noise either weak (WN, 109 dB re 1 μPa at 125 Hz; approx.10-100m away from the wind farm) or strong (SN, 138 dB re 1 μPa at 125 Hz; nearby the wind farm) conditions for 1, 3 and 7 days. Comparing to the control group (CC, 80 dB re μPa at 125 Hz), the SN fish had higher plasma cortisol levels than other groups in the first day. Subsequently, the plasma cortisol levels of those fish returned to the resting levels quickly, but the noise exposure increased head kidney *cyp11b1* (11β-hydroxylase, which converted 11-deoxycortisol to be cortisol) mRNA levels at both 3rd and 7th day. Nevertheless, there was no difference was found neither in plasma cortisol levels nor in *cyp11b1* mRNA levels between CC and WN at all times point. Moreover, noise exposure did not change hypothalamus *crh* (Corticotropin-releasing hormone) mRNA levels. This study showed that the continuous noise may up-regulate the gene that related to cortisol biosynthesis, which possibly made the fish more susceptible to other stressors. However, *crh* expression may not involve in the regulation pathway. The influences of continuous ambient sound were, of course, depending on the sensory ability of the animals. As far as earlier results are shown, sound pressure of the offshore wind farm noise of the WN condition was far lower than the hearing thresholds of milkfish.

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Zebrafish locomotion profiling as a tool to study behavioural toxicity of endocrine disrupting chemicals

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The presence of endocrine disrupting chemicals (EDCs) in the aquatic environment can have severe effects on the health of aquatic organisms. Numerous anthropogenic EDCs, such as plasticizers, fire retardants and antibacterial agents, enter the aquatic ecosystems via effluents from wastewater treatment plants and land runoff. Many of these man-made chemicals are emerging pollutants and have been shown to affect the development of fish, affecting brain formation, hormonal disruption, exerting neurodevelopmental disorders, among other adverse effects. Previous studies have mainly focused on single compound exposures or simple mixtures at high doses which does not reflect the complex mixtures of EDCs at low concentrations present in the aquatic environment. As a consequence, the risk may be underestimated and the effects of low doses of EDCs and mixtures of these compounds need further evaluation. The aim of this project was to examine the behavioural toxicity of low concentrations of EDCs on developing zebrafish (*Danio rerio*). Since EDCs can have impacts on fish behaviour through acting on endocrine pathways, behavioural effects can potentially be used as sensitive indicators for sublethal toxicity of these compounds. We tested a number of single compounds (BPA, BPS, 4-nolylphenol, TBP, DBP) as well as a complex mixture consisting of phthalate metabolites, triclosan and polyfluorinated alkyl substances in order to examine the effects on fish behaviour. Zebrafish larvae were exposed to the selected toxicants during early developmental stages and the locomotion and sensory integrity of the larvae were studied using an automatic tracking system with a custom-made protocol. At low and environmentally relevant exposure levels, behavioural disruption (hyperactivity, hypoactivity, loss of sensorimotor integrity) was detected. With this study, we present a behavioural screening approach that may be used as a non-invasive alternative to animal testing and as a sustainable tool for high throughput toxicity screening of emerging environmental pollutants, such as EDCs. Once coupled with established biomarkers, it can also be used to provide mechanistic understanding of the observed effects. This framework could potentially be used to improve existing risk assessment procedures with endpoints of biological integrity, higher ecological relevance and sensitivity.

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Differential cis-regulatory element in promoter of duplicated somatolactin genes is related to estrogen response of *slβ* in *Cyprinus carpio.*

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Somatolactin (SL),a piscine hypophyseal hormone belonging to growth hormone (GH) and prolactin (PRL) superfamily, is involved in background adaptation, osmoregulation, reproduction and fatty acid metabolism which might be affected by estrogenic endocrine disruptor compounds. Two somatolactin genes were identified *in silico* and transcripts of both were detected in pituitary of *Cyprinus carpio*. Only *slβ* but not *slα* responded with increased expression in pituitary of male adult carp to 17β-estrogen treatment respect to control as shown by RT-qPCR analyses. In addition, the proximal promoter region of *slα* and *β* was cloned using inverse PCR, transcription start site was determined with 5'RACE for 5´-UTR. Indeed, in the proximal promoter of slβ, but not in slα, an estrogen response element (ERE) was predicted with Tfscan software and specific binding was confirmed in vitro by electromobility shift assays (EMSAS) with pituitary nuclear extract. To clarify the role of this ERE in SLβ response to estrogen in a functional assay, two constructs were assembled, one containing wild type proximal *slβ* promoter and another with the same sequence but mutated ERE (EREmut) in front of *firefly* luciferase coding sequence. The assay was performed in rat pituitary cells GH3/BH6 cotransfected with a control *renilla* luciferase plasmid. Cells were treated with 17β-estrogen, and *firefly* luciferase was measured and normalized with *renilla* luciferase. Clearly, wild type *slβ* promoter plasmid showed increased luciferase activity in response to estrogen correlating perfectly with the *in vivo* expression data. However, *slβ* promoter with EREmut plasmid showed no significant variation in luciferase activity, indicating that this particular ERE is related with the differential expression of *slβ* in response to 17β-estrogen. Taken together these data suggest that SL could serve as early indicator of neuroendocrine disrupting effects to assess biologically relevant changes in the aquatic environment.

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Stanniocalcin secretion is mediated by a soluble adenylyl cyclase in seabream *Sparus aurata*

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Stanniocalcin (STC) is an endocrine factor first identified in fish and recently identified in mammals, and several phyla including invertebrates. STC actions in fish have been described as hypocalcemic and, a link with plasma calcium regulation is undeniable. However, recent reports provide evidence for the involvement of STC in the regulation of acid-base balance. To date, there is extremely limited information regarding the regulation and control of STC synthesis and secretion, mainly due to the ubiquitous nature of the STC- producing cells. However, fish happen to be unique models for the study of both processes, as they present a specific STC-secretory gland known as the corpuscles of Stannius (CS). Here we investigated several of the synthesis and release pathways of STC by *ex-vivo* and *in-vitro* incubations of the CS of a teleost fish, the seabream. In isolated cultured CS, increased levels of either calcium or bicarbonate, alone, increased STC secretion. However, when both treatments were combined high levels of calcium and bicarbonate revealed a synergic massive secretion of the hormone (up to ~20 fold increase). This secretion stimulation is hereby confirmed to occur through cAMP pathway produced by a soluble adenylyl cyclase (sAC). Manganese, which acts as a selective stimulator of sAC also results in an increase of cAMP levels in the CS. The use of sAC inhibitors exposed differentiated mechanisms of action regarding the already known sAC enzymes, highlighting a feasible new distinctive fish sAC. These results suggest that bicarbonate directly stimulates STC synthesis and release through the mediation of a soluble adenylyl cyclase.

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PO-55

Aquatic acidification: a comparison of the behaviour of marine and freshwater stickleback under elevated pCO_2

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Ocean acidification, the decrease in ocean pH caused by anthropogenic emission of carbon dioxide, can cause behavioural disturbances in marine teleost species. The proposed mechanism is that CO_2 interferes with the functioning of the major inhibitory neurotransmitter GABA. When fish compensate for the CO_2-induced acidosis they take up bicarbonate ions (HCO_3^-) and in exchange extrude chloride ions (Cl^-) into the water. This reduces the electrochemical gradient for Cl^- influx, such that when GABA binds to the GABA-A receptor, Cl^- ions flow out of the neuron causing depolarization rather than the normal hyperpolarization. Whether freshwater fish are also affected by end-of-the-century pCO_2 is only starting to be investigated. Freshwater pH and pCO_2 vary more over space and time and it has previously been argued that freshwater fish therefore might have evolved greater tolerance to pH fluctuations. However, freshwater-raised pink salmon show much the same alterations in anti-predator behaviour and anxiety as marine species. I will compare the results of behavioural tests on activity and anxiety between two closely related species of stickleback, nine-spined stickleback (*Pungitius pungitius*) from a freshwater population and three-spined stickleback (*Gasterosteus aculeatus*) from a marine population. The results will provide a greater understanding of whether CO_2-induced acidification can impact freshwater fish in the same way as marine species.

PO-56

Evaluation of gemfibrozil effects in a marine fish combining ecotoxicogenomic tools with conventional endocrine and biochemical biomarkers

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The information on the potential hazardous effects of gemfibrozil (GEM) in marine fish is extremely scarce. In the current study, molecular, endocrine and biochemical parameters were assessed in *Sparus aurata* after 96 h waterborne exposure to a range of GEM concentrations. Hepatic mRNA abundance of target genes known to be regulated via peroxisome proliferator-activated receptor α (PPARα) in mammals, such as apolipoprotein AI (APOA1) and lipoprotein (LPL) was significantly increased even without a concomitant activation of the PPAR pathways. Furthermore, an environmentally relevant concentration of GEM (15 $\mu g.L^{-1}$) induced an upregulation in the mRNA levels of interleukin 1β (IL1β), tumour necrosis factor-α (TNFα) and caspase 3 (CASP3). These results suggest that waterborne exposure to environmental levels of GEM may lead to an activation of proinflammatory processes in *S. aurata* liver. However, the transcriptional of genes related with the antioxidant defence system and cell-tissue repair was unaltered under the present experimental conditions. Higher levels of GEM induced a cortisol rise, an indication that it is recognized as a stressor by *S. aurata*. Cortisol levels and the mRNA levels of IL1β, TNFα and CASP3 may be suggested as potential biomarkers of GEM effects in marine fish.

PO-57

The influence of environmental pollution in the vitellogenesis of *Astyanax fasciatus* (Characiformes: Characidae)

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Chemicals present in the aquatic environment can alter the physiological processes of fish, such as reproduction. The aim of this study was to investigate the influence of environmental pollution on vitellogenesis of *Astyanax fasciatus* in two reservoirs from the same basin, with different degrees of eutrophication, located in São Paulo State (Brazil). Adult females were sampled during two years in the Ponte Nova (PN, reference site) and Billings (Bil, polluted site) reservoirs. Water samples were collected to evaluate physical and chemical parameters. Ovarian histology; relative fecundity (RF); gonadosomatic index (GSI); estradiol plasma levels (E2); VTG plasma absorbance; VTG and estradiol receptor (ER) gene expression were analysed. The water analysis confirmed the presence of pollutants in Bil, mainly high metal levels. Ovaries sampled in both reservoirs were mature throughout the year. Females from PN maintained a seasonal variation in GSI, FR, plasma E2 and plasma VTG, while these variables remained almost unchanged in animals from Bil. Females living in Bil showed higher GSI, FR, and plasma E2 than PN ones, mainly during the non-breeding season. VTG and ER gene expression did not change neither during the year nor comparing both reservoirs, except for an unexpected VTG peak in Bil during summer. *A. fasciatus* showed a high allostatic capacity to adjust the physiological set-points, allowing vitellogenesis in an impounded environment, at the expense of high E2 levels.

PO-58

Estrogen-Related Receptor α Participates in H^+ Secretion in Zebrafish

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Estrogen-related receptor α (ERRα), an orphan nuclear receptor, is an important regulator for adaptive metabolic responses under conditions of increased energy demands. ERRα was found to be expressed in zebrafish embryonic skin showing the “pepper and salt” distribution pattern of skin ionocytes, which conduct energy-consuming ionic and acid-base regulation for zebrafish. The present study aims to test a hypothesis if ERRα plays some roles in ionic and acid-base regulation mechanisms in zebrafish. In situ hybridization indicated that ERRα mRNA was located in H^+-pump rich cell, a group of ionocytes responsible for Na^+ uptake and acid-base regulation. ERRα expression was significantly stimulated by acidic (pH 4) water, but not by water with different Na^+/Cl^- concentrations, suggesting its possible relevance to acid-base regulation. Knockdown of ERRα by antisense morpholino oligonucleotides significantly impaired H^+ secretion at the yolk sac of embryos. Expressions of proton secretion-related transporters and metabolic genes were reduced in ERRα morphants, suggesting that ERRα affects the ability of H^+ secretion through regulating abundance of transporters as well as energy metabolism. Taken together, this study demonstrated, for the first time that ERRα is required for transepithelial H^+ secretion for systemic acid-base homeostasis.

PO-59

Effects of different light wavelengths from LEDs on oxidative stress and apoptosis in olive flounder (*Paralichthys olivaceus*) at high water temperatures

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We investigated how different light spectra affect thermal stress in olive flounder (*Paralichthys olivaceus*), using light emitting diodes (LEDs; blue, 450 nm; green, 530 nm; red, 630 nm) at two intensities (0.3 and 0.5 W/m^2) at relatively high water temperatures (25 and 30 °C, compared to a control condition of 20 °C). We measured the expression and activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and the levels of plasma hydrogen peroxide (H_2O_2) and lipid peroxidation (LPO). Furthermore, the levels and mRNA expression of caspase-3 were measured, and terminal transferase dUTP nick end labeling (TUNEL) assays and comet assays were performed. The expression and activity of antioxidant enzymes, as well as plasma H_2O_2 and LPO levels were significantly higher after exposure to high temperatures, and significantly lower after exposure to green and blue light. Caspase-3 levels and mRNA expression showed a similar pattern. The TUNEL assay showed that apoptosis markedly increased at higher water temperatures, compared with the 20 °C control. In contrast, green light irradiation decreased apoptosis rate. Furthermore, the comet assays showed that nuclear DNA damage was caused by thermal stress, and that green light irradiation played a role in partially preventing this damage. Overall, these results suggest that light with green and blue wavelengths can reduce both high temperature-induced oxidative stress and apoptosis, and that particularly green light is efficient for this. Therefore, green light can play a role in protecting in olive flounder from thermal stress damage.

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PO-60

Intensity-dependent effects of green light on the growth performance and endocrine properties of barfin flounder *Verasper moseri*

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We investigated the effects of green light on the growth and endocrine systems of juvenile barfin flounder *Verasper moseri*. The fish, reared in white tanks in a dark room, were irradiated with light from light-emitting diodes (LEDs) with a peak wavelength of 518 nm (green) under a controlled photoperiod (10.5:13.5, light:dark cycle; 06:00–16:30, light). Fish were reared for three weeks under three levels of photon flux density (PFD) — 2.0 (low), 7.0 (medium), or 21.0 (high) $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the surface of the water — at controlled water temperatures 8.1–12.1 °C (average: 10.2 °C) between November 26, 2013 and December 16, 2013. Fish that were irradiated with the high PFD had the highest specific growth rates (% body weight·day^{-1}), followed by fish reared in the medium, then the low PFD. In addition, fish reared under the medium PFD had higher condition factor than those reared under the low or high PFD. The highest average values for voluntary feed intake and feed efficiency ratio were observed in fish reared under the high PFD. Melanin-concentrating hormone (MCH) 1 mRNA was higher in the brains of fish reared under the medium and high PFD than in those reared under the low PFD. Similar results occurred in *mch2* mRNA. No difference was observed in the amount of mRNA for proopiomelanocortin (POMC)-C, orexine, or neuropeptide Y. However, *pomc-a* mRNA content was higher in the pituitaries of fish reared under the low and medium PFD than in those reared under the high PFD. The content of *pomc-c* mRNA was higher in fish reared under the medium PFD than in those reared under the low or high PFD, whereas no difference was observed for *pomc-b* mRNA content. Fish reared under the low PFD contained higher amounts of growth hormone (*gh*) mRNA than fish reared under the medium or high PFD. These results suggest that the endocrine systems of barfin flounder are modulated by a specific wavelength of light that stimulates somatic growth. Notably, the extent of expression of *mch1* and *mch2* in the hypothalamus, and of *pomc-a*, *pomc-c*, and *gh* in the pituitary are closely linked to the intensity of green light.

PO-61

Effects of neuropeptide and sex steroid treatment on pituitary-ovarian axis of pre-pubertal wreckfish (hāpuku) Polyprion oxygeneios in vivo

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Hāpuku (*Polyprion oxygeneios*), also known as groper, is a species with considerable aquaculture potential. In captivity, the species has a generation time of five years, corresponding to an average body mass of 8.6 kg (range 4.6-16.0 kg). From the perspectives of breeding programme design and fish husbandry, a reduction in generation time is desirable to accelerate genetic gain while reducing space and feed requirements for each breeding fish. Puberty advancement by manipulating the brain-pituitary-gonadal axis can potentially be useful in this regard. Thus, we investigated the effectiveness of combining one of either Kisspeptin2 isoform 12 (Kiss2_12) or gonadotropin-releasing hormone analog (GnRHa) peptides with one of three steroid hormones (11-ketotestosterone (11KT), 17β-estradiol (E2) or methyltestosterone (MT)), or each compound singly, in advancing puberty in pre-pubertal (3 year-old) Hāpuku. Each compound was administered via intramuscular slow-release cholesterol-cellulose implants. There was no effect of the treatments on gonadosomatic index. However, a significant interaction effect was found whereby E2 implant seemed to inhibit oocyte growth, but not when in combination with GnRHa. In terms of pituitary mRNA abundance, females were found to have significantly lower copy numbers of the target transcripts, gonadotropin subunits follicle-stimulating hormone-β (fshβ), luteinizing hormone-β (lhβ) and glycoprotein-α (gpα), and GnRH-receptor (gnrhr). Apart from an effect of GnRHa in decreasing pituitary fshβ abundance, there was no other statistically significant effect of the tested peptides on any of the target gene mRNA levels. Pituitary target gene mRNA levels were found to be significantly depressed in fish given androgen (11KT or MT) but not E2 implants relative to control fish. This pattern was consistent regardless of whether androgen was co-implanted with control or one of the two peptides. In conclusion, we were not able to advance puberty in Hāpuku using the combination of peptides and sex steroids above. Instead we may have found evidence for an inhibitory effect of exogenous androgens on pubertal development in this species.

PO-62

Alteration of mRNA Expression Patterns Associated with Post-stripping Oocyte Ageing in Goldfish (*Carassius auratus*)

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A decrease in egg viability and subsequently over-ripening phenomenon is well documented following prolonged oocyte storage *in vitro*, but little is known about the molecular mechanisms involved in the progress of oocyte ageing. To evaluate the maternal mRNA levels in Goldfish (*Carassius auratus*) unfertilized eggs, during post-stripping ageing, changes in gene expression were quantified in oocytes incubated *in vitro* at 20°C for (0-3, 3-6, 6-9, 9-12, and 12-18 h). Additionally success of fertilization in relationship with mRNA expression profiles during oocyte post-stripping ageing for each ageing time was monitored. mRNA levels of each candidate transcripts was measured by real time quantitative PCR. Complete loss of egg viability was observed after 18 h post-stripping ageing of oocytes displaying eightfold decrease in egg fertilizing ability when compared with eggs obtained at the time of ovulation. Differentially expressed genes were related to oxidative injury and stress response (*hsp70*, *cox1*, *sod*) cell cycles (*cyclina* and *jnk*), apoptosis (*ctpb*), reproduction (*vasa*) and developmental competence (*igf2*). Relative mRNA expressions of *hsp70* and *vasa* significantly decreased from 9-12 to 12-18 hours, whereas the levels of *cyclina* and *ctpb* increased as storage was prolonged ($P < 0.05$). Furthermore, the levels of *sod* were significantly reduced, and *igf2* quickly increased from 6-9 to 9-12 hours in aged fish oocytes ($P < 0.05$). We identified several transcripts as potential molecular markers of egg quality associated with post-stripping oocyte ageing.

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PO-63

Effect of GnRHa therapy on spawning performance in Atlantic halibut (*Hippoglossus hippoglossus* L.)

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Wild-captured Atlantic halibut females mature and release eggs of good quality in captivity. However, sometimes females of the F1/F2 generation have been reported to display reproductive dysfunctions, including irregular spawning cycles, low and unstable fertilization, low gamete survival and lower realised fecundity than wild females. In the present study, we tested GnRH agonist (GnRHa) therapy as a means to improve reproductive performance in F1 female breeders. Long-term release implants of GnRHa were implanted into maturing females in two experiments, one pilot study and one commercial trial. In the pilot study, ovarian biopsies were taken from 12 maturing females before treatment, to assess stage of maturity. Four females were implanted with 50 µg GnRH kg^{-1}, and 4 females were implanted with 100 µg GnRH kg^{-1}, while Control fish were sham-injected. All fish were checked regularly for ovulation. All females implanted with GnRHa ovulated within 2 weeks of implantation. One control fish had entered oocyte maturation before implantation, as seen by the occurrence of transparent, hydrating oocytes in the pre-treatment biopsy. This fish spawned spontaneously, at the same time as the implanted females, while 2 of the other control fish released their first egg batch 3-4 weeks later than GnRHa-treated fish. The fourth Control fish did not mature during the experiment. Fertilization sucess was generally low, and only a few batches with >50% fertilization were obtained. In the commercial trial, 5 females were implanted with 75 µg GnRHa kg^{-1} and 5 Control fish were sham-injected. Females given GnRHa completed spawning before the Controls, with acceptable fertilization success. There was some overlap in the timing of the first ovulation between GnRHa-implanted and Control females, due to a naturally high individual variation in the onset of spawning in this species.

PO-64

Social structure affects reproductive development and sex change in giant grouper (*Epinephelus lanceolatus*)

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Giant grouper (*E. lanceolatus*) is a protogynous hermaphrodite that can grow up to 400 kg. Sex change has been reported to be as early as 15 kg and 5 years old. To address commercial need to secure more males of smaller size, we assessed the efficacy of aromatase inhibition (AI) as a method of sex reversal. We implanted ~3 kg giant grouper with Fadrozole (1 mg and 3 mg/kg BW) every 50 days for six months. We followed the levels of circulating sex steroids, and at termination, samples were collected for gene expression and histology. The results showed that within each tank within treatment groups, a hierarchical structure developed. One fish per tank (n=6) exhibited more advanced gonadal male development relative to others. Gonadal histology revealed that the level of transition to masculinity was more pronounced in the AI treatment groups than in the control group. These results were complemented by the levels of plasma sex steroids, gene expression profile and gonado-somatic indices. Traditionally, MT is used to induce sex change in grouper females, however, it is not environmentally friendly and unsuitable for recirculating systems. Previous studies in other grouper species, using similar dosages of AI, showed masculinisation within one lunar cycle. We hypothesise that the slower rate of sex change observed in this study is due to the immaturity of the fish as they had not progressed through maturation to females yet. The clear developmental hierarchy within each tank suggests that giant grouper follow diandric male development, and the AI treatment enhanced the masculinisation of the dominant fish. Diandric development has been reported in a few other grouper species recently[1,2]. The understanding of the effect of social hierarchy in giant grouper reproductive development may change the approach to sex change manipulations in aquaculture operations.

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PO-65

In vivo and *in vitro* effects of genistein on the regulation of autophagy and lipid metabolism in rainbow trout (*Oncorhynchus mykiss*)

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Although several studies have provided important information about the valuable application of soybean products in human healthcare, its use has become controversial in recent years. Soybean products are rich and a primary dietary source of isoflavones, such as genistein (GE). As a food component, GE may have a potential impact on growth, hormonal regulation and lipid metabolism. In this framework, the aim of the present study was to investigate the *in vivo* and *in vitro* effects of GE on lipid metabolism and autophagy regulation in white muscle, liver and adipose tissue, as well as adipocytes in culture from rainbow trout (*Oncorhynchus mykiss*). *In vitro* results from primary cultured adipocytes showed that GE (100 µM) significantly reduces cell viability and lipid accumulation via estrogen receptor-independent mechanisms. Regarding the *in vivo* experiment, plasma parameters showed a differential response after a high (50 µg/g body weight) or a low (5 µg/g body weight) concentration of GE, indicating that it does not have a dose-response relationship. Among the changes, a significant reduction in plasma triglyceride levels was observed with the high concentration of GE in comparison to a control condition in the absence of GE, suggesting a lipolytic or anti-obesogenic effect. With regards to the gene expression data, the high concentration of GE up-regulated hormone sensitive lipase (HSL) and peroxisome proliferator-activated receptor alpha (PPARα) in liver and white muscle, while increased mRNA levels of fatty acid synthase (FAS) and PPAR beta (PPARβ) were found in adipose tissue and white muscle. These results indicated increased lipid turnover especially in white muscle. Moreover, the high concentration of GE also up-regulated the expression of the autophagy-related gene 4b (atg4b) in all tissues, suggesting a clear effect of GE on autophagy and tissue atrophy. Altogether, these findings contribute to improve our knowledge on the regulatory role of GE on lipid metabolism and autophagy in rainbow trout, and indicate the importance of investigating the effects of soybean products in order to improve its inclusion in aquafeeds towards the sustainability of aquaculture.

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PO-66

Effects of intraperitoneal administration of leptin on voluntary feed intake, appetite signalling pathways and metabolism in Atlantic salmon, *Salmo salar*

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Atlantic salmon is commercially a key species in the global aquaculture industry, and serves as a model species for teleost breeding, particularly regarding physiology and nutrition. We have validated an experimental protocol that incorporates a tank-based system for analysis of individual feed intake in Atlantic salmon (N=16), which allows handling and experimental treatments like intraperitoneal (IP) injections and assessments of leptin (Lep) on voluntary feed intake and appetite in Atlantic salmon post-smolts. Individual salmon were placed in small tanks and feed intake monitored until it stabilized at a high level, after which each individual was IP injected with recombinant salmon leptinA1 (rsLepA1). The fish were re-stocked into the tanks and feed intake (hand-feeding 4 times daily) was monitored for up to 4 more days. For all experiments, salmon brain, liver and stomach for qPCR analysis of appetite related genes (*lepa1, 2; lepr1, 2; pomca1, a2, 2s, b; agrp1, 2; cart; npy; pyy; cckl)* were sampled 4 h after the last meal. Plasma was collected and analyzed for levels of the energy-related metabolites triglycerides, glucose, free fatty acids, lactate and D-3-hydroxybutyrate. Lep caused a significant reduction in feed intake for up to 3 days as well as a reduction in specific growth rate, compared to pre-injection and sham controls. Brain *pomca1* and *pomca2* mRNA levels was significantly ($p<0.05$) up-regulated in the Lep-treated fish, consistent with previous findings linking *pomc* to reduced appetite and feed intake. Brain *pyy* expression was significantly ($p<0.05$) down-regulated in fish with reduced feed intake, suggesting that PYY may be involved in the central Lep signaling pathway. Hepatic *lepa1* and *lepa2* mRNA expression was up-regulated in Lep-treated fish, but no significant differences in energy related metabolites in the plasma were found. In summary, a validated IP administration protocol for rsLepA1 has been established, demonstrating regulatory effects of homologous Lep on voluntary feed intake in Atlantic salmon.

PO-67

Establishing ultrasound as a non-invasive method for maturation monitoring in Atlantic salmon (*Salmo salar*)

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Increased use of recirculation facilities to produce Atlantic salmon (*Salmo salar*) smolts has lead to a year-round demand for eyed eggs, which the natural spawning period of Atlantic salmon does not cover. Therefore, controlling and manipulating the time of maturation of broodstock is necessary to extend the spawning period and maintain good quality eggs. The use of gonado-somatic index (GSI) to monitor maturation in females requires the sacrifice of potentially valuable broodfish or use of deceased fish. At final maturation, eggs are ovulated into the abdominal cavity, and the softness of the belly at palpation is used to categorize females as either mature and ready to be stripped, or not yet mature. This method can be subjective and depends on the experience of the person performing the procedure. The aim of this study is to establish use of ultrasound technology as a non-invasive tool for maturational control and sorting of Atlantic salmon broodstock in a commercial aquaculture setting. This requires knowledge of the relationship between ultrasound images and other measures of sexual maturation, such as sex hormones, oocyte development and GSI. Beginning at one year before expected stripping until complete maturation, 20 salmon females were sacrificed once a month. Body weight and length was registered, and a blood sample was drawn for plasma sex steroid analysis. The left ovary was examined with ultrasound by measuring the length and making 3-4 cross-section ultrasound images for later calculation of ultrasound-based volume. Ovaries were then dissected out and weighed, and length and volume of the left ovary was measured. Ovary samples for histological analysis were also taken. The length of the ovary measured by ultrasound will be correlated to the estimated volume of the ovary based on ultrasound images, and used as the basis for a non-invasive volume-GSI (vGSI). The vGSI will be related to measured standard GSI, left ovary volume, sex hormone profiles (T, E2, FSH, LH, MIH, 11-KT) and oocyte developmental stages. The detailed overview of sexual maturation and its relation to ultrasound measurements can be considered a potential standard against which other, off-season, broodfish groups can be compared. This method may also provide a more accurate grading of the final maturation in broodfish leading to fewer gradings and less handling-related stress for the female fish and the handling personnel.

PO-68

Application of recombinant follicle stimulating hormone to manipulate reproduction in giant grouper, *Epinephelus lanceolatus*

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The follicle stimulating hormone (FSH) is classically known to stimulate the early stages of gonadal development and sexual maturation in gonochoristic teleosts. This role of FSH has been demonstrated in a number of species of fish treated with a recombinant form of the hormone. In addition to its well-established function, recent studies show that FSH may have a role in sex change. In the protogynous hermaphrodite *Epinephelus merra*, FSH treatment induced sex change from female to male (Kobayashi et al. 2010). In zebrafish, masculinization was induced when the FSH receptor was disrupted (Zhang et al. 2015). Data from our sex change study in giant grouper using an aromatase inhibitor showed an FSH expression profile pointing to its role during transition from female to male. We are producing a recombinant single chain giant grouper FSH with the aim of using it to manipulate gonadal development in this species, which is known to mature first as females in 3-4 years and >10 kg body weight. Furthermore, we also aim to confirm whether FSH has a role in sex change of this protogynous hermaphrodite whose reproductive biology has not yet been characterised.

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PO-69

Effect of temperature on gonadal development and maturation in the cultured yellowtail *Seriola quinqueradiata*

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The yellowtail *Seriola quinqueradiata* is a very important and popular species for aquaculture in Japan. In recent years, the production of artificial seed has been promoted in order to encourage efficient and stable aquaculture production. However, the effective temperature for gonadal development and spawning is not clear. Therefore, we examined the effect of temperature on ovarian development and endocrine change. Exp. 1) To reveal gonadal development and endocrine change related to the seasonal variation of water temperature, the annual changes in gonadal development, plasma levels of sex steroids, and gonadotropins (GtHs) mRNA expression in pituitary were examined in the cultured female yellowtail. Exp. 2) To understand the effects of temperature on gonadal development and spawning, the yellowtail females were kept at three different temperatures (18, 22, and 26 ^{o}C) for 60 days. Blood, pituitary, and gonad were collected from experimental fish regularly. These samples were taken to measure of plasma sex steroids levels and gonadotropins mRNA (*fsh*β and *lh*β) expression, and to conduct histological observation of gonadal development. In Exp.1, the accumulation of yolk protein into the oocyte (vitellogenesis) accelerated with a rise in water temperature. Final oocyte maturation and spawning were confirmed in April when the temperature reached approximately 18 ^{o}C. The expression pattern of *fsh*β and *lh*β indicated closely correlation with gonadal development. In Exp. 2, gonadal development proceeded gradually over two months under 18 ^{o}C as with the case of wild fish, while the maturation was completed in only one month in fish reared at 22 ^{o}C. However, the vitellogenesis and maturation were not observed in fish at 26 ^{o}C during experimental period. In addition, steroids synthesis and *fsh*β and *lh*β expression were inhibited at 26 ^{o}C. These results suggest that gonadal development and maturation in the yellowtail are regulated by the suitable temperature.

PO-70

Brain serotonin, stress, and agonistic behaviour outcome modulation in a cichlid fish fed with an L-tryptophan supplemented diet

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Serotonin (5-HT) is a neurotransmitter and neuromodulator known to be associated with aggression, stress and reproduction, beyond others. This monoamine synthesis depends on the amino acid L-tryptophan (trp), and so, brain 5-HT levels can be indirectly augmented by incorporating trp in the diet. In a first study we evaluate the effects of a 4 week trp-supplemented diet (TRP) on brain serotonergic activity, and cortisol and sex steroid hormone plasma levels in isolated specimens of the highly social cichlid fish *Cichlasoma dimerus*. Results showed that animals fed with TRP exhibited 1.6 times higher forebrain serotonergic activity and a 3.3 times higher reduction in their relative cortisol levels, with no effects on sex steroid plasma levels. Given that the dietary protocol succeeded in producing changes on brain serotonin, and considering its known relationship with aggression, we secondly focused on the possible effects of the aforementioned diet delivered for 2 weeks on the outcome of 1 hour male dyadic agonistic encounters, and the resultant hormonal profiles. Even though the winner of the contest was always the largest male, independently on the feeding protocol, results showed an increase in the latency to the first attack when at least one of the two males had received a TRP diet, and a higher number of total aggressive displays in the same condition. However, these increase consisted in a higher frequency of non-contact (threatening) displays, with a concomitant reduction of contact ones. At the hormonal level, testosterone and 17β-estradiol plasma concentrations did not differ between feeding conditions or among winners and losers. On the other hand, 11-ketotestosterone plasma levels were 4.5 times higher in winners than losers, independently of the diets. Finally, cortisol levels were always higher in fish that lost, and lower in those winners fed with TRP, with respect to winners fed with a non-enriched diet. These results indicate an effect of trp in the diet on brain serotonin, which probably results in the modulation of the stress response and the development of an agonistic encounter.

PO-71

Mussel meal can substitute fish meal in rainbow trout diet

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Blue mussels are a locally available resource with a large potential as raw material for fish feed, with nutritional characteristics similar to those of fish meal. Rainbow trout were fed three test diets: fish meal (FM) based diet, mussel meal (MM) based diet, or a combination of FM and MM (FMM, 50:50%) based diet. Samples were taken at the onset of the study and after 3, 6 and 9/10 weeks of *ad libitum* and restricted feeding. Plasma growth hormone (GH), insulin-like growth factor I (IGF-I) and cortisol levels were measured and intestinal nutrient transport and integrity studied. There was a general decrease in plasma GH levels throughout both trials, concurrent with an increase in plasma IGF-I levels, an indication of good growth conditions. Although the fish on MM diet had higher plasma GH levels, and the fish on the FM diet had lower IGF-I levels, than the other groups, overall all three diet groups appeared to have similar status of endocrine growth stimulation. The assessment of the intestinal epithelial integrity using the Ussing chamber technique revealed no major differences between fish fed fish meal or mussel meal. The tendency towards decreased transepithelial potential difference in the distal region in combination with higher diffusion rate of ^{14}C-mannitol indicates that MM may cause a certain degree of disturbance of the intestinal barrier. However, this does not appear to have any significant negative effect on the health and welfare of the fish as the growth and condition factor of the fish was similar in all diet groups. Nutrient transport in the anterior intestine, measured as transepithelial L-lysine transport rate, was not affected by diet. Thus, an over-all assessment is that rainbow trout thrived on all three diets, showing active endocrine growth stimulation and rapid growth, not only when fed *ad lib*, but also on a restricted ration. In those terms, mussel meal, either as the sole protein source (MM diet) or as a partial protein source (FMM diet) appears to be a promising replacement for fish meal.

PO-72

Gonadal development of captive F1 wreckfish (hāpuku) *Polyprion oxygeneios* under two different temperature regimes

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A captive breeding programme for *Polyprion oxygeneios,* a wreckfish locally known as hāpuku or groper, has been established in New Zealand. This species is considered a candidate for aquaculture but a number of bottlenecks remain a challenge, including highly variable egg quality and deficient data on the growth and reproduction of F1 broodstock. Thus, a core aim of the programme is to gain insights into the key factors controlling the reproductive physiology of captive hāpuku. In this study, we examined the role of rearing temperature and age on reproductive development by evaluating gonadal growth and estimating plasma levels of estradiol-17β (E2) in F1 broodstock (females) during their transition from immaturity to sexually maturing adults. The relative expression of several genes associated with oogenesis (ovarian aromatase (*cyp19a*) and gonadotropin receptors; luteinizing hormone receptor (*lhr*) and follicle-stimulating hormone receptor (*fshr*)) were also traced. Information obtained will provide insights into the age at which F1 hāpuku obtain spawning competency, reveal any reproductive dysfunctions that may rise throughout the reproductive cycle and help develop appropriate broodstock rearing temperatures. Pre-pubertal 4-year-old F1 were divided between two cohorts maintained under the same simulated natural photoperiod but under different water temperature regimes in semi-recirculating systems. One cohort of fish was maintained at constant 17ºC while the other was held at temperatures that changed in a circannual fashion between 10ºC and 17 ºC during two breeding seasons (~ 20 months). Gonad biopsies, morphometrics and blood samples were collected every two months from fish under each temperature regime until females (~10 per group) reached late stages of vitellogenesis. Total RNA was extracted from biopsies and reverse-transcribed to quantify the relative expression of target genes by quantitative RT-PCR. Our findings have identified that female F1 hāpuku obtain spawning competency at the age of 5 years. Relative levels of all target genes remained consistently low in immature fish at the age of four years but increased as oogenesis progressed in five-year-old fish. Estimates of plasma E2 also followed this pattern. The observation that a higher proportion of females reached late-vitellogenesis in the varying temperature group compared to the constant 17ºC temperature group suggests cooler temperatures may be important during oogenesis in this species.

PO-73

Developing a low cortisol responsive line of channel catfish, *Ictalurus punctatus*

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Channel catfish, *Ictalurus punctatus* is the most important farm-raised aquacultured species in the USA. Stressors in aquaculture are unavoidable and stressors exacerbate disease susceptibility of cultured fish. Developing mitigating strategies to overcome stress in aquaculture conditions is a strategy to develop low cortisol responding fish to standardized stressors. The goals of the study were to determine cortisol responsiveness was a heritable trait in channel catfish and to determine if a relationship exist between cortisol responsiveness and performance traits. Low responding (LR) and High responding (HR) parental fish were identified by periodic stress tests. Four pairs of LR and four pairs of HR parental fish were strip-spawned to produce 64 families with four categories of progeny: LRxLR, LRxHR, HRxLR and HRxHR. All the families were produced within a 3-hour interval and hatched in individual aquarium under optimal conditions. After documenting percent fertilization, neurulation and hatch, the density of fish were reduced to 200 fish per aquarium. At the end of six weeks, the fish density was further reduced to 10 fish per aquarium. Excess fish were stocked equally in two 0.25 acre ponds. At 4 months post-hatch, catfish fingerlings raised in individual aquaria were subjected to standardized stress condition to measure plasma cortisol concentration (CORT) and body weight (BW) of individual fish. Traits, BW and CORT were analyzed by a linear animal model in a bivariate setup using ASREML. Heritability estimates were low for body weight (h^2=0.12 $\pm$0.06), and moderate for cortisol (h^2=0.42$\pm$0.09), and the genetic correlation (r_g) between body weight and cortisol was negative (rg=-0.64$\pm$0.24). The cortisol responsive trait was heritable in channel catfish and negatively correlated with body weight. At 6 months post-hatch, 1600 fish raised in ponds were fin-clipped, pit-tagged, weighed (g), measured (mm), and were stocked equally in three 0.1 acre ponds. Progeny are presently genotyped with DNA microsatellite markers to identify parental fish. Cortisol responsiveness of the families will be compared with performance traits of pond-raised channel catfish.

PO-74

Pluripotentiality towards adipogenesis of bone-derived cells from gilthead sea bream

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In the recent years the relevance of adipose tissue participating in both endocrine and physiological processes is becoming clear. In fish, excessive fat accumulation may have negative effects on growth and metabolism. Previous studies have evidenced the pluripotentiality of mesenchymal stem cells (MSCs) towards adipogenesis, while reducing osteogenesis, when induced with a differentiation medium. Thus, it is of great importance to better understand the capability of cells from different origin, especially bone, to become adipocytes. In this sense, we recently established an *in vitro* model of primary MSCs derived from vertebra of gilthead sea bream (*Sparus aurata*) with adipogenic potential. The main objective of this study was to determine their gene expression profile during induced adipogenesis. Bone-derived cells were capable of differentiating into adipocyte-like cells and to accumulate lipids in their cytoplasm after incubation with an adipogenic medium for 4 days. Gene expression levels of bone extracellular matrix components like fibronectin 1-alpha (Fib1α), Matrix Gla Protein (MGP) and osteopontin (OP) remained low and stable during adipogenesis. Contrarily, some bone metabolism-related genes were up-regulated after 20 days, such as bone morphogenetic protein 2 (BMP2), collagen type 1-alpha (Col1α) and runt-related transcription factor 2 (Runx2). Moreover, some of the lipid metabolism-related genes analyzed were also down-regulated during adipogenesis such as: fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), hormone sensitive lipase (HSL), peroxisome proliferator-activated receptor alpha (PPARα), beta (PPARβ) and liver X receptor alpha (LXRα). Overall, the use of an adipogenic medium in these fish bone-derived cells decreases the expression of some bone-related genes, but also most of the lipid metabolism-related genes. This down-regulation can be related to the intracellular accumulation of lipids occurred during the apparent differentiation into mature adipocytes; an issue to be further explored in future experiments. The knowledge acquired on the genes participating in the control of Bone MSCs pluripotentiality towards adipogenesis and their regulation, can help to modulate lipid accretion in fish species in order to improve aquaculture sustainability.

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PO-75

Farming intensification- impacts on the mental and physiological robustness of post-smolt Atlantic salmon (*Salmo salar*)

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It is currently of interest to develop new technology for farming post-smolt Atlantic salmon in closed-containment systems in the sea. The commercial feasibility of these systems relies on maximizing fish density and minimizing specific water flow. Knowledge is needed on the biological limits for salmon in these controlled environments to avoid situations that reduce the fish`s capacity to respond to new challenges and compromise welfare. While, physiological homeostasis can be maintained under chronic mild stress, an additional challenge might result in an allostatic overload impairing physiological and/or cognitive function. An acute challenge test is proving to be a sensitive test of the latent welfare and robustness of fish, and more reliable than acute or chronic stress studies alone. In this study, post-smolts were stocked in sea water tanks at 5 different densities (25, 50, 75, 100 and 125 kg m^{-3}) with a specific water flow of 0.6 L $kg^{-1}min^{-1}$ or in 4 different specific water flows (0.5, 0.4, 0.3 and 0.2 L $kg^{-1}min^{-1}$) with a set density of 75 kg m^{-3}. After 8 weeks fish from each treatment were subjected to an acute crowding challenge. Post-smolts stocked in 125 kg m^{-3} for 8 weeks had decreased growth, osmo-regulatory disturbances and increased plasma cortisol levels. After the acute challenge test, fish in the 75 kg m^{-3} group upregulated important normal stress and neural responses, whereas at the highest and lowest densities fish showed reduced capacity to respond, hence effecting the ability of post-smolts to cope with new challenges and adapt to changing environments. Reducing specific waterflow to 0.3 L $kg^{-1}min^{-1}$ and below induced a typical physiological regulatory response seen with increased water CO_2. However, reduced water flow did not affect growth or the ability to upregulate important neural responses after an acute challenge. This indicates that, within the constraints of this experiment, post-smolts have sufficient physiological and behavioural adaptions to reduced water flow and are able to elicit responses in order to cope with new challenges. Further studies in large scale systems should take these findings as a reference to verify fish density and specific water flow limits for commercial rearing of post-smolt Atlantic salmon.

PO-76

Early onset of puberty at elevated rearing water temperature in red spotted grouper, *Epinephelus akaara*

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It takes quite a long time for the grouper to spawn. In the case of red spotted grouper (*Epinephelus akaara*), at least three to four years of rearing is usually required to reproduce them for the first time. Reproductive control techniques can be applied to repress, delay or advance the onset of puberty. Thus, they can be used to accelerate the process of selective breeding in this species. The present study investigated whether alterations of rearing water temperature (WT) can advance the onset of puberty in the red spotted grouper. Juvenile red spotted grouper (110 DAH, 7.25±0.5 cm, 6.45±1.5 g) were randomly divided into 4 groups and reared for approximately 10 months (from Nov. 2014 to Aug. 2015) at four different WT: natural (12.6-19.5℃), 20℃, 24℃ and 28℃. When they were reared at 24 or 28℃ WT, sexually mature individuals appeared within 12 months after hatching during their breeding season (Jul. to Aug.). The mRNA levels of reproduction-related genes such as Kisspeptin, GnRH, FSHβ and LHβ were higher at these rearing WT than at natural or 20℃ WT ($P<0.05$). Mature yolk stage oocytes (≥400 μm diameter) were found in the ovaries of female red spotted grouper reared at 24 or 28℃ WT, while only oogonia were found at natural WT and peri-nucleolus stage oocytes were observed at 20℃ WT, respectively. Moreover, males were found to produce sperm only at 24 or 28℃ WT. The one-year-old mature females ovulated 6-10 ml of eggs that corresponded to 10% of their body weight. In artificial fertilization performed at 24℃ WT, the fertilization and hatching rates were determined to be 95% and 97%, respectively. This is the first report demonstrating that rearing at 24 or 28℃ WT can significantly advance the onset of puberty in the red spotted grouper.

PO-77

Welfare, Health and Individuality in Farmed FISH: The WIN-FISH project

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In modern aquaculture, fish are exposed to farming-inherent stressors that can be detrimental to animal health and welfare. However, it is increasingly clear that stress reactions are different for each individual and therefore, individuality should be included in the concept of animal welfare. Individual differences often take the form of suites of traits, or stress coping styles (SCS), where traits like sympathetic reactivity, aggression and the tendency to follow and develop routines show positive relationships. In addition, these traits often show a negative relationship with plasma cortisol levels and are also associated with differences in immune function. The main aim of the WIN-FISH project is to investigate the relevance of fish individuality when assessing fish welfare and performance under different culture conditions. The WIN-FISH consortium, consisting of 6 partners in 5 countries, will validate behavioral and physiological welfare indicators for sea bass, sea bream and rainbow trout at the individual and rearing unit level. This will generate new information about responses to environmental factors, knowledge that can be applied to improve husbandry and management practices. Modern recirculating aquaculture systems (RAS) related-stressors such as higher rearing densities and water quality parameters may challenge the welfare of fish. In WIN-FISH, health, welfare and production related effects of RAS rearing of sea bass and sea bream kept at different densities will be monitored. In order to account for individual variation, these studies will be performed on fish screened for SCS. Similarly, in flow through systems, health, welfare and production related effects of rearing densities will be further investigated in sea bream differing in SCS. It is also known that, in general, environmental enrichment has positive effects on animal welfare. WIN-FISH will investigate effects of environmental enrichment on rainbow trout with contrasting SCS. In an attempt to generate genetic markers for selective breeding of farmed Atlantic salmon, a genome-wide association analysis will be performed on salmon with divergent SCS, focusing on proactive fish differing in aggressive behavior. Finally, zebrafish will be used as a model to gain additional knowledge on mechanisms underlying SCS and aggressive behaviour.

PO-78

Analysis of steroidogenic pathway key transcripts in interrenal cells isolated by laser microdissection (LMD) in stressed rainbow trout

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An assessment of the key transcripts expression of the steroidogenesis-related genes in rainbow trout subjected to either acute or chronic stress was performed in both interrenal cells and whole head kidney tissue. The analysis of interrenal cells was possible thanks to the use, for the first time in this specific type of cells, of the technique of laser microdissection (LMD) which allows to isolate specific cells and process them independently of other surrounding cells in the tissue. The results indicated that both acute and chronic stressors induced a significant up-regulation of the steroidogenesis-related genes with a higher but expected degree in the isolated cells. In addition, under acute stress a delay between cortisol levels and transcript expression was found, whereas under chronic stress a clear relation between plasma cortisol levels, mRNA transcription and interrenal tissue area was observed, since all parameters were concomitantly increased at day 5 after stress. Moreover results also indicated that the LMD technique allowed ascertaining with more precision and accuracy whether and when the steroidogenesis-related genes were significantly expressed, disregarding the noise produced by other cells present in the head kidney. Results also showed a typical physiological response in plasma parameters and a positive relationship between plasma cortisol data and transcript abundance in isolated cells. The present results may help to better understand the mechanisms behind the interrenal response to stress challenges in fish.

PO-79

Characterization of the transcriptional coactivator Ncoa7 and its role during the onset of puberty in European sea bass (*Dicentrarchus labrax*)

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Puberty comprises the developmental period that leads to the first successful reproduction. In European sea bass (*Dicentrarchus labrax*) intensive rearing results in a high percentage of precocious maturation one year before the physiological onset. Recent studies in European sea bass using an hemigonadectomy approach and transcriptomic analyses have revealed several genes potentially involved in the onset of puberty in female gonads. In these studies histological observation of the developmental stage achieved by the non-extracted gonad and plasma profiles of different reproductive hormones were used to classify the fish into precocious or non-precocious. RNA samples from precocious and non-precocious extracted ovaries were subsequently hybridized on a sea bass specific microarray for the identification of differentially expressed genes. One of these genes is *ncoa7* that encodes a nuclear receptor co-activator that, in mammals, increases the transcriptional activity of the estrogen receptor. This study is aimed at the molecular and functional characterization of the *ncoa7* gene. We have cloned the complete cDNA of sea bass *ncoa7* and studied its expression in different tissues showing high expression in brain and gonads. Functional analyses using HEK293 cells co-transfected with Ncoa7 together with the estrogen, androgen and progesterone receptors, and exposed to E2, Testosterone or 17,20β-P, show that Ncoa7 is able to increase the transcriptional activity of all the different receptors, suggesting that can control different processes in early gametogenesis. Finally, quantification of the expression of *ncoa7* in the ovary and testis of adult sea bass during a whole reproductive cycle showed different expression patterns among stages, reinforcing the hypothesis that Ncoa7 could be involved in the onset of puberty in sea bass.

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PO-80

The role of Jagged-Notch signalling for the development of *ff1b/nr5a1a*-expressing interrenal tissue in the zebrafish

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In the zebrafish, developmental processes of the pronephros and the interrenal tissue, counterparts of the kidney and the adrenal cortex respectively, are temporally and spatially highly parallel. While interrenal precursors expressing *ff1b/nr5a1a*, the equivalent of mammalian SF1, are derived from *wt1b*-expressing pronephric primordia at about 21-somite stage; how the *ff1b/nr5a1a*-expressing interrenal precursors are specified within and then segregated from the pronephric field remains unclear. The Notch signaling controls cell-fate choices and is implicated in various aspects of kidney development and disease. Our results demonstrated that both the size and the extent of functional differentiation of interrenal tissue were significantly increased upon the inhibition of the Notch signalling, and the specified interrenal lineage was unable to be segregated from the pronephros. While the Notch pathway was conditionally activated during the interrenal specification, functional differentiation but not specification of interrenal tissue was drastically compromised. In embryos deficient for the Notch ligand Jagged molecules, which were localized at both *wt1b*-expressing and *ff1b/nr5a1a*-expressing cells, segregation between pronephric podocytes and steroidogenic interrenal cells was defective, suggesting that local Notch activity ensures proper parallel development of kidney and interrenal tissue. In summary, our results indicate that Jagged-Notch signalling is required for (1) the segregation of interrenal tissue from the pronephros; and (2) modulating the extent of functional differentiation in the steroidogenic interrenal tissue.

PO-81

Identifying the effects of leptin in rainbow trout metabolism

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Metabolism is defined as the overall physical and chemical processes that either exchange or use energy that occur within a body. The 16KDa peptide hormone leptin is involved in multitude of processes such as growth, maturation, stress signaling as well as appetite and energy balance regulation. In some fish species such as rainbow trout, plasma leptin levels tend to increase during prolonged periods of fasting. In order to investigate the effect of leptin in metabolism, measurements of the O_2 consumed by fish implanted with control, low and high leptin dose cholesterol implants were performed. From these measurements, resting metabolic rate (MO_2) was obtained. Leptin implanted fish at both concentrations showed an overall lower MO_2 than the control group, indicating that leptin reached its peak suppressor effect at 0.6 ng leptin g fish^{-1}. % wet weight loss was higher in the control than in the low dose group. Altogether these results indicate that leptin acts as a metabolic suppressor, reducing energy expenditure during times of food scarcity. Further analyses are being performed on the enzymes involved in lipid metabolism such as lipoprotein lipase (LPL), fatty acid synthase (FAS), 3-hydroxyacyl CoA dehydrogenase 2 (HOAD2), carnitine palmitoyl CoA transferase 1 (CPT1) and adipose triglyceride lipase (ATGL) in the liver.

PO-82

The integration of growth and immunity in salmonids: a transgenic and proteomics approach

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Energetic resources are finite in nature and must be balanced across different physiological systems. For salmonid fishes, the reallocation of energetic resources stored in skeletal muscle occurs routinely, for example during migration, fasting, sexual maturation and host defence responses to pathogens. However, the mechanisms underlying such processes remain poorly characterized. We are exploiting immune-challenged Coho salmon transgenic for growth hormone (GH) – a central anabolic pathway in vertebrates. We hypothesize that GH-transgenic fish have an attenuated scope to reallocate resources away from growth into immunity, owing to ubiquitous growth signalling. We compared skeletal muscle responses of GH-transgenic and wildtype Coho salmon undergoing immune responses to a viral mimic (PolyIC). Label-free protein extracts were analysed on a Q-Exactive-Plus mass-spectrometer (n=5 for four treatments: GH-transgenic: control and PolyIC; wild fish: control and PolyIC). Data was analysed using MaxQuant, with protein identification performed against a complete salmonid protein database. Over 1,000 proteins were identified, representing diverse functions such as growth, metabolism, signalling, intracellular transport, structural roles, and immunity. A marked proportion of muscle proteins were significantly regulated by GH-transgenesis. However, we detected only minor modifications to the muscle proteome owing to PolyIC, despite verification of a strong immune response at the mRNA level. GO enrichment and KEGG pathway analysis are currently being performed and initially point towards major changes in the expression of ribosomal proteins in GH-transgenic fish. This work is part of our wider project aiming to elucidate the molecular mechanisms allowing resources to be managed between growth and immune phenotypes in salmonids.

PO-83

Chronic ghrelin treatment stimulates food intake and growth in Atlantic salmon

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Ghrelin modulates food intake in fish, but experiments investigating its potential long term effects, as well as possible environmental influence on its effects, are limited. The aim was to clarify if ghrelin has a long term effect on food intake, hypothalamic appetite regulatory genes, food conversion, and nutrient uptake and growth of Atlantic salmon reared in different salinities. Subgroups of fish were implanted with cholesterol-based ghrelin implants or with sham implants. The fish were acclimated to and kept for 4 weeks post-implantation in three salinities, 12, 22 or 34 ppt, until sampling. Food intake was measured on tank basis and the data show that ghrelin-treated fish had a higher appetite which was reflected in a 20% higher specific growth rate in comparison with the control fish. To investigate potential mechanisms behind the stimulatory effect of ghrelin on growth and appetite the gene expression of appetite genes (inhibitory: *crf, crfr1, pomca1, pomca2*) (stimulatory: *npy, agrp*) in the hypothalamus was analysed with qPCR. The results show that the expression of *npy* was significantly influenced by salinity, being lower at 34ppt than at 12 ppt. There was trend for an interaction effect of treatment and salinity on *pomca1* mRNA expression ($p=0.055$) and a similar but weaker trend for *pomca2* ($p=0.01$). The effect of ghrelin on body weight gain was independent of salinity. Ghrelin did not influence adiposity of the fish, as measured by condition factor and liver somatic index (LSI). Food conversion efficiency was analysed since that could be an alternative mechanistic explanation for possible effects of salinity or ghrelin treatment on growth, but no differences between groups were detected. We also carried out Ussing chamber measurements to assess any effect of ghrelin and salinity, alone or in combination, on intestinal nutrient uptake but no such effects were observed. The ghrelin receptor could be found in the apical membrane of the enterocytes in both the proximal and distal intestine. This implies that ghrelin has other intestinal functions than regulating nutrient uptake. Our conclusion is that ghrelin increases food intake in Atlantic salmon during chronic treatment, but the underlying mechanisms of action need to be further investigated.

PO-84

Characterization of appetite-regulating factors in dourado (*Salminus brasiliensis*): cloning, tissue distribution and effects of fasting and feeding on expression.

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In fish as in other vertebrates, food intake regulation involves intricate networks of appetite regulating hormones, produced by both brain and peripheral tissues that affect feeding centers in the brain to either stimulate or inhibit feeding. These include orexins (OX) and CART (cocaine and amphetamine regulated transcript). Dourado, *Salminus brasiliensis* (Characiforme, Characidae) are freshwater subtropical/tropical fish that are targeted by fisheries and have a non-negligible economical value. Its rapid growth and excellent flesh makes the species a good potential candidate for aquaculture. Despite their importance, the endocrine regulation of feeding and growth has been little examined in these fish. The aim of this study was to characterize appetite-regulating hormones in dourado and examine their tissue distributions. Fragments of cDNAs encoding for these peptides were isolated and their mRNAs were shown to have widespread peripheral and brain distributions, suggesting a variety of physiological roles. To examine the possible role of these peptides in the regulation of feeding and energy status, mRNA expression levels of these peptides were compared between fed and fasted fish and at different times around feeding time. Our results show that both fasting and feeding affect the expression of appetite-regulating hormones in dourado. Our studies could provide new insights on the feeding physiology of dourado, which might lead to significant advances in their farming.

PO-85

Physiological mechanisms behind increased nutrient uptake in salmonids reared in fresh- and brackish water

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The intestine is a key organ for nutrient uptake and osmoregulation in fish. Freshwater (FW) and brackish water (BW) acclimated Atlantic salmon had higher transport of L-lysine and D-glucose than salmon reared in full-strength seawater (SW). Na^+/K^+-ATPases (NKA) located in the basolateral membrane of the enterocytes create the main driving force for both ion-coupled intestinal fluid uptake and nutrient absorption. In SW there may be an increased competition for the sodium gradient so that more of the gradient is directed towards osmoregulation at the cost of nutrient transport. The main utilizers of the Na^+- gradient for osmoregulation in SW are the Na^+, K^+, $2Cl^-$-co-transporter (NKCC2) and Na^+Cl^--co-transporter (NCC), while the transporter rBAT (SLC6A14), amongst others, utilize the Na^+- gradient for amino acid uptake. The first aim of the this study was to clarify if osmoregulatory mechanisms and nutrient uptake compete for the Na^+-gradient in the enterocytes of SW adapted rainbow trout. The intestinal nutrient transport was assessed *in vitro* in Ussing chambers. L-lysine transport was compared between FW and SW rainbow trout and it presented similar differences in nutrient uptake in relation to salinity as previously seen in Atlantic salmon. Thereafter, the relative importance of the utilisation of the Na-gradient for ion coupled fluid uptake versus amino acid uptake was assessed by inhibiting the NKCC2 with bumetanide and NCC with thiazide. None of the inhibitors used resulted in any significant increase in L-lysine transport. Thus, our study does not support that the increased nutrient transport observed in FW and BW in Atlantic salmon is a result of less competition for the Na^+-gradient between osmoregulation and nutrient uptake. The second aim was to investigate possible regulation of the nutrient transport in salmonids, with focus on the growth hormone (GH)-insulin-like growth factor (IGF-1)-system. Atlantic salmon was acclimated to four salinities and growth rate was measured along with sampling of intestinal tissue for gene analysis of GH and IGF-1 receptor. In rainbow trout, Ussing chamber experiments were done to assess a possible direct effect of GH on L-lysine uptake across the intestinal epithelium. Gene expression of GH and IGF-1 receptors in the Atlantic salmon intestine were not significantly affected by salinity. There was no indication that GH influences L-lysine uptake in rainbow trout. This suggests that the GH-IGF-1-system is not likely a main factor regulating nutrient uptake at the intestinal level in salmonids.

PO-86

Daily expression of orexigenic/anorexigenic neuropeptides in Senegalese sole is affected by light spectra, photoperiod and feeding regimes.

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Feeding in fish is a rhythmic process regulated by appetite stimulating (orexigenic) and inhibiting (anorexigenic) endocrine factors secreted in telencephalic, preoptic and hypothalamic brain regions, and influenced by environmental light and temperature. Feeding rhythms have been observed in a wide range of commercial species including Senegalese sole, although information on daily profiles of appetite-regulating factors is scarce. Interestingly, this species undergoes a light-dependent switch from diurnal to nocturnal in feeding behaviour at the onset of metamorphosis. In order to investigate how different light spectra, photoperiods and feeding regimes influence the daily expression of orexigenic and anorexigenic neuropeptides in sole, two different experiments were carried out. In the first experiment, sole larvae were maintained under 12h light: 12h dark cycles of white (LDw), blue (LDb) or red light (LDr) until the completion of metamorphosis. In the second experiment, adult specimens were reared under LD cycles or constant darkness (DD) and different feeding schedules were applied. Daily gene expression profiles of orexigenic (neuropeptide Y and agouti-related protein) and anorexigenic factors (corticotropin-releasing hormone and pro-opiomelanocortin) were analysed using real time quantitative PCR and significant daily rhythms were determined by cosinor analysis during pre- middle- and post-metamorphic stages and in central areas (telencephalon, diencephalon, optic tectum) and pituitary of adult specimens. The expression peaks of appetite-regulating factors were shifted during development from diurnal (pre-metamorphosis) to nocturnal (post-metamorphosis) under LDw and LDb conditions, but were maintained at night under the LDr regime. Moreover, under LD conditions daily rhythms of expression were revealed in some central areas including the telencephalon and the optic tectum as well as in the pituitary. Most of them appeared to be synchronized to the LD cycle, peaking in the light-dark transition or early during the night, irrespective of the feeding time. Under DD, feeding time significantly influenced daily expression rhythms by shifting the acrophases in specimens maintained under different feeding schedules. The persistence of circadian rhythms of transcript levels for some genes under DD conditions in fish fed at random suggests an endogenous control of their expression in certain brain regions. These results could be useful to optimize feeding strategies in sole and contribute towards improving fish farming conditions in this species.

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PO-87

Nesfatin-1-like peptide (NLP) encoded by nucleobindin-1 suppresses food intake and is modulated by steroids, nutrients and daily rhythm in goldfish

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Nesfatin-1 is an 82 amino acid anorexigen encoded in a secreted precursor nucleobindin-2 (NUCB2). NUCB2 was named so due to its high sequence similarity with nucleobindin-1 (NUCB1). It was recently reported that NUCB1 encodes an insulinotropic nesfatin-1-like peptide (NLP) in mice. The first objective of this research was to identify goldfish NLP, its tissue specific expression, and the regulation of NUCB1 in goldfish. Second, we determined whether exogenous NLP has any effects on food intake in fish. Abundance of NUCB1 mRNA expression was detected in several tissues including the hypothalamus, midbrain, hindbrain, muscle and pituitary of goldfish. Western blot analysis found NUCB1 at 55 kDa in goldfish whole brain samples. NUCB1/NLP immunofluorescence was detected in the pituitary, gastric mucosal cells, testicular Leydig cells, and ovarian theca/follicular cells of goldfish. NUCB1 mRNA expression in goldfish pituitary and gut displayed a daily rhythmic pattern of expression. NUCB1 mRNA expression was downregulated by estradiol in goldfish pituitary, while testosterone upregulated its expression in female goldfish brain. High carbohydrate and fat diet suppressed NUCB1 mRNA expression in the brain and gut. A single intraperitoneal injection of synthetic rat NLP or goldfish NLP at 10 and 100 ng/g body weight doses caused potent inhibition of food intake in goldfish during 1-hour post-administration. Synthetic rat and goldfish NLP injection also downregulated the expression of preproghrelin and orexin-A mRNAs, and upregulated cocaine and amphetamine regulated transcript mRNA in goldfish brain. Collectively, these results provide the first set of results supporting an anorectic role for NLP.

PO-88

Establishing muscle specific miRNAs differentially regulated during Methionine Restriction

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As the prevalence of obesity continues to increase in the industrialized world researchers and clinicians alike are working towards novel therapeutic interventions. Metabolic syndrome is an obesity related set of risk factors (i.e. glucose intolerance and abdominal adiposity) that significantly raise the risk of heart disease, diabetes, and stroke. Models of metabolic disorders have shown that dietary interventions, such as methionine restriction (MR), can decrease the risk of these disease processes. Methionine is a concomitant methyl donor in the body; its depletion in the diet is shown to reduce amounts of available S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) leading to decreased global methylation and the MR phenotype (i.e. increased fat oxidation and glucose tolerance). Micro-RNAs (miRNAs) have been shown to be strongly regulated by methylation and are known to regulate both fat oxidation and glucose homeostasis. This project explores micro RNAs (miRs) mediated by methionine restriction *in vitro* using next generation sequencing (NGS), miR array analysis, and qPCR verification of targets. This study utilized cultured Rainbow Trout (*Oncorhynchus mykiss*) myogenic precursor cells (MPCs) depleted of methionine for 48 or 72h. Using a miR array, multiple candidates for regulation were established and initial data was compared to existing NGS sequencing data of the rainbow trout miRNA transcriptome. Combining both of these large data sets a list of 15 potential candidates was created to target largely muscle specific miRs regulated by the depletion of methionine, and these 15 candidates were further explored using qPCR. Candidates found to be heavily regulated by methionine depletion were entered into TarBase v7.0 to explore gene clusters known to be regulated by these miRs. This is the first study to explore the connection between miRs and methionine restriction.

PO-89

The peripheral leptin system in growth hormone (GH) transgenic coho salmon

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GH-transgenic coho salmon have increased growth rate over non-transgenic coho salmons as well as increased appetite. This study aimed to elucidate if these differences are linked to changes in the peripheral leptin system. Three weight-matched (but differing in age) groups were studied; non-transgenic salmon fed to satiation, GH-transgenic salmon given same ration and growing at the same rate as the non-transgenic salmon, and GH-transgenic salmon fed to satiation (the fish in this group are a year younger than the fish in the other two groups to allow stage matching). All groups were sampled before being fed to satiation as well as 1 and 4 hours after feeding. The groups differed in the amount of visceral adipose tissue, with the food-restricted GH-transgenic group having the least amount adipose tissue, while the GH transgenic salmon fed to satiation had the highest visceral adipose storage. Prior to feeding, the feed-restricted transgenic group had lower blood glucose levels than the other groups. When fed to satiation, both the transgenic groups had higher feed consumption than the non-transgenic group. The fully fed GH-transgenic group exhibited a reduction of blood glucose levels 1h after feeding. Plasma leptin levels did not differ among the three groups, neither before nor after feeding. However, there was a difference in hepatic *lepa1* expressions between the non-transgenic group and the food-restricted transgenic group. Furthermore there was a negative correlation between hepatic *lepa1* expression and blood glucose levels. These results indicate that plasma leptin levels in the coho salmon are not directly related to the amount of visceral adipose tissue, and that differences in appetite are likely to be primarily driven by GH. The food-restricted transgenic salmon appear to prioritize energy allocation to length growth, and while the satiation-fed transgenic salmon also grows fast, it has excess energy which is allocated to visceral adipose stores. Blood glucose regulation appears to differ somewhat between the GH transgenic salmon groups, possibly related to differences established in energy stores, and this could be correlated with hepatic *lepa1* expression.

PO-90

Effect of orexin on glycometabolism of the orange-spotted grouper

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Orexin is a very important hypothalamic neuropeptide which regulates feeding behavior, metabolism as well as other physiological activities. We have identified prepro-orexin and two kinds of orexin receptor type 2 (OX2R), OX2RL and OX2RS, from the orange-spotted grouper (*Epinephelus coioides*) in our previous study. In the present study, we try to understand the role of orexin on glycometabolism in teleost. After intraperitoneal injection of orexin-A in the dose of 10ng/g.BW, 100ng/g.BW and 1000 ng/g.BW, it was found that orexin-A could increase the mRNA level of GLUT4 in the liver and muscle of grouper. And the increase of GLUT4 expression level induced by co-injection of orexin-A and glucose was inhibited by SB334867 the antagonist of OXR. It suggested that OXR was involved in the GLUT4 expression regulated by orexin-A. The transcription levels of GS and PK were up-regulated while G6P down-regulated in the liver after co-injection of orexin-A and glucose, on the other hand, orexin-A could significantly block the increase of blood glucose induced by glucose injection. These observations indicated that orexin-A might play a role in the regulation of blood glucose level via its involvement in the glycometabolism in the liver. Treatment of primary cultured hepatocytes with either orexin-A or glucose alone had no effect on the expression of GLUT4, while co-treatment with orexin-A and glucose significantly increased the expression of GLUT4, which was partially blocked by the ERK1/2, JNK or p38 MAPK inhibitors and further blocked by orexin receptor antagonist, the data indicated that orexin-A could stimulate the expression of GLUT4 in a glucose dependent manner in primary hepatocytes via ERK1/2, JNK and p38 signaling. Our results suggested that orexin-A could play a pivotal role in stimulating glucose utilization in grouper, for a long-term goal, which might be useful in reducing the cost of aquaculture industry.

PO-91

Integrated analysis of miRNA and mRNA transcriptomes reveals miRNA targeted pathways during onset of puberty in testis of Atlantic salmon (*Salmo salar* L).

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In Atlantic salmon, a species of great relevance for aquaculture in Northern Europe, precocious puberty in males increases susceptibility to disease, affects flesh quality, reduces growth and cause hypo-osmoregulatory problems (Taranger et al. 2010). It is therefore of high importance to understand the underlying causative molecular processes involved in puberty. Putative molecular regulators of puberty are microRNAs (miRNAs), short 18-20nt RNA species acting as post transcriptional repressors of gene expression. By imperfect basepairing to the 3-prime untranslated regions (3'-UTRs) of targeted mRNAs, miRNAs control the abundance of large sets of mRNAs. In order to detect miRNA-regulated pathways at the onset of puberty in Atlantic salmon testis, we studied the expression of miRNAs and their potential cognate mRNA targets. To identify miRNA controlled processes, we searched for miRNA and cognate predicted mRNA sets that were differentially expressed at the onset of testis maturation and showed opposite expression changes. With this outcome, we performed pathway enrichment analyses in order to reveal integrated miRNA-mRNA targeted pathways that are potentially relevant during the onset of testis maturation in salmon. Our analyses suggest that a miRNA subset might control mRNA targets in several different pathways involved with, amongst others, cell cycle regulation, metabolism, pentose phosphate pathway, Notch, Hedgehog and TGF-beta -signaling as well as targets involved in retinoic acid metabolism and adherens junctions. Future studies will reveal how these miRNA associated pathways may interact to induce puberty In Atlantic salmon.

PO-92

Assessing behavioural toxicity of a mixture of persistent organic pollutants (POPs) using larval zebrafish

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Persistent organic pollutants (POPs) are widespread in the environment, and some are suspected of being endocrine disruptors and neurotoxic. As animals and humans are exposed to complex mixtures of POPs daily, it is reasonable to assess how these pollutants could interact with neurological development. Our aim is to investigate how a POP(s) mixture constructed from human blood data, affects the behaviour of early life stages in zebrafish. The POP mixtures consisted of different compounds, the ratio of which is based on the respective concentration in human blood of the Scandinavian population. Zebrafish embryos/larvae were used to investigate changes in behaviour following exposure to a POPs mixture as well as sub and individual mixtures using a high-throughput behavioural screening test. Zebrafish embryos were housed in 96 well plates and were exposed to a series of sub-lethal doses. Exposure was carried out at 4 – 6 hours post fertilization (hpf) and continued under static conditions until 96 hpf when behavioural tests were undertaken. The distance travelled, time spent active, and swimming speed were recorded during light/dark periods. In order to determine which phase of embryogenesis is most sensitive to the mixture, embryos were also exposed at different time points during embryogenesis. Results indicate that the POPs mixture increases the swimming speed of larval zebrafish, and this is related to exposure between 48 and 96 hpf. This behavioural effect was associated with the perflourinated compounds within the mixture, more specifically with PFOS (Perfluorooctane sulfonate). The findings of this study showed that the POPs mixture affected zebrafish locomotor activity. Further studies will be carried out to determine the POPs mixture/PFOS role in expression of relevant genes involved in behaviour of early life stages in zebrafish.

PO-93

Transcriptional profiles of HPG-axis related genes in female, male and intersex thicklip grey mullets (*Chelon labrosus*)

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The participation of the hypothalamus-pituitary-gonad axis (HPG-axis) in the development of the intersex condition in fish is not understood, although the axis is known to be affected by endocrine disrupting chemicals. On the other hand, the expression profile of certain genes in those organs differs in females and males during the gametogenic cycle. In this work, genes belonging to this neurohormonal system and playing key roles in gametogenesis control were sequenced and analyzed by qPCR in female, male and intersex *Chelon labrosus* thickip grey mullets from the Pasaia harbour (Bay of Biscay), at different gametogenic stages. Partial sequences for gonadotropin releasing hormone-1 (*gnrh1*), gonadotropin hormone common α-subunit (*gth-α*), luteinizing hormone β-subunit (*lh-β*), follicle stimulating hormone β-subunit (*fsh-β*), kisspeptin-2 (*kiss2*) and kisspeptin-1 receptor (*gpr54*) and for luteinizing hormone receptor (*lh-r*) and follicle hormone receptor (*fsh-r*) were successfully obtained in brain and gonads, respectively. Gametogenic stage dependent differences in transcript levels were detected, but without marked sex related differences. Transcription levels of *gnrh1*, *gth-α*, *kiss2* and *gpr54* increased during gametogenesis in females and males. At late gamete maturation stages transcript levels decreased regardless of sex and maintained low during spawning and post-spawning stages in females. However in male mullets transcription levels increased after spawning. Intersex mullets showed a female-like transcript profile during early gametogenic stages, but at maturation, spawning and post-spawning stages they resembled males. In conclusion, transcriptional profiles of genes in the HPG-axis change during gametogenic cycle in *C. labrosus*; both males and females. Intersex mullets showed female-like transcription during gametogenesis but thereafter they behaved like males. Thus, results suggest the participation of neurohormonal signalling in the promotion of intersex condition in mullets. Ongoing work on gonadotropin regulation will offer a better understanding of oocyte formation in testis.

PO-94

Endocrine disruption of phenanthrene in the protogynous dusky grouper *Epinephelus marginatus*

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The dusky grouper, *Epinephelus marginatus* (Serranidae: Perciformes) is a protogynous hermaphrodite, living on rocky bottoms. These fish mature first as female and then ovaries are replaced by testis, based on a complex social structure. As a hermaphrodite species, they maintain high levels of plasma steroids, even when juveniles, as substrates for sex inversion. In many tropical and temperate regions, overfishing and environmental degradation have been depleting wild dusky grouper populations. These fish are exposed to marine pollution, from the vessel traffic and oil spill during cargo handling. Polycyclic aromatic hydrocarbons (PAH) such as phenanthrene (Phe) are the main crude oil components and are toxic to fish, acting as endocrine disruptors (ED), negatively impacting reproduction. This study investigated the effect of Phe as ED on gonadal steroidogenesis in *E. marginatus* juveniles. After the LC_{50} determination, two experiments were designed to evaluate the effects of Phe in *E. marginatus* steroidogenesis. An *in vivo* sublethal exposure (96 h) to Phe was carried out at two concentrations (0,1mg/L and 1 mg/L); exposure to the vehicle (ethanol; EtOH) was also performed. Plasma levels of 17B-estradiol (E2), testosterone (T) and 11-ketotestosterone (11-KT) were measured by ELISA. Gonads, liver and spleen were processed for histological analysis. In an *in vitro* bioassay, gonad fragments were incubated with Phe (50µM) or EtOH. Steroid levels in the culture media were measured by ELISA. The *in vivo* exposure to Phe triggered an increase of the area of the hepatocytes, increased number of melanomacrophagic centers and hemosiderosis in the spleen; EtOH also induced similar effects on spleen. E2 and T levels did not change neither in plasma nor *in vitro* media. Phe sharply reduced 11-KT levels *in vitro* and *in vivo*. In plasma, EtOH also decreased 11-KT levels. Considering the importance of 11-KT in fish developing ovaries, phenanthrene seems to disrupt steroidogenesis in juvenile grouper, possibly being able to cause dysfunctions during sex change.

PO-95

Use of transgenic zebrafish models to study the endocrine effects of natural and synthetic progestins

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As compared to (xeno-)estrogens, natural and synthetic ligands of the progesterone receptor (PR) have been scarcely studied on aquatic organisms while the potential risk posed by these compounds has been recently pointed out. However, there is still a lack of data to accurately characterize the hazards posed by these compounds. Our strategy combines mechanism-based zebrafish bioassays that use new transgenic models expressing Green Fluorescent Protein (GFP) under the control of steroidogenic genes. The *cyp19a1b* gene is ER-regulated and encodes the enzyme Aromatase B, expressed in radial glial cells and responsible for the biosynthesis of estradiol. The *cyp11c1* gene encodes the enzyme 11β-Hydroxylase, involved in the hypothalamus-pituitary-interrenal axis through cortisol biosynthesis. A selection of 26 nPR ligands derived from testosterone, progesterone or spironolactone was screened on the *cyp19a1b*-GFP zebrafish model to assess their potential estrogenic effect. Some of these compounds were further tested on the *cyp11c1*-GFP zebrafish model to characterize their effect on *cyp11c1* expression. We showed that neither progesterone nor progestins structurally related to progesterone had an effect on GFP expression in the *cyp19a1b*-GFP zebrafish model. However, all the testosterone-derived progestins tested so far such as levonorgestrel (LNG) and norethindrone (NET) induced *cyp19a1b* expression in a ER- and concentration-dependent manner. Furthermore, we showed that some of the pro-estrogenic progestins were also able to affect the expression of *cyp11c1* in the interrenal cells. Parallel experiments have shown that *cyp11c1* is up-regulated by dexamethasone, a GR agonist ligand. Our data thus show that some progestins are capable to affect the tissue-specific expression of genes involved in estradiol and cortisol synthesis in developing embryo and larvae. This study demonstrates the usefulness of combining different mechanism-based bioassays that use transgenic fish to characterize the endocrine disruptive potency of emerging aquatic contaminants raising further questions regarding their developmental and reproductive effects

PO-96

Southern California's urban ocean fish show alterations in protein expression within the endocrine disrupted, cortisol-producing interrenal tissue

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Wild fish residing near wastewater treatment plants (WWTPs) located on the coast of southern California have previously been shown to be associated with broad physiological impairments. This recent work has revealed a correlation between exposure to environmental contaminants and an impairment in the production of the stress and metabolic hormone cortisol. This form of endocrine disruption is observed as an inability to activate a normal neuroendocrine response to stress and has been detected in several species, including English sole, hornyhead turbot, and California scorpionfish. When interrenal tissue samples from WWTP exposed English sole were subjected to an in vitro ACTH challenge, it was found that individuals sampled from WWTP outfall areas had impaired cortisol responses. Additionally, the inability of impacted fish to produce cortisol was significantly related to a corresponding drop in expression of key mRNAs in the interrenal, including for steroidogenesis-activation regulator (StAR) and P450-11β hydroxylase. The inability to respond to ACTH stimulation and a reduction in important steroidogenic mRNAs pointed directly to a dysfunctional interrenal gland. Thus, the proteomes of impacted English sole interrenals were analysed to determine alterations in protein expression associated with this form of endocrine disruption. Analyses have revealed that proteins have a varied relationship with cortisol response, with nine proteins showing a negative correlation while nineteen proteins show a positive correlation ($p<0.05$). Protein identification includes 4-aminobutyrate aminotransferase, protein disulfide-isomerase A3, ATP synthase subunit-α, glyceraldehyde-3-phosphate dehydrogenase, and malate dehydrogenase. Multivariate analyses were also utilized to determine the implications of systematic changes in the interrenal proteome. These collective data are beginning to elucidate the molecular mechanisms underlying disruption in this form of interrenal impairment.

PO-97

Di-isononyl phthalate affects the endocannabinoid system and induce fatty liver in zebrafish

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Phthalates, used as plasticizers, have become a ubiquitous contaminant and have been reported to potentially induce toxicity effects in organisms (Oehlmann et al., 2009, Carnevali et al, 2010). Di-isononyl phthalate (DiNP), can interfere as Endocrine Disruptor (EDC) (Chen et al., 2014) but few information are available on its effects. The aim of our study was to assess the effects of DiNP in the Endocannabinoid System (ECS) in zebrafish liver and brain and its effect on hepatic lipid storage. Adult female zebrafish were exposed to DiNP (0.42; 4.2; 42, 420 and 4200 µg/L) for 3 weeks. Quantification of the expression of genes belong to ECS in brain and liver. H&E histology and Image Analysis in liver. Western blot analysis. Statistical analysis using GraphPad Prism 6. The lower concentrations of the DiNP were the most detrimental in the hepatosteatosis, increasing the lipid vacuoles area. The hepatic gene transcription of EC receptors (*gpr55*, pparγ and *trpv1*) were inhibited while *cb1* and *cb2* were not affected. *Dagla*, involved in 2-AG synthesis was increased in fish exposed to lower concentrations, while *nape-pld*, involved in AEA synthesis decreased, as well as *Lptr*. In the brain, the lower DiNP concentrations increased cb1 and *npy* synthesis. No changes in *fasn*. In the last years, ECS has emerged as essential player in energy balance and lipid metabolism. Recently, numerous plasticizers were classified as metabolic disruptors (Migliarini et al, 2011) and few information available on the effects of EDCs on ECS on teleost are limited (Martella et al., 2016). The hepatosteatosis developed with the lower DiNP doses appeared not regulated by the local ECS since the gene expression of the EC receptors were inhibited or not influenced. The hepatic increase of lipids is related with the increase of *cb1* and *npy* in the brain, the latter a powerful enhancer of appetite associated to the down regulation of *Lptr* key gene codifying for satiety molecule (Piccinetti et al, 2010). Further studies are in progress to understand the lack of involvement of *ppary* and *fasn* in the steatosic liver induction by DiNP.

PO-98

Effects of various LED light spectra on antioxidant and immune response in juvenile rock bream, *Oplegnathus fasciatus* exposed to bisphenol A

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Bisphenol A (BPA) is a monomer used in plastics and plasticizers. As an environmental toxin included in industrial wastewater, it contaminates the aquatic environment and is known to cause endocrine disruption in fish. Particular wavelengths of light-emitting diodes (LEDs) are known to affect the endocrine regulation of fish. The present study aimed to investigate the effects of green and red LED light on the antioxidant and immune systems in rock bream (*Oplegnathus fasciatus*) exposed to BPA. We used green and red LED exposure at two intensities (0.3 and 0.5 W/m^2) for 1, 3, and 5 days. We measured liver mRNA expression and plasma levels of antioxidant enzyme superoxide dismutase (SOD) and caspase-3. Furthermore, we measured plasma levels of hydrogen peroxide (H_2O_2), lipid peroxidation (LPO), melatonin, and immunoglobulin M (IgM). DNA damage and apoptotic activity were measured using comet and terminal transferase dUTP nick end labeling (TUNEL) assays, respectively. We found that SOD, H_2O_2, and LPO increased significantly, whereas melatonin and IgM decreased significantly, suggesting that BPA induces oxidative stress and reduces immune function. Likewise, both DNA damage and apoptotic activity increased following BPA exposure. However, we found that exposure to green LED light effectively reduced the detrimental effects induced by BPA, including decreasing DNA damage, apoptotic activity, SOD mRNA expression, and plasma levels of SOD, H_2O_2, and LPO. Likewise, the plasma levels of melatonin and IgM increased. Thus, our results indicate that green light conditions effectively reduces oxidative stress and promotes the immune function.

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PO-99

Exposures of *Solea solea* through diet to persistent organic pollutants induce endocrine disruption not only in parents but also in their offspring

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Aquatic ecosystems in general and littoral areas in particular are submitted to ever growing anthropic pressures. Coastal ecosystems have a high economic and ecological value as they host a large number of species during their life cycle. These species are thus exposed to anthropogenic aggression from very early and highly sensitive stages. This is the case of the common sole *Solea solea*, which reaches coastal nursery one month after hatching, and then settles for a year or two. Indeed, it has been shown that juvenile soles caught in the Seine Estuary are highly contaminated by polychlorinated biphenyls (PCB). Furthermore, soles fed diets spiked with selected persistent organic pollutants (POPs) (PCB or polybrominated diphenyl ethers [PBDE]) not only displayed physiological disruption but also transmitted these POPs to the eggs. Within the frame of the project "Fish'n'POPs", an interest was placed on the neuroendocrine status of contaminated soles and offspring. To tackle this question, animals were exposed through diet to environmentally relevant PCB or PBDE congeners. Exposure started at 6 months of age and ended 5.5 years later. The expression of five genes of the hypothalamo-pituitary axis was monitored by mRNA quantification in the exposed genitors (F0) and offspring (F1). The genes studied included luteinizing (LH) and follicle stimulating (FSH) hormones, thyroid stimulating hormone (TSH), pro-opiomelanocortin (POMC) and growth hormone (GH). We also studied the expression of the genes encoding the arylalkylamine *N*-acetyltransferase (AANAT) and aromatic L-amino acid decarboxylase (AAAD), involved in the biosynthesis pathways of, respectively, melatonin (the hormonal time keeper) and norepinephrine (the neurotransmitter). The pituitary endocrine status appeared disrupted by chronic exposure to PBDE: a dramatic decrease in TSH gene expression was noticed in F0 and unexposed F1. No significant change was seen in the expression of genes of the reproduction axis in F0, while a dramatic and significant decrease in the expression of all pituitary genes tested was observed in F1 from POPs exposed adults. In addition to these disorders, a noticeable decrease in the expression of AANAT and AAAD was also detected in F1. Our data demonstrate the toxic effects of the contaminants and their endocrine disrupting impact in sole genitors as well as offspring. The threat was noticeably severe in the latter. TSH of F1 was particularly affected, which anticipates impact on their growth, metamorphosis and central nervous system development, *i.e.* on their survival.

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PO-100

Turning light into hormonal signaling: mapping the melatonin-receptive cells in the brain of the juvenile Atlantic salmon (*Salmo salar*), and circadian and seasonal variations through smoltification

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Light perception is central for detecting environmental changes and keeping track of the circadian and seasonal status. When specific conditions are met, the integration of the biological signals resulting from this perception can induce physiological and developmental changes, and trigger life history transitions. This process is thus key for adaptation, flexibility and survival. Melatonin is typically a hormone that cycles in the organism according to visual and non-visual light perception, and translates circadian rhythms into hormonal signaling. The seasonal changes in the photoperiod lead to variations in the daily melatonin release and eventually are integrated as a signal for seasonal transitions. When juvenile, freshwater-living parr salmonids reach a critical size, they become responsive to seasonal photoperiod fluctuations, and spring induces a large set of morphological, physiological and behavioral transformations called smoltification. The resulting young smolt is ready to migrate towards the sea and adapt to a very different environment. One of the main driving hormonal systems is the thyroid hormone system. A very important part of this smoltification process concerns the brain, which undergoes a large amount of remodeling and results in changes in behavior, perception, cognition, and neuroendocrine responsiveness. One of our main focuses is to investigate what cells are directly responsive to melatonin signaling in the parr and the smolt brain, how they molecularly and cellularly respond to it, and whether melatonin receptor signaling can be upstream of local gene expression modifications leading to increased responsiveness to thyroid hormone signaling (in particular the thyroid hormone deiodinases). Here we present a map of melatonin-receptive cells in the brain, detected using an antibody against the type 1 melatonin receptors (MT1), and colocalization with different cell type and developmental markers. We compare day and night parr samples and day and night smolt samples.

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PO-101

Photoperiodic preadaptation suppresses the acute transcriptional response to seawater exposure in Atlantic salmon

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The Atlantic salmon (*Salmo salar*) has an anadromous life cycle in which juvenile fish migrate to sea in the second or third year of life. The process of transformation from a freshwater resident "parr" to a migratory "smolt" (known as smoltification) encompasses substantial behavioral, physiological, morphological and metabolic changes, including changes in body conditions, ion- and osmoregulation, buoyancy, color and behavior. The temporal coordination of these changes, ensuring that smolts enter the sea in a spring smolt window, depends on seasonal changes in photoperiod. To investigate the influences of light and seawater exposure on the hypo-osmoregulatory capacity of smolts and associated changes in gill physiology, we subjected groups of salmon parr to three different photoperiod regimes: continuous light (LL control), LL followed by short photoperiod (SP) for 16 weeks and LL followed by SP for 8 weeks, then LL for 8 weeks (LLSPLL). We compared their development and hypo-osmoregulatory ability at sampling points throughout the experiment by analyzing plasma chloride concentration and osmolality after 24 h seawater challenge tests. RNA profiling of gills in all samples was conducted by high throughput sequencing. Our data demonstrate that although LL control fish are able to hypo-osmoregulate over a 24-hour period, they undergo massive changes in gill transcription following SW exposure. Contrastingly, fish taken through the LLLSPLL smoltification sequence hypo-osmoregulated with minimal changes in transcription. This difference in response reflects photoperiodic induction of a key group of transcription factor pathways in the first week following return of SP fish to LL, and then subsequent reprogramming of downstream gene expression. Defining the transcriptional pathways involved in this photoperiodic response cascade will provide unprecedented insight into the temporal control of smolt gill physiology.

PO-102

Gonadal development and expression of sex-specific genes during sex differentiation in Japanese eel

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It has been proposed that gonadal development in eel is related more to body size than to age, but there are different views in the process of gonadal development; moreover, the mechanisms involved in sex differentiation in eel are unclear. The objectives of this study were to investigate the gonadal development and the expression pattern of sex-specific genes during sex differentiation in Japanese eel, *Anguilla japonica*. The elvers were reared from 8-10 cm in total length and for feminization, oral treatment with estradiol-17β (E2) were conducted for 6 months in the elvers. The E2 treated eels were 100 % females and the control (untreated) eels were 100 % males under our experimental condition. We classified the gonads of control eels up to 20 cm long, and E2 treated eels with 15 cm were undifferentiated. Differentiating control eels with 20-40 cm and gonads exhibited PGCs, primary oocytes and/or degenerating oocytes. Fully differentiated testes were found in control eels greater than 40 cm. In E2 treated eels, gonads in 15-20 cm were differentiating eels greater than 20 cm exhibited ovarian lamellae containing oogonia and primary oocytes. Gonad histology revealed that male eel seems to differentiate through an intersexual stage, and female eel would differentiate directly from an undifferentiated gonad. The *vasa*, *figla* and *sox3* transcript levels in gonads were significantly increased during sex differentiation in eels. High *Vasa* expression was in males; in contrast, *figla* and *sox3* favor ovarian differentiation. Transcripts of *dmrt1* and *sox9* were sex-dimorphically expressed in the males during donadal differentiation of eels and showed significant increase during testicular development. The transcript levels of *cyp19a1* were significantly increased during testicular development, but did not show differential expression pattern between male and female eels. These results suggest that *dmrt1* and *sox9* are suitable markers for the testicular differentiation in eel. C*yp19a1* seems not important for ovarian development in E2 induced feminization eels, but may play a role in testicular development.

PO-103

Characterisation of the γ-aminobutyric acid signalling system in the zebrafish (*Danio rerio* Hamilton) central nervous system by real-time quantitative polymerase chain reaction

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In the vertebrate brain, inhibition is largely mediated by γ-aminobutyric acid (GABA). This neurotransmitter comprises a signalling machinery of $GABA_A$, $GABA_B$ receptors, transporters, glutamate decarboxylases (*gad*s) and 4-aminobutyrate aminotransferase (*abat*), and associated proteins. Chloride is intimately related to $GABA_A$ receptor conductance, GABA uptake, and GADs activity. The response of target neurones to GABA stimuli is shaped by chloride-cation co-transporters (CCCs), which strictly control Cl^- gradient across plasma membranes. This research profiled the expression of forty genes involved in GABA signalling in the zebrafish (*Danio rerio*) brain, grouped brain regions and retinas. Primer pairs were developed for reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The mRNA levels of the zebrafish GABA system share similarities with that of mammals, and confirm previous studies in non-mammalian species. Proposed $GABA_A$ receptors are $\alpha_1\beta_2\gamma_2$, $\alpha_1\beta_2\delta$, $\alpha_{2b}\beta_3\gamma_2$, $\alpha_{2b}\beta_3\delta$, $\alpha_4\beta_2\gamma_2$, $\alpha_4\beta_2\delta$, $\alpha_{6b}\beta_2\gamma_2$, $\alpha_{6b}\beta_2\delta$. Regional brain differences were documented. Retinal hetero- or homomeric ρ-composed $GABA_A$ receptors could exist, accompanying $\alpha_1\beta_y\gamma_1$, $\alpha_1\beta_y\delta$, $\alpha_{6a}\beta_y\gamma_2$, $\alpha_{6a}\beta_y\delta$. Expression patterns of α_{6a} and α_{6b} were opposite, with the former was more abundant in retinas, the latter in brains. Given the stoichiometry $\alpha_{6w}\beta_y\gamma_z$, α_{6a}- or α_{6b}-containing receptors likely have different regulatory mechanisms. Different gene isoforms could originate after the rounds of genome duplication during teleost evolution. This research depicts that one isoform is generally more abundantly expressed than the other. Such observations also apply to $GABA_B$ receptors, GABA transporters, GABA-related enzymes, CCCs and $GABA_A$ receptor associated proteins, whose presence further strengthens the proof of a GABA system in zebrafish.

PO-104

Activation of brain steroidogenesis and neurogenesis during the gonadal differentiation in protandrous black porgy, *Acanthopagrus schlegelii*

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Black porgy is a protandrous hermaphroditic fish with a natural model of monosex juvenile stage. At the time of testicular differentiation (in fish between 90 to 150 days after hatching), brain neurogenesis was raised: increases in the number of both Nissl-stained total brain cells and Pcna-immunostained proliferative brain cells were observed, revealing brain cell proliferation in specific area of the diencephalon, such as ventromedialis thalami and posterior preoptic area. Significantly higher expressions of the radial glial cell marker *blbp* and neuron marker *bdnf* were detected in the brain during gonadal differentiation. Strong immunohistochemical staining of Blbp and extended cellular projections were further observed. A peak expression of aromatase (*cyp19a1b*) and increased concentration of estradiol (E_2) were also detected in the early brain. The data demonstrated that during the gonadal differentiation, the early brain had higher activity of E_2 synthesis, cells proliferation, and neurogenesis. To investigate the role of E_2 in the early brain, E_2 or aromatase inhibitor (AI) were given to undifferentiated fish. E_2 treatment stimulated progenitors including up-regulation of *cyp19a1b* and *blbp* expression, and enhanced brain cell proliferation. Conversely, AI reduced brain cell proliferation. The analytic results in castration experiment confirmed gonad removing had no influence on the brain gene patterns and brain cell number. Our data clearly support E_2 biosynthesis in the early brain, and that brain E_2 induces neurogenesis. These peak activity patterns in the early brain occur at the time of gonad differentiation but are independent of the gonads.

PO-105

Identification of two *amh* paralogues and their expression profiles during gonadal sex differentiation of cobaltcap silverside *Hypoatherina tsurugae*

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Our group has recently shown that the master sex-determining gene *amhy* (Y-linked anti-Müllerian hormone) is conserved in two Atherinopsidae (Atheriniformes) species, *Odontesthes hatcheri* and *O. bonariensis*. However, little is known on the distribution and evolution of this gene in other Atheriniforms. In this study, the presence of an *amhy* homologue and its role on testis determination was examined in cobaltcap silverside *Hypoatherina tsurugae,* an Atherinid fish from the northwest Pacific Ocean. As a first step, the full sequence of the coding and non-coding regions of an *amha* homologue was obtained using testicular cDNA and genomic DNA from wild adult animals. Several primer sets were then designed in coding regions and those that gave a pattern with more than 1 fragment were selected and amplified in a large number of wild females and males to check for sex linkage. All fragments were sequenced and the transcription levels of *amha* and the putative *amhy* during gonadal sex differentiation of larvae reared at 22ºC (average temperature of spawning season) were measured by qRT-PCR. All remaining individuals from this experiment were sampled at 11 weeks after hatching (wah) and their *amhy*-based genotype and phenotypic sex was determined. Homologues of *amha* and *amhy* were successfully cloned in cobaltcap silverside. The deduced proteins for Amha and Amhy comprised 511 and 340 amino acids, respectively. The small size of Amhy protein is due to the absence of exons II, III, and V in relation to Amha isoform. PCR analysis with genomic DNA from wild adults and from the progeny reared in captivity at 22ºC revealed a linkage with male sex in 95% and 96% of individuals, respectively. This incomplete linkage between *amhy*-genotype and phenotypic sex, i.e., presumable sex-reversed animals, could be due to environmental effects such as of water temperature on gonadal sex determination. mRNA expression analyses using *amhy*-positive larvae revealed that *amhy* transcripts increased at 6 wah, a period encompassing the presumed time of sex determination and histological differentiation of the gonads. *amha* expression, in contrast, was low during this period.These results suggest that *amhy* is closely linked to sex and likely important for testicular development in cobaltcap silverside. It may be useful as a genetic marker for sex in this species in studies addressing the effects of environmental factors on sex determination and reproduction (see Miyoshi et al.; this symposium).

PO-106

Do non-thermal environmental factors also affect sex determination in the temperature-sex determined pejerrey *Odontesthes bonariensis*?

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The pejerrey *Odontesthes bonariensis* is a gonochoristic species that presents a combination of genotypic sex determination (GSD) and environmental sex determination (ESD) in the form of strong temperature-dependent sex determination (TSD). Temperatures of 17 and 29°C during the first 5 weeks after hatching lead to sex reversal of XY and XX individuals to female and male, respectively, whereas at intermediate temperatures (24-25°C) there is a high concordance between genotypic and phenotypic sex. Masculinization by high temperatures in pejerrey is associated with thermal stress as indicated by increasing cortisol titers. Stress and cortisol have been implicated also in the ESD of other species in response to salinity, background color or the social environment, so it is plausible that pejerrey may show additional forms of ESD. We are conducting a systematic study of the effects of environmental factors other than temperature on the sex determination of pejerrey. Here we report the preliminary results for two factors, tank background color and rearing density. The background color (white, black, light blue, dark blue, green, grey, red) effect was examined using the progeny of two pairs of known genotype (XX-XY), at an intermediate temperature (24±0.5°C) and low density (15 larvae/L). The sex reversal rate of each group was inferred from the results of histological analysis of the gonads and detection of *amhy* gene as the genotypic sex marker. Background color was not associated with any specific trend in sex reversal rates although there were significant differences in survival rates and fish body color. In the rearing density experiment, progeny from one XX-XY pair was stocked in three tank volumes (6.1, 1.6 and 0.4 L) at three different densities (15, 62 and 250 larvae/L) and reared at 24±0.5°C. The tank walls were meshed to allow water exchange between all groups. The results point to an interaction of volume (available space) and rearing density with a higher frequency of masculinization at the smallest volumes and/or higher densities. Also, a trial with a mirror wall tank resulted in increased sex reversal, suggesting that the fish perceive and are stressed by the excessive proximity of conspecifics. These preliminary results suggest that pejerrey has other forms of ESD in addition to TSD. Cortisol levels and gene expression profiles are now being examined to determine the endocrine and molecular bases of the observed effects.

PO-107

Genetic and molecular markers for reduced aggression in Atlantic salmon (*Salmo salar* L.).

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As other vertebrates, teleost fish display divergent stress coping styles ("personality traits"), similar to what has been described as proactive and reactive stress coping in rodents. The routine based behaviour of proactive fish should be an advantage in the confined environment of aquaculture. In addition, proactive fish respond to stress with a more modest elevation of plasma cortisol than reactive fish. However, aggression, which is often a serious problem in commercial aquaculture, is also part of the proactive behavioural profile. This project aims at identifying genetic markers for proactive stress coping and aggression, markers that could allow selective breeding for non-aggressive proactive salmon. So far, we have performed an initial pilot study to evaluate behavioural assays to be used for screening stress coping styles of individual salmon. Behavioural tests were done on both group and individual level in order to more accurately derive a behavioural true profile of each individual. At the end of the test series individual fish were subjected to confinement stress after which blood plasma and brain tissue were sampled. Relationships between neuroendocrine stress responses and behavioural profiles will be investigated.

PO-108

In situ observation of DLK1 and DIO3 tumorsupressor genes in zebrafish

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The 15% of the intracranial tumors are pituitary adenomas. In young adults this is the second most common tumor type. Pituitary tumors can be either hormone producing or hormonally inactive. Adenomas usually are bening, and their symptoms are mostly related to the pressure exerted by the growing tumor on the surrounding tissues: the optic nerve, pituitary gland and hypothalamus. The exact molecular background for the development of adenomas is not yet clear, however the role of several tumor suppressor genes, oncogenes and miRNAs have been revealed in tumor formation. Of these regions, one locus containing the DLK1-MEG3 genes together with several miRNAs have been implicated in tumor formation. This region contains multiple tumor suppressor and miRNA's encoding genes, whose expression were decreased in tumors compared to healthy pituitary. In adult fish adenohypophysis consists of distinct regions and the hypothalamic-pituitary system (HPS) of fish shows great similarity with the mammals, it consists of 3 parts: the hypothalamus, neuro- and adenohypophysis. Anatomic location of the zebrafish pituitary glands of adult is the same with the mammals. The aim of our study was to evaluate whether the zebrafish could be suitable for modelling pituitary adenomagenesis through expression of genes of the DLK1-MEG3 locus. In silico and in situ hybridization were used to evaluate the expression of DLK1-MEG3 locus in zebrafish pituitary. Using in silico study the most important 14 human miRNAs and 6 genes that are known to play role in the development of pituitary adenoma and the oncogene PTTG (pituitary tumortransforming gene) were tested in zebrafish genome. Our results showed that while in human and mouse there is a large genomic conservation in the selected genes which show a high degree of sequence similarity (i.e. Dlk1: 76%; Dio3: 86%; Rtl1: 78%; Pttg1: 81%; Meg3: 79%; Rian: 69%), in zebrafish this was less pronounced (dlk1: 74%; dio3: 71%; pttg1: 91%) with some genes having no zebrafish corresponding genes including miRNAs encoding genes. Expression of the DLK1 (delta-like 1 homolog (*Drosophila*); ID: 101883341, Chromosome 17, NC_007128.6 (1354942.1363588, complement) and DIO3 (deiodinase, iodothyronine type IIIb; ID: 798872, Chromosome 20, NC_007131.6 (54014081.54014983) tumor supressor genes by in situ hybridization in zebrafish larvae (5 dpf) and adult brain sections (6-9 month old) confirmed their expression. Zebra danio may be a useful in vivo model for pituitary tumorigenesis regarding to DLK1 and DIO3 but not for miRNAs.

PO-109

Effects of temperature on sex determination of cobaltcap silverside *Hypoatherina tsurugae* and its usefulness as a bioindicator species of the effects of abnormal water temperatures on fish sex determination

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Sex determination in several Atherinopsid species (New World Atheriniforms) is strongly dependent on the water temperature experienced in early life (temperature-dependent sex determination, TSD) but this phenomenon has not been documented so far in Atherinid species (Old World Atheriniforms). Water temperature changes due to global warming and climate change are of concern world-wide and may affect the reproduction of species that have TSD (for example, by causing extremely unbalanced sex ratios). We have recently shown that the cobaltcap silverside, an Atheriniform from the Northwest Pacific Ocean, possesses the Y-chromosome-linked anti-Müllerian hormone (*amhy*). Species that present both TSD and a chromosomal sex marker may be useful indicators of the effects of climate change on fish reproduction, for example by monitoring mismatches (sex-reversal) between genotypic and phenotypic sex. In this context, the primary goal of this study was to clarify the effects of water temperature on sex determination of cobaltcap silverside. For this purpose, fertilized eggs were collected and incubated at 22°C until hatching. Newly hatched larvae were divided in three temperature groups and reared until 13 weeks after hatching (wah) at 18, 22, or 26°C. All larvae in the three groups were collected at the end of the experiment, individually labeled, fin-clipped for genomic DNA extraction and PCR using *amhy*-specific primers to determine the genotypic sex, and fixed for histological analysis of phenotypic sex. Finally, a field study to assess the presence of sex reversals in wild populations were conducted. The sex ratio at 18°C was female-biased whereas that at 26°C was male-biased. In contrast, the sex ratio of fish reared at 22°C did not differ significantly from 1:1. Comparison of phenotypic and genotypic sex of each individual at 11 wah revealed that the percentage of XX-males and XY-females at 18, 22 and 26°C were 0, 11, and 42%, and 67, 10, and 8%, respectively. Screening of wild *H. tsurugae* (n=337) collected in Tokyo Bay, Japan, in 2014 revealed the presence of of 152 XX females, 13 XY females, 25 XX males and 147 XY males. The results of this study indicate that low and high water temperatures induce the formation of sex reversed XX-males and XY-females, respectively, and proves the occurrence of TSD in Atherinidae. Because of co-occurrence of TSD and *amhy* this species may be useful for monitoring the effects of abnormal water temperatures on sex determination in fish.

PO-110

Spatiotemporal coordination of *amh* and *cyp19a1a* gene expression and apoptosis during testicular differentiation in pejerrey *Odontesthes bonariensis*

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The pejerrey is a gonochorist teleost with strong temperature-dependent sex determination (TSD) whereby female and male populations are formed when larvae develop at intermediate temperatures. The histological gonadal differentiation follows a characteristic cephalocaudal, left-to-right gradient regardless of sex whereas intense apoptosis in the anterior region of the right gonad is associated with testis differentiation. The sex-related genes *amh* and *cyp19a1a* are thought to play important roles in the sex differentiation process of pejerrey, but their expression profiles and relation to gonadal apoptosis are still unclear. The objective of this study was to investigate the spatiotemporal patterns of apoptosis, *amh* and *cyp19a1a* gene expression and their interplay during testicular differentiation in pejerrey. The progeny from a cross of a XX female and a XY male was reared for 14 weeks at 25°C. Sampling was performed weekly between 1 and 10 weeks after hatching (wah) for the analyzes of apoptosis by TUNEL assay, *amh* and *cyp19a1a* mRNA expression by *in situ* hybridization, and the onset of histological gonadal differentiation and sex ratios by light microscopic histology. Ovarian and testicular differentiation began at 4 and 7 wah, respectively; XX individuals developed as 67% female and 33% male whereas XY individuals were all-male. *amh* mRNA transcripts were detected from 1 wah in all XY and part of the XX; they were clearly more abundant in the left gonads and generally weak or absent from the anterior region of the right gonads between 4 and 10 wah. *cyp19a1a* expression was observed from 2 wah in both genotypes but disappeared by 5-6 wah in putative males beginning from the right gonad. Apoptosis was detected only in putative males (all XY and part of the XX) from 3-4 wah; it was stronger in the anterior region of the right gonad compared to the left gonad. In summary, all putative males showed early and constant *amh* expression; transient *cyp19a1a* expression began shortly after and disappeared concomitantly with the appearance of apoptosis. The location and timing of these events seem to be highly coordinated. Overall, these results suggest that apoptosis might be implicated in removing *cyp19a1a* expressing cells, setting the ground for successful testicular differentiation. Interestingly, *amh* expression is reduced or disappear completely from areas where apoptosis is more intense, perhaps as “collateral damage”.

PO-111

Identification of a specific microRNA expression signature during follicle activation in zebrafish

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As one of the most dynamic physiological and developmental processes in vertebrates, ovarian folliculogenesis has always been the major focus for reproductive biologists. However, the molecular mechanisms that control follicle development, particularly the early phase of follicle activation or recruitment, still remain poorly understood. In an attempt to decipher the gene networks and signaling pathways involved in the transition, we have conducted a transcriptomic analysis (RNA-seq) on zebrafish PG (primary growth, stage I; inactive) and PV (pre-vitellogenic, stage II; activated) follicles by using HiSeq 2000 platform (Illumina). A total of 118 unique miRNAs (11 up-regulated and 83 down-regulated candidates in PG) and 56711 unique mRNAs (1839 up-regulated and 7243 down-regulated candidates in PG) were identified. Upon qPCR validation, we confirmed the preferential expressions of 46 miRNAs from 66 candidates (66.67%). Among which, we chose to focus on 13 miRNAs (dre-let-7a, -7b, -7c-5p, -7d-5p, -7h, -7i; dre-miR-21, -23a, -27c-3p, -107a-3p, -125b-5p, -145-3p, -202-5p) which exhibited significant differential expressions between PG and PV follicles ($p \leq 0.045$). With this 13-miRNA expression signature alone, PG follicles can be well differentiated from PV follicles by hierarchical clustering, which is highly suggestive of their functional relevance during PG-to-PV transition. By simply overlaying gene lists of the online predicted targets and the RNA-seq derived differentially expressed transcripts with a reciprocal expression pattern, we have successfully shortlisted a panel of miRNA downstream targets for luciferase reporter validation. We postulated that these potential miRNA-regulated genes may have important roles implicated in follicle activation as well as oocyte development.

PO-112

Transcriptional regulation of *amhy* and *amha* in temperature-dependent sex determination of pejerrey and their regulation by cortisol

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Sex determination in pejerrey is characterized by a strong temperature dependence (TSD). However, we recently identified a homologue of a testis determinant, *amhy*, and demonstrated that at an intermediate temperature its presence (XY/YY) or absence (XX) can favor the formation of males and females, respectively. In this study, we investigated the transcriptional profiles of *amhy* and the autosomal *amha* at feminizing and masculinizing temperatures during early larval development with the aim to evaluate their relationship with TSD and testis formation. The regulation of *amhy* and *amha* by the stress hormone cortisol was also analyzed as cortisol has been implicated in the TSD of pejerrey. XY and XX larvae were reared at 17ºC and 29ºC (female- and male-promoting temperatures, respectively) during the critical period of thermolabile sex determination and used for transcriptional analyses of *amhy* and *amha* by qRT-PCR. In addition, a luciferase reporter assay with the presumptive promoters (~3kb 5' upstream fragment) of both *amh* paralogues was performed to investigate the regulation of these two genes by cortisol *in vitro*. The glucocorticoid receptor expression plasmid was co-transfected with luciferase reporter plasmids containing *amhy* or *amha* promoter into endothelial progenitor cells. Transcriptional activity was measured 48 hours post-transfection in cells exposed to different cortisol doses. The *in vivo* expression analyses showed that *amhy* mRNAs were highly expressed in XY larvae from both 17ºC and 29ºC groups at the beginning of sex determination period but declined thereafter. *amha* was upregulated during the sex determination period in a few XY larvae at 17ºC and in both genotypes at 29ºC and was highly correlated with maleness. Transcriptional activity analyses showed that the *amhy* promoter did not respond to any cortisol doses whereas *amha* transcription increased with cortisol in a dose-dependent manner. This study shows that although *amhy* is considered the genotypic sex determinant, the autosomal *amha* might hold a key role in testis formation in pejerrey. This study also revealed cortisol signaling as an important transcriptional regulator of *amha* gene during the process of masculinization, especially at high temperatures.

PO-113

Development of methods to identify the sex chromosomal genotype (XX, XY, YY) in pejerrey and its application in a field survey of sex-reversals and super-males

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The pejerrey *Odontesthes bonariensis* is known for its strong temperature-dependent sex determination (TSD). However, we have also shown that this species possess a major, if not master, testis determining gene, the Y-chromosome-linked anti-Müllerian hormone (*amhy*). This finding makes possible to monitor wild pejerrey populations for sex-reversals and may prove instrumental for field studies addressing the effects of abnormal temperatures on reproduction. It is also possible that super-male YY individuals occur in the wild, for example, by mating of sex-reversed (XY) females to normal XY males, but current assays cannot distinguish XY from YY individuals. Because of the possible impact that sex-reversed and YY individuals might have on the sex ratios of wild populations, we artificially produced YY individuals to test their survival and fertility and developed a molecular method to discriminate fish with single (XY) and double (YY) copies of *amhy*. Finally, we carried out a field study to assess the presence of sex reversals and YY individuals in wild populations. First, captive-reared broodstock were genetically screened for *amhy* and a sex-reversed XY female was mated to an XY male. Their progeny was reared until sexual maturity and screened by genomic DNA PCR analysis using *amhy*-specific primers. In this screening, 66% (53 out of 80) of the progeny was *amhy*-positive, which suggested the presence of YY fish assuming Mendelian segregation (XX 1:3 XY+YY). We then performed a qPCR analysis on genomic DNA which detected 16 individuals, presumably YY individuals, with *amhy* values twice higher than the other 37 *amhy*-positive fishes. Progeny tests with *amhy*-negative (XX) females showed that the presumed YY individuals were in fact YY as their offspring was 100% *amhy*-positive (XY). We then used these molecular tools to screen wild pejerrey (n=158) collected in Lake Chascomus, Argentina, in 2014. The analysis revealed the presence of 35 XX-females, 4 XY-females, 12 XX-males, 105 XY-males, and 2 YY-males. The results of this study reveal that YY pejerrey are both viable and fertile and confirm the occurrence of sex reversal in both directions, probably as a result of TSD, and even YY individuals in a wild pejerrey population. The molecular tools developed in this study may be useful for surveying the effects of temperature and other factors on sex determination of wild populations.

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