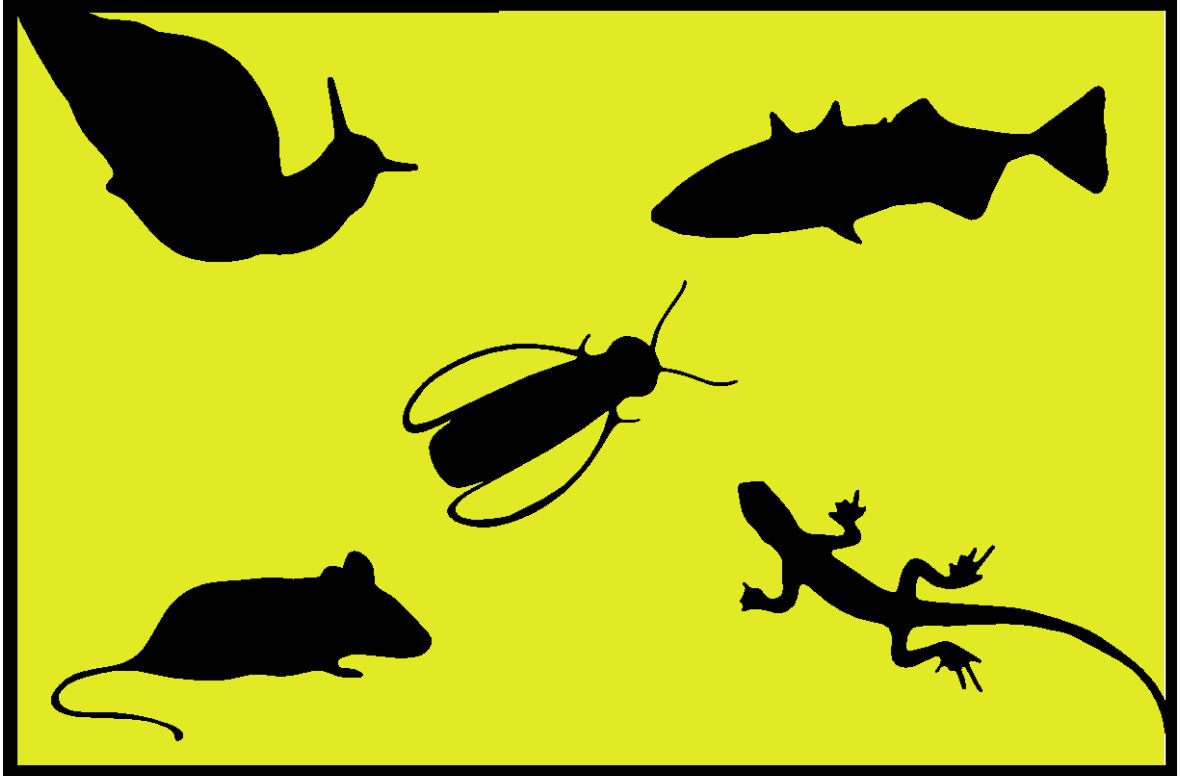
30th CECE

9th ISFE

2022

4 - 8 September 2022



ABSTRACT BOOK

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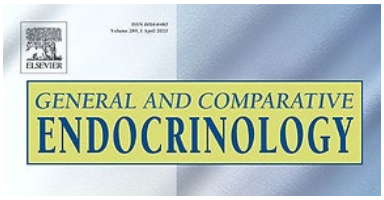

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ASOCIACIÓN IBÉRICA DE ENDOCRINOLOGÍA COMPARADA

Asociación Ibérica de Endocrinología Comparada
Associação Ibérica de Endocrinologia Comparada
Iberian Association for Comparative Endocrinology

HUBEL Grupo

MADREfruta Algarve, Portugal

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visit Algarve Portugal

30th CECE & 9th ISFE

Joint Conference of the European Society for Comparative Endocrinology and of the International Society for Fish Endocrinology

Welcome!

We are delighted to welcome you to the 30th CECE & 9th ISFE, our first in person meeting since the Covid 19 pandemic and we hope it will be a memorable experience scientifically and socially as we have planned a diverse programme and will have participants from all over the world. The 30th CECE and 9th ISFE should have taken place separately in 2020 but were postponed due to the pandemic. By joining forces, the European Society for Comparative Endocrinology, the International Society for Fish Endocrinology, and the International Federation of Comparative Endocrinology Societies, made it possible for us to assemble this magnificent programme. This was possible through the dedication of the symposium organizers, invited speakers and comparative endocrinologists and I thank them all. We feel sure you will enjoy the conference and will take advantage of the quiet and beautiful environment around Faro and the Algarve region.

Adelino Canário

Organizing Committee:

- **Adelino VM Canário**
- **Deborah M Power**
- **Pedro M Guerreiro**
- **João C Cardoso**
- **Patrícia Pinto**
- **Liliana Anjos**
- **Rute Felix**
- **Rute Martins**

ESCE

The European Society for Comparative Endocrinology (ESCE) brings together European and international researchers in the field of comparative endocrinology. Our objective is to promote interdisciplinary knowledge in the fields of Biology and Medicine, specifically concerned with the morphological, functional, and evolutionary aspects of endocrinology.
http://www.escendo.info/

ISFE

The mission of the 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 growth, adaptation, reproduction, stress, immunity, behaviour and endocrine disruption in fishes. In particular, the ISFE ambitions to encourage and foster career development of junior members. The ISFE will do its best to favor the participation of junior members to the ISFE by providing financial support.
https://www.isfendo.com/

Scientific and Executive Committees

30th CECE

- **Deborah Power, Portugal**
- **Jozef Vanden Broeck, Belgium**
- **Encarnación Capilla, Spain**
- **Julian Dow, UK**
- **Maurice Elphick, England**
- **Elisabeth Eppler, Switzerland**
- **Adrien Fonagy, Hungary**
- **Hamid Habibi, Canada**
- **Angela Lange, Canada**
- **Heather Marco, South Africa**
- **Florian Raible, Austria**
- **Samantha Richardson, Australia**
- **Liliane Schoofs, Belgium**
- **Rüdiger Schultz, Netherlands**

9th ISFE

- **Chair: V.L. Trudeau (Canada)**
- **Co-chair: O. Carnevali (Italy)**
- **Treasurer: A. Servili (France)**
- **Communications: R. Fontaine (Norway)**

Members:

- **Thrandur Björnsson (Sweden)**
- **Ana Gomez (Spain)**
- **Olivier Kah (France)**
- **Wensheng Li (China)**
- **Subash Peter (India)**
- **Paula Vissio (Arg)**
- **Renata Guimarães Moreira Whitton (Bras)**

INDEX

SCIENTIFIC PROGRAM

LIST OF ABSTRACTS

SCIENTIFIC PROGRAM

Sunday, September 4th	
15:00	REGISTRATION DESK OPEN
16:00	WELCOME CEREMONY
16:30-17:15	PLENARY I - *The multifaceted role of growth hormone in salmonid physiology and behavior* Thrandur Bjornsson Chair: Mark Sheridan (*Vincent Wigglesworth room*)
17:15-18:30	WELCOME COCKTAIL

Monday, September 5th			
08:00	REGISTRATION DESK OPEN		
08:30-09:15	PLENARY II- *Neuroendocrine mechanisms of seasonal life-history transitions* **Deborah I. Lutterschmidt** (Pickford Medal – IFCES award) Chair: James Carr *(Vincent Wigglesworth room)*		
Parallel Sessions	S1- *Invertebrate hormones and behaviours* Chairs: Angela Lange & Ian Orchard *(Vincent Wigglesworth room)*	*S2- Stress Axis: Molecular and Cellular Regulation of the HPI/HPA Axis* Chairs: Bob Dores & Matt Vijayan *(Grace Pickford room)*	S3- *Cell Plasticity, Stemness, Differentiation* Chairs: Yong Zhu, Ramji Bhandari & Yonghua Sun *(Howard Bern room)*
09:15-09:45	SOTA 1 - *Discovering missing links in neuropeptide evolution and function: insights from echinoderms.* Maurice Elphick	SOTA 3 - *Novel insights into the action of corticosteroid receptors from knockout zebrafish.* Matt Vijayan	SOTA 5 - *Regulation of cellular plasticity by IGF and calcium signaling: New insights from fish ionocytes.* Cunming Duan
09:45-10:00	*O1- Localisation of relaxin-like gonad-stimulating peptide expression in starfish: are the gonoducts the physiological source for its role as a regulator of spawning?* Yuling Feng	O7- *Multifactorial hypophysiotropic regulation of the HPI axis in Atlantic salmon.* Nicholas Bernier	O13- *Egg thermal regime modifies the endocrine response to food deprivation of the European seabass* Ana Patrícia Mateus
10:00-10:15	O2- *An Ancient thyrostimulin-like signaling system regulates growth in C. elegans.* Signe Kenis	O8- *Regulation of fish neural melanocortin receptors by melanocortin-2 receptor accessory protein 2.* Ya-Xiong Tao	O14- *Adamts9 is critical for the development of primary ovarian follicles.* Jonathan Carver
10:15-10:30	O3- *Knockdown of Halloween genes spook, shadow and shade affect the length/width ratio of oocytes and eggs in the desert locust, Schistocerca gregaria.* Sam Schellens	O9- *Trends in the evolution of the melanocortin-2 accessory protein, MRAP1.* Robert M. Dores	O15- *Regenerative angiogenesis and neurogenesis after zebrafish telencephalon injury: deleterious effects of chronic hyperglycemia.* Danielle Fernezelian
10:30-11:00	COFFEE BREAK		
11:00-11:30	SOTA 2 - *Advances in understanding juvenile hormone biology.* Fernando G. Noriega	SOTA 4 - *Evolution of ligand selectivity for the melanocortin-2 receptor: implication for the hpa/hpi axis of vertebrates.* Ciaran Shaughnessy	SOTA 6 - *Molecular modules that create eggs after sex determination of germ cells.* Tanaka Minoru
11:30-11:45	O4- *Mechanisms of neuropeptidergic regulation of reproductive physiology in starfish.* Victor Manuel Piñon Gonzalez	O10- *Osmoregulatory response induced by hypersaline challenge is modulated by corticosteroids, cortisol and dexamethasone, in the gilthead seabream.* Andre Barany Ruiz	O16- *Molecular mechanism of sexual plasticity in fish: a viewpoint from a natural sex changing fish.* Tapas Chakraborty
11:45-12:00	O5- *The role of the bivalve calcitonin (CALC) peptides in shell building and recovery.* Zhi Li	O11- *Impact of ocean acidification on the neuroendocrine response to an acute stress in a teleost fish.* Arianna Servili	O17- *Characterization of early events of puberty in female European sea bass (Dicentrarchus labrax) and the role of the transcriptional coactivator ncoa7.* Cinta Zapater
12:00-12:15	O6- *Evolutionarily conserved neuropeptidergic signalling systems in nematodes.* Luca Golinelli	O12- *Effect of climatic and estrogenic stress on the life cycle and physiology of an estuarine fish.* Jimmy Devergne	
12:15-13:30	LUNCH		

AFTERNOON

13:45-14:30	**PLENARY III**- *Reflections on fundamental and translational research in fish reproductive biology* **Yoni Zohar** (RE Peter Lecture – ISFE award) Chair: Abigail Elizur (*Vincent Wigglesworth room*)		
Parallel Sessions	S4- *Thyroid hormone actions in vertebrate development* Chairs: Aurora Vidal, Veerle Darras & Hamid Habibi (*Vincent Wigglesworth room*)	S2- *Stress Axis: Molecular and Cellular Regulation of the HPI/HPA Axis* Chairs: Bob Dores & Matt Vijayan (*Grace Pickford room*)	S3- *Cell Plasticity, Stemness, Differentiation* Chairs: Yong Zhu, Ramji Bhandari & Yonghua Sun (*Howard Bern room)*
14:30-15:00	**SOTA 7-** *How early are T3 effects upon central nervous system myelination?* **Aurea Orozco**	**SOTA 9-** *Across the metamorphic divide: developmental mechanisms underlying the stress-induced susceptibility hypothesis in amphibians.* **Erica Crespi**	**SOTA 11-** *Epigenetics in the diversity of sexual systems and sex determining mechanisms in fish.* **Francesc Piferrer**
15:00-15:15	**O18-** *Thyroid hormone (T3) is involved in intestinal development during SW acclimation of Atlantic salmon.* **Vilma Duarte**	**O24-** *Itch and its Molecular/Functional Evolution.* **Keiko Takanami**	**O30**- *GDNF acts as a germ cell growth factor and regulates zebrafish germ stem cell niche in autocrine- and paracrine-dependent manners.* **Lucas Doretto**
15:15-15:30	**O19-** *The thyroid axis participates in temperature-induced sex reversal through its activation by the stress axis in the medaka.* **Juan Fernandino**	**O25-** *CRHR1 integrates the temperature-induced hormonal and metabolic response in zebrafish.* **Zachary Shvartsburd**	**O31-** *Steroidogenic activity of anti-müllerian hormone in European sea bass (Dicentrarchus labrax) males*. **Alessia Mascoli**
15:30-15:45	**O20-** *Maternal thyroid hormones affect zebrafish embryo development.* **Maira da Silva Rodrigues**	**O26-** *Stress response in the silver catfish Rhamdia quelen to the interaction stocking density – feeding regimen.* **Juan Miguel Mancera**	**O32-** *Molecular characterization of Siberian sturgeon ovarian sex differentiation.* **André Lasalle**
15:45-16:15	COFFEE BREAK		
16:15-16:30	**SOTA 8-** *Role of thyroid hormones in the multifactorial control of reproduction and spermatogenesis in fish.* **Hamid Habibi**	**SOTA 10**- *The stress of subordination: impacts of chronic social stress on HPI axis function in rainbow trout.* **Kathleen Gilmour**	**SOTA 12 -** *Single cell transcriptomics in zebrafish with natural chromosomal sex determination.* **John Postlethwait**
16:45-17:00	**O21-** *Identification of biomarkers of Thyroid Disruption in Xenopus laevis by transcriptomic analysis.* **Alicia Tribondeau**	**O27-** *Serotonin plays a key role in the activation of the hypothalamic pituitary interrenal axis during high environmental ammonia exposure in the teleost model rainbow trout.* **Mauro Chivite**	**O33-** *Two genes coding for gonadal soma-derived factors act in early gametogenesis in European sea bass.* **Ana Gómez**
17:00-17:15	**O22-** *T2 effects upon gill remodelling during axolotl metamorphosis. what do transcriptomic analysis reveal?* **Aurora Olvera Vidal**	**O28-** *The role of cortisol in the intestinal bicarbonate secretion of Atlantic salmon.* **Pasqualina Gaetano**	**O34**- *Transcriptome analysis of a protandric hermaphrodite, the common snook (Centropomus undecimalis), during gonadal differentiation under sex steroid treatments.* **Juliana Morena Bonita Ricci**
17:15-17:30	**O23**- *Deciphering the dialog between the environment and the brain during the metamorphosis of the clownfish Amphiprion ocellaris.* **Mélanie Dussenne**	**O29**- *CART and CRH mediated responses in the brain of Euphlyctis cyanophlyctis tadpoles subjected to natural pond drying.* **Swapnil Shewale**	**O35-** *Increased biomaker discovery through a twist on the analysis of transcriptomic data during sexual development in the European sea bass, mouse, and humans.* **Núria Sánchez Baizán**
17:30-18:15	POSTER SESSION		

Tuesday, September 6th			
08:00	REGISTRATION DESK OPENS		
08:30-09:15	PLENARY IV- *Endocrine control of seawater adaptation in teleost fishes: How they gain water in the hypertonic environment* **Yoshio Takei** (Bargmann-Scharrer Lecture – IFCES award) Chair: Robert M. Dores *(Vincent Wigglesworth room)*		
Parallel Sessions	S5- ***Brain-pituitary interactions*** Chairs: Paula Vissio & Ignacio Fernandino *(Vincent Wigglesworth room)*	S6- ***Neuropeptides: new and emerging concepts*** Chairs: Vance Trudeau & Hervé Tostivint *(Grace Pickford room)*	S7- ***In memory of Christopher Cheng*** Chairs: Deshou Wang, Chi Bun Chan & Jianzhen Li *(Hybrid session)* *(Howard Bern room)*
09:15-09:45	SOTA 13 - *Emerging questions on the teleost hypothalamic-pituitary system during developmental and adult stages.* **Paula Vissio**	SOTA 15- *Evolution and functions of urotensin II-related peptides.* **Hervé Tostivint**	SOTA 17- *Gonascin: a germline stem cell-derived hormone with glucogenic, orexigenic, and gonadal activities.* **Jianzhen Li**
09:45-10:00	O36- *The effect of day length on the regulation of pituitary gonadotrope cells in the teleost fish, medaka.* **Muhammad Rahmad Royan**	O42- *EFLa-type neuropeptide is produced by alternative trans-splicing in some insects.* **David Dolezel**	SOTA 18- *Spexin as a satiety factor: the story from fish to mouse model.* **Anderson O. L. Wong**
10:00-10:15	O37- *Neuroendocrine effects of 17α-ethynilestradiol during the early developmental stages of sea bass.* **Salima Aroua**	O43- *Neuropeptide precursors and G-protein coupled receptors in the bivalve mantle.* **João Cardoso**	
10:15-10:30	O38- *Exploring central and peripheral appetite-regulating peptides in the brain and gastrointestinal tract in grow-out Atlantic halibut.* **Endre Lygre**	O44- *Somatostatin signalling regulates germ cell number, fecundity, pancreatic cell proliferation and metabolism in zebrafish.* **Jie Chen**	O48- *Inhibin in fish reproduction – a molecule that should not be forgotten.* **Wei Ge**
10:30-11:00	COFFEE BREAK		O49- *Tilapia as a good model for studying comparative endocrinology.* **Deshou Wang** O50- *Neuropeptide Y in tilapia: Emphasis on the role of food intake.* **Wensheng Li**
11:00-11:30	SOTA 14- *Pituitary plasticity in the teleost fish.* **Romain Fontaine**	SOTA 16- *Looking for the true love hormone: evidence that secretoneurin controls reproduction.* **Vance Trudeau**	O51- *The roles of neuropeptides in fish reproduction.* **Yong Zhang** O52- *Dendrimer-small RNA drugs targeting renin-angiotensin system for cancer therapy.* **Leo Tsz On Lee**
11:30-11:45	O39- *Investigations on the role of brain aromatase in mediating behavioral defects following exposure to estrogenic chemicals in zebrafish.* **Mélanie Blanc**	O45- *Relaxin-like gonad-stimulating peptide family in asteroidea.* **Masotoshi Mita**	O53- *Early Gonadal Development, Expression Profile and Regulation of Sex-Related Genes and Hormonal Induction of Sex Reversal Mechanism in Scatophagus argus.* **Dong-Neng Jiang**
11:45-12:00	O40- *Genotype-dependent activation of CRH-family genes during heat-induced masculinization in pejerrey Odontesthes bonariensis.* **Aaron Torres Martínez**	O46- *Rethinking the regulation of pre-ovulatory LH surge in female zebrafish: is the hypophysiotropic GNRH (gnrh3) dispensable?* **Sakura Tanaka**	O54- *In vivo drug discovery for increasing incretin-expressing cells identifies dyrk inhibitors that reinforce the enteroendocrine system.* **Lianhe Chu**
12:00-12:15	O41- *Impact of night shift work on brain function and morphology.* **Horst Werner Korf**	O47- *Impact of CRISPR/Cas 9 complete Secretogranin-ii gene knockout on neuropeptides and reproduction in zebrafish (Danio rerio).* **W.K.C Udeesha Erandani**	O55- *Multifaceted brain-derived neurotrophic factor – a growth factor in brain, a myokine, or an endocrine hormone?* **Chi Bun Chan**
12:15-13:30	LUNCH		

AFTERNOON

13:30-13:40	General Comparative Endocrinology, Elsevier Mark Sheridan (Editor) *(Vincent Wigglesworth room)*		
13:45-14:30	PLENARY V- *Analyses of enhancers of non-model fishes provide the reason for the differentiation of GnRH1/3 neurons and FSH/LH cells* Shinji Kanda Chair: Vance Trudeau *(Vincent Wigglesworth room)*		
Parallel Sessions	S8- *Endocrine regulation of metabolism and growth* Chairs: Joaquim Gutierrez & Elizabeth Jonsson *Sponsored by AIEC* *(Vincent Wigglesworth room)*	S9- *Integrative action of hormones* Chairs: Subhash Peter & Samantha Richardson *(Grace Pickford room)*	
14:30-15:00	SOTA 19- *Swimming and Growth.* Arjan P. Palstra	SOTA 20- *From lampreys to multiple sclerosis and back again.* Samantha Richardson	
15:00-15:15	O56- *The absence of light and feeding-related synchronizers differently affects energy balance in goldfish.* Nuria Saiz	O62- *Leptin signaling promotes epimorphic regeneration in Xenopus laevis.* Robyn E Reeve	
15:15-15:30	O57- *Diabetes impairs reactive gliosis and increases extracellular matrix synthesis after ischemic stroke in mice.* Julien Clain	O63- *"The Snail, the Shark and the Whale": From Evolution to Endocrine Disruption.* Filipe Castro	
15:30-15:45	O58- *A multidisciplinary approach to investigate probiotic mitigation against chronic Bisphenol A exposure effects at hepatic and gut levels in Danio rerio.* Christian Giommi	O64- *Discovery of a prolactin-like in lampreys reveals the divergence of prolactin and growth hormone in jawless vertebrates.* Ningping Gong	
15:45-16:15	COFFEE BREAK		
16:15-16:30	SOTA 21- *The gut-brain axis in fish: from the detection of nutrients in the gut to the modulation of central appetite-regulatory systems.* Ayelén M. Blanco	SOTA 22- *Lessons from amphibians: TH action at metamorphosis.* Nicolas Buisine	
16:45-17:00	O59- *Therapeutic potential of the medicinal plant Hypericum lanceolatum lam. on metabolic disorders in zebrafish (Danio rerio) models.* Laura Gence	O65- *CART dynamics during croaking in the brain of anurans.* Ketaki Chaturbhuj Shetye	
17:00-17:15	O60- *Metabolic disorders impair brain homeostasis and neurogenesis.* Nicolas Diotel	O66- *Maternal transfer of microplastics in the yolk of loggerhead sea turtles (Caretta caretta) embryos and their correlation with development.* Giorgia Gioacchini	
17:15-17:30	O61- *Muscle and bone interaction after an injury in Gilthead Sea bream: implications of endocrine and regulatory factors in muscle regeneration.* Joaquim Gutiérrez	O67- *Evolutionary analysis of temperature receptor TRPV (transient receptor potential vanilloid) family with a special focus on "fish".* Marina Morini	
17:30-19:00	POSTER SESSION		

Wednesday, September 7th			
08:00	REGISTRATION DESK OPENS		
08:30-08:40	Journal of Experimental Biology, The Company of Biologists Kathleen Gilmour (Editor) *(Vincent Wigglesworth room)*		
08:45-9:30	PLENARY VI- *Use of non-mammalian animal models for biomedical research* Hiroko Nishimura (Barrington-Kobayashi Lecture) Chair: Dan Larhammar *(Vincent Wigglesworth room)*		
Parallel Sessions	S10- *AKH and GnRH-related peptides in Metazoa – a tribute to Gerd Gäde* Chairs: Gerd Gäde, Heather Marco & Jean-Paul Paluzzi *(Vincent Wigglesworth room)*	S11- *Endocrine and paracrine regulation of gonad physiology* Chairs: Rüdiger Schulz & Chun Peng *(Grace Pickford room)*	
09:30-10:00	SOTA 23 -*The long and short of the adipokinetic hormone/red pigment-concentrating hormone peptide family in insects.* Heather G. Marco	SOTA 25- *Nuclear progestin receptor mediated linkage between coagulation and ovulation.* Yong Zhu	
10:00-10:15	O68- *The unique C-mannosylated glycopeptide adipokinetic hormone of the indian stick insect.* Gerd Gäde	O74- *Impact of light pollution on male reproductive success in Japanese medaka.* Lauren Closs	
10:15-10:30	O69- *Distribution and functional insight into adipokinetic hormone/ corazonin-related peptide in the human disease vector, Aedes aegypti* . Jean-Paul Paluzzi	O75- *Putative role of the cerebellum and the vagal lobe as oscillators in goldfish.* Aitana Alonso Gómez	
10:30-10:45	O70- *Chromactivating neuropeptides in crabs: neuroarchitectures, receptors, established and novel functions.* Simon Webster		
10:45-11:15	COFFEE BREAK		
11:15-11:45	SOTA 24-*The Adipokinetic hormone - gonadotropin releasing hormone family of peptides: role during postembryonic development of the desert locust.* Jozef Vanden Broeck	SOTA 26- *Endocrine and paracrine regulation of spermatogenesis.* Diego Crespo	
11:45-12:00	O71- *Characterization of invertebrate gonadotropin-releasing hormone/corazonin in the mollusc Lymnaea stagnalis: evolutionary and functional implications.* István Fodor	O76- *Impaired leptin signalling disrupts oocyte maturation and ovulation in female zebrafish.* Emmanouil Tsakoumis	
12:00-12:15	O72- *AKH signaling in the prothoracic gland alters development in response to larval nutritional stress.* Bryon N. Hughson	O77- *Searching for the relationship between body size and maturity in female European sea bass.* Laura Sempere	
12:15-12:30	O73- *(antiviral) RNAi pathways in insects.* Dulce Santos	O78- *Expression analysis of receptors for glycoprotein hormones and of the thyrostimulin during spermatogenesis in the catshark, Scyliorhinus canicula.* Fabian Jeanne	
12:30-13:45	LUNCH		

AFTERNOON

13:45-14:30	PLENARY VII - *Shape Shifting: thyroid hormones and developmental ontogeny in a changing world* **Deborah Power** Chair: Veerle Darras (*Vincent Wigglesworth room*)		
Parallel Sessions	S12- *Endocrine Biotechnology in Aquaculture* Chairs: Ana Gómez & Abigail Elizur (*Vincent Wigglesworth room*)	S13- *Endocrine disruption: current status and challenges* Chairs: Joachim Sturve & Patrícia Pinto (*Grace Pickford room*)	
14:30-15:00	SOTA 27- *Uses of biotechnology to control reproduction in Atlantic salmon.* **Anna Troedsson-Wargelius**	SOTA 29- *The challenge of evaluating endocrine disrupting chemicals for marine life.* **Ioanna Katsiadaki**	
15:00-15:15	O79- *A comparison of growth performance and hormone production of precocious and immature prepubertal female sea bass, Dicentrarchus labrax.* **Alicia Felip Edo**	O85- *Systematic evidence map of the effect of endocrine-disrupting chemicals on thyroid hormone measurements in mammals.* **Isabel Forner-Piquer**	
15:15-15:30	O80- *Can fish be artificially equipped with a secondary functional gonad?* **Issei Yahiro**	O86- *New endpoints for thyroid hormone system disruptor testing with fish.* **Lisa Baumann**	
15:30-15:45	O81- *Unveiling the potential of probiotics to mitigate the toxic effects of perfluorooctanoic acid on zebrafish development.* **Marta Lombó**	O87- *Past exposure effects on future generations' health: are future generations organisms safe at all?* **Ramji Bhandari**	
15:45-16:15	COFFEE BREAK		
16:15-16:45	SOTA 28- *Biotechnology in Aquaculture - Lessons from recombinant Hormones.* **Joseph Aizen**	SOTA 30- *Environmental estrogens interact with the growth hormone-insulin-like growth factor system to reduce seawater adaptation and retard growth of rainbow trout.* **Mark A. Sheridan**	
16:45-17:00	O82- *Reanalysis of gonadal transcriptome using sex markers reveals new genes involved in male sex differentiation of Siberian sturgeons.* **Denise Vizziano-Cantonnet**	O88- *Effects of atorvastatin and 17α-ethinylestradiol on blood and liver lipids contents in brown trout juveniles.* **Tiago Lourenço**	
17:00-17:15	O83- *Transcriptome analysis of endocrine genes during sea bream (S. aurata) and sea bass (D. labrax) larval development in hatchery condition.* **Babak Najafpour**	O89- *What do ribosomes tell us about fish oocyte development during sex differentiation, oogenesis and xenoestrogenic exposure.* **Ibon Cancio**	
17:15-18:00	O84- *Transcriptomics points to an association between the HPT-axis and immune system maturation before and during Senegalensis sole larvae metamorphosis.* **Sandra C Silva**	O90- *A structuring approach using bioassays to assess endocrine disruption activity in Quebec's effluents.* **Valerie S Langlois**	
17:30-18:00	GENERAL ASSEMBLIES		
18:15-18:45	CLOSING OF CONFERENCE		
20:00-23:00	CONFERENCE DINNER (EVA SENSES - HOTEL)		

Thursday, September 8th	
09:00-12:00	SATELLITE WORKSHOP (Registration required) MODEL-EDC: 2nd Edition Threats and tools for Endocrine Disrupting Pollutants in Marine Organisms *Organized by: Deborah Power, Patrícia Pinto, Ioanna Katsiadaki, Joachim Sturve, Tiphaine Monsinjon* *(Vincent Wigglesworth room)*
09:00-10:00	*Overview on monitoring and testing of EDCs: position paper on gaps and priority areas*
10:00-11:00	*Discussion groups on monitoring and testing of EDCs*
11:00-11:30	COFFEE BREAK
11:30-12:00	*Summary notes and conclusions*
12:00-12:45	LUNCH

POSTER LIST

Nr #	Author	Title
P1	Noriyoshi Sakai	*Genetic characterization of a zebrafish inbred strain generated through full sib-pair mating.*
P2	Samyar Ashoori	*Possible role of faeces in chemical communication in the Mozambique tilapia (Oreochromis mossambicus).*
P3	Paula Vissio	*The enigmatic saccus vasculossus. Characterization of this structure in Cichlasoma dimerus.*
P4	Alessia Mascoli	*A multidisciplinary approach to investigate the reproductive biology of European hake (Merluccius merluccius): the study case of males.*
P5	Ana Maria Coimbra	*Inhibition of Eisenia fetida reproduction during urban sewage sludge vermicomposting.*
P6	Francisco Prat	*Molecular characterization and expression of gdnfa and gdnfb and their putative receptors, gfra1a, gfra1b and ret during testicular development of the European seabass (Dicentrarchus labrax L.).*
P7	Jose Antonio Paullada Salmerón	*Spexin in the European sea bass, Dicentrarchus labrax: characterisation, brain distribution and interaction with gnrh and gnih neurons.*
P8	Zsolt Pirger	*Studies on the great pond snail highlight weaknesses in two lines of evidence that vertebrate steroids have a hormonal role in the reproduction of molluscs.*
P9	Germán Benech	*GSDF is the only major pro-male gene activated during the molecular sex differentiation period of Siberian sturgeon.*
P10	Sara Filippi	*Reproduction is not altered by environmental stress: new insight in the swordfish (Xiphias gladius) females.*
P11	Amrutha Bagivalu Lakshminarasimha	*Leptin modulates oocyte maturation via central and a direct pathway in zebrafish.*
P12	Amanda Guerreiro	*Carbamazepine on Astyanax lacustris females.*
P13	Katherine Shaw	*Brain aromatase mutation affects zebrafish sexual behaviour and reproductive health.*
P14	Ivana F. Rosa	*Thyroid axis is activated in the female-to-male sex reversal induced by higher temperature in medaka.*
P15	Valerie S Langlois	*Genomic and physiological mechanisms of androgen signalling: steroid-5α-reductase type 2 knockout investigation in frogs.*
P16	Renato Massaaki Honji	*Preliminary data on reproductive genes of the hypothalamic-pituitary-gonads axis of the dusky grouper Epinephelus marginatus (Perciformes: Serranidae).*
P17	Giulia Chemello	*A study on folliculogenesis in loggerhead sea turtle (Caretta caretta): structure and biochemical characterization through histological analyses and fourier transform.*
P18	Tapas Chakraborty	*Oct4 and sexual plasticity in fish gonad.*
P19	Valerie Langlois	*Gonadal development in THR alpha or beta knock-out Silurana tropicalis.*
P20	Fabian Jeanne	*Functional annotation of the testicular proteome during spermatogenesis in the catshark, Scyliorhinus canicula, with a focus related to steroidogenesis.*
P21	Tatsuyuki Takada	*Effects of the nonylphenol on 'in vitro' spermatogenesis of the critically endangered cyprinid Gnathopogon caerulescens.*
P22	Simon Webster	*Neuropeptide cascades during ecdysis in the shore crab, Carcinus maenas. BURS and CCAP neurons simultaneously release Allatostatin-C and -CC, during ecdysis.*

P23	Maricela Luna Muñoz	*Growth hormone (GH) induces neuroprotective effects in the embryonic chicken cerebellum exposed to hypoxic injury, in vitro and in vivo.*
P24	Shan-Ru, Jeng	*Gonadal sex differentiation in Japanese eel: Expression profiles of cyp19a1, ers and gths in the brain/pituitary.*
P25	Mercedes Blázquez	Pubertal development in European sea bass: evaluation of germ cell molecular markers.
P26	María Isela García Martínez	*T3 effects upon myelination depend on the developmental stage of zebrafish.*
P27	Stijn Van den Brande	*Identification and profiling of stable microRNAs in hemolymph of young and old Locusta migratoria fifth instars.*
P28	Hamed Abdollahpour	*Preliminary results regarding the role of thyroid hormone on growth performance, and morphology of stellate sturgeon (A. stellatus) larvae during metamorphosis.*
P29	Naghmeh Jafari	*Effects of temperature manipulation on growth and skeletal development of sterlet sturgeon (A. ruthenus) during the first two months of life.*
P30	Isabel García-Pérez	*Regulation of growth performance, gh/igfs axis and muscle development markers by diet and exercise in juveniles of Gilthead sea bream.*
P31	Santiago M Pech Pool	*Detection of myelin rich regions in the brain of axolotl (Ambystoma mexicanum)*
P32	Jie Chen	*Maternal loss of somatostatin 2 impairs embryonic development in zebrafish*
P33	Enrique Rosell-Moll	*An hydroxytyrosol-rich extract from olive juice ameliorates the obesogenic effects of a high-fat diet in Gilthead sea bream.*
P34	Ayelén M. Blanco	*Evidence for the presence of fatty acid sensing mechanisms based on changes in intracellular metabolic pathways in the rainbow trout gastrointestinal tract.*
P35	Szymon Chowański	*Muscarinic system regulates the expression of insect insulin-like peptide genes.*
P36	Paweł Marciniak	*The influence of myotropic insect neuropeptides on mammalian cardiomyocytes – Preliminary results.*
P37	Malgorzata Slocinska	*Identification of sulfakinin receptors (SKR) in Tenebrio molitor beetle and the influence of sulfakinins on carbohydrates metabolism.*
P38	Jessica Calo Rodríguez	*Impact of dietary vegetable protein content on amino acid sensing and feed intake regulation in rainbow trout juveniles.*
P39	María Alborja Valado	*Serotonin rhythms in rainbow trout intestine. circadian influence and role of photoperiod and feeding.*
P40	Sunil Koli	*Diurnal neuropeptidergic oscillations in the brain of an anuran Euphlyctis cyanophlyctis.*
P41	Paolo Cocci	*Association between microplastic occurrence and cytokine signaling in the gastrointestinal tract of commercial fish species.*
P42	Nuria Saiz	*Anxiogenic effects of different feeding conditions in goldfish.*
P43	Sipra Mohapatra	*Autophagy: an important player in germ cell maintenance and gonadal sexuality.*
P44	Andre Barany Ruiz	*Stress response induced by hypersaline challenge is modulated by corticosteroids, cortisol and dexamethasone, in the gilthead seabream (Sparus aurata).*
P45	Jan Černý	*Defence reactions against honeybee venom.*
P46	Rubaiyat E Sania	*Ionotropic glutamate receptors are possible mediators of pheromone.*
P47	Cármen Sousa	*Immunoendocrine response of the head-kidney to pathogen challenges in N. rossii.*

P48	Juan Miguel Mancera	*Putative role of DNA methylation in the gh response of Gilthead seabream (Sparus aurata) to osmotic challenge.*
P49	Jo Alexander	*Can crustacean ecdysis be 'triggered'?: Exploring the eclosion hormone (eh) and ecdysis triggering hormone (eth) system in Carcinus maenas.*
P50	Dan Larhammar	*Evolution of oxytocin and vasotocin receptor genes in vertebrates.*
P51	Rute Félix	*In vitro responsiveness of male and female skin fibroblast cells under the influence of estradiol or testosterone.*
P52	Jozef Vanden Broeck	*The 'crustacean cardioactive peptide' signaling system is crucial for ecdysis and inhibits ecdysteroidogenesis in the desert locust.*
P53	Célia Lopes	*Testing the trout liver rtl-w1 cell line potential to study the influences of temperature on the effects of endocrine disruptors.*
P54	Tânia Vieira Madureira	*The effects of two progestins and 17α-ethinylestradiol on cultured hepatocyte spheroids of brown trout (Salmo trutta).*
P55	Rodrigo F. Alves	*Brown trout primary hepatocyte spheroids – a model to assess estrogenic effects.*
P56	Filipe Andrade de Godoi	*Effects of anti inflammatories drugs diclofenac and ibuprofen on the responses of reproductive, metabolic and stress hormones of Astyanax lacustris (Teleostei:Characiadae).*
P57	Salima Aroua	*Assessment of endocrine disrupting chemicals by using pituitary cell culture of European sea bass.*
P58	Patricia Pinto	*Disruption of the sea bass skin-scale barrier by antidepressant fluoxetine and estradiol: in vivo and in vitro evidence.*
P59	Marta S Monteiro	*Single and combined effects of triclosan and ultraviolet radiation in metamorphosing sole.*
P60	Francesca Maradonna	*Glyphosate exposure induces sex-specific outcomes in zebrafish adult: insights on hepatic and gonadal toxicity.*
P61	Valerie S Langlois	*The steroidal growth promotant, melengestrol acetate, may act through the hypothalamic-pituitary-interrenal axis to disrupt metamorphosis in Silurana tropicalis.*
P62	Ana S. Gomes	*Effects of swimming exercise on performance of Atlantic salmon.*
P63	Elisabeth Eppler	*A comparative approach to measure sex and age-related differences in shoulder morphology and body size.*

PLENARY LECTURES

PLENARY I
THE MULTIFACETED ROLE OF GROWTH HORMONE IN SALMONID PHYSIOLOGY AND BEHAVIOR

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The pluripotent role of growth hormone (GH) in fish is clearly demonstrated in salmonids where it has important osmoregulatory and growth-promoting roles. Briefly, as an osmoregulatory hormone, GH promotes seawater tolerance during salmon smoltification, with photoperiod as a *zeitgeber*, through a signaling route identified already in the 1970's as the "light-pituitary-axis". As growth is the end result of multiple processes, the question how growth hormone (GH) promotes growth is complex. Growth is dependent on food intake, which is dependent on appetite. However, appetite does not always guarantee increased food intake, and complex changes in behavior need to take place. In the field, GH-treatment most often, but not always, induces growth, through increased foraging activity within territory, as well as through changes in pray selection. Thus, when growth-enhanced fish enter the wild, they can have multitrophic ecosystem impacts. GH may stimulate appetite indirectly through IGF-I stimulation of metabolism and nutrient levels, which trigger appetite-stimulating neural or endocrine signals to appetite regulating centers. However, GH can also pass the salmonid blood-brain barrier and affect central appetite regulation directly. Salmonids are temperate species that are likely to experience seasonal lack of food availability, especially when in freshwater over the winter months. As foraging is energetically costly, stimulation of appetite and growth would likely become detrimental to long-term survival. While fast-growing fish have relatively low plasma GH and high IGF-I levels, fasting/starvation leads to an endocrine condition of acquired GH resistance, defined by high plasma GH levels and low IGF-I levels. Similar syndrome in induced when salmon juveniles are transferred prematurely to seawater, resulting in growth-retarded "stunts". While the root cause for acquired GH resistance has been postulated to be the downregulation of GH receptors, the syndrome can occur without such receptor changes. Although established that plasma Lep levels increase during catabolic conditions in salmonids, it has been unclear whether the GH-IGF-I and Lep parts of the endocrine system are responding independently to catabolism-induced physiological changes, or if they are functionally linked. Recently, Lep treatment has been found to increase GH and decrease IGF-I in plasma, shifting the GH-IGF-I system toward a state of acquired GH resistance. These interrelated changes present a mechanism for long-term survival under catabolic conditions, by inhibiting appetite as well as saving energy by suppressing growth and hepatic metabolism.

PLENARY II- PICKFORD MEDAL
NEUROENDOCRINE MECHANISMS OF SEASONAL LIFE-HISTORY TRANSITIONS.

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Examples of life-history transitions are readily apparent in nature and include seasonal changes in reproductive behavior, migratory activity, foraging and hibernation. Intriguingly, both sex and phylogeographic differences in the timing of life-history events occur in many species, yet the neuroendocrine factors underlying these differences remain enigmatic. Using common garter snakes (*Thamnophis sirtalis*) as a model system, research in my lab focuses on the neuroendocrine mechanisms that mediate the seasonal transition from winter dormancy to spring mating behavior and from spring mating to migration and/or summer foraging. We recently found exciting evidence that the neuroendocrine gonadotropin-releasing hormone (GnRH) system is strongly modulated by environmental temperature during winter dormancy. Moreover, temperature-induced increases in GnRH were sexually dimorphic, varied among different garter snake species, and were associated with changes in neural thyroid hormone metabolism (via changes in deiodinase expression) within the hypothalamus. Our results further indicate that melatonin and glucocorticoid hormones play a role in mediating the effects of temperature on GnRH and both reproductive and feeding behaviors, with concomitant effects on the neuromodulators arginine vasotocin and neuropeptide Y. For example, low-temperature winter dormancy increases plasma glucocorticoid concentrations, which in turn is associated with decreased appetite and feeding behavior. Experimentally decreasing glucocorticoids directly activates feeding behavior and also increases neuropeptide Y immunoreactivity in the brain, a potent regulator of appetite and feeding in all vertebrates. Together, these results indicate the neuroendocrine mechanisms that mediate seasonal rhythms are conserved among vertebrates and also modulated by environmental temperature. As we continue to investigate the neuroendocrine factors that regulate life-history transitions, comparative analyses among different populations, species, and environments will help reveal how organisms orchestrate seasonal rhythms and cope with a changing environment

PLENARY III- RE PETER LECTURE
REFLECTIONS ON FUNDAMENTAL AND TRANSLATIONAL RESEARCH IN FISH REPRODUCTIVE BIOLOGY

Yonathan Zohar

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R.E. Peter was a pioneer in integrating basic and translational research in fish endocrinology and reproduction, whose scientific rigor and excellence inspired many of us in the field. Concomitantly to his elegant studies on the GnRH system in cyprinids, several groups demonstrated the multiplicity of GnRHs in fish, which led the comparative endocrinology community to also recognize the existence of two GnRHs in primates. Fish reproductive endocrinologists then used the ever-evolving technology and molecular platforms to better understand the functional significance of GnRH multiplicity in different fish models over the span of a couple of decades. Despite major advances, we still do not fully understand the respective roles of the 2 or 3 GnRH isoforms in fish or, quite surprisingly, whether GnRH is indispensable for fish reproduction at all. Progress in this intriguing field of research will be discussed, as well as the translation of our basic understanding of the GnRH system toward developing GnRH-based therapies for the induction and synchronization of captive reproduction in commercially important fish, thus enabling their hatchery production and, in turn, their intensive aquaculture. Other examples of fundamental research in reproductive biology leading to important applied outcomes will also be discussed, such as: how the knowledge gained regarding gonadotropin functions, associated with biotechnology platforms, is applied to controlling the onset of puberty and how understanding mechanisms and processes involved in the early establishment of fish gonads led to germ cell transplantation and surrogate broodstock technology (Yoshizaki et al.) and to the disruption of primordial germ cell development for generating reproductively sterile farmed fish (Wong et al.).

PLENARY IV-BARGMANN-SCHARRER LECTURER
ENDOCRINE CONTROL OF SEAWATER ADAPTATION IN TELEOST FISHES: HOW THEY GAIN WATER IN THE HYPERTONIC ENVIRONMENT

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As teleosts lose water osmotically in seawater (SW) through the gill, they must gain water to counter dehydration. To this end, they drink copiously and absorb more than 80% of imbibed SW by the digestive tract. It is now well established that hormones play critical roles in the regulation of drinking and intestinal ion/water absorption. Because of the life in water, fish can drink buccal water only by swallowing without searching for water. Accordingly, dipsogenic hormones such as angiotensin II (Ang II) and antidipsogenic hormones such as atrial natriuretic peptide (ANP) act on the area postrema in the medulla oblongata, a circumventricular organ (CVO) devoid of blood-brain barrier, to stimulate or inhibit swallowing. This is in contrast to mammals, in which Ang II and ANP act on the forebrain CVOs such as subfornical organ to arouse or depress thirst that motivates a series of drinking behavior. It is noteworthy that the antidipsogenic hormones are much more potent and efficacious than the dipsogenic hormones in fishes, while the reverse is true in mammals. The dominancy of the inhibitory mechanisms in fishes is most likely to prevent excess drinking of environmental water. Imbibed SW is desalinated in the esophagus and the Na^+ and Cl^- concentrations are decreased to half when it enters the stomach. The osmolality of luminal fluid is further decreased in the anterior intestine by removal of Ca^{2+} and Mg^{2+} as carbonate precipitate, which are contained at high concentrations in SW. The precipitation is achieved by active secretion of HCO_3^- into the lumen. Water is absorbed from isotonic luminal fluid together with Na^+ and Cl^- that are actively absorbed by Na+-K+-2Cl- cotransporter 2 (Slc12a1) and other transporters located on apical and basolateral membrane of enterocytes. Most transporters involved in ion and water absorption have been elucidated at the isoform level. Like dominancy of antidipsogenic hormones, inhibitory hormones are predominant in the control of ion/water absorption by the intestine of teleosts. Most potent are ANP and guanylin, which almost completely stop the absorption through accumulation of cytosolic cGMP and cAMP. Some hormones that decrease cytosolic cAMP is weakly stimulatory, but the potency and efficacy are much lower than ANP and guanylin. Investigations on the hormonal control of esophageal desalination and intestinal HCO_3^- secretion is underway.

Acknowledgements: The work presented here is realized by collaboration with many, scientists, postdocs and graduate students. I do not name each of them but deeply appreciate it. I also thank Japan Society for the Promotion of Science for financial supports.

PLENARY V
ANALYSES OF ENHANCERS OF NON-MODEL FISHES PROVIDE THE REASON FOR THE DIFFERENTIATION OF GNRH1/3 NEURONS AND FSH/LH CELLS.

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There is no doubt that GnRH and gonadotropins (FSH and LH) are the key players of the reproduction in teleosts and other vertebrates. However, there are strange phenomena in their use of paralogous genes of these systems. Hypophysiotropic GnRH neurons regulates LH release, which induces final oocyte maturation and ovulation. As demonstrated in many comparative studies of GnRH neurons, there is a strong tendency that species that possess both *gnrh1* and *gnrh3* genes show the hypophysiotropic projection of fibers originated of GnRH1 neurons rather than GnRH3 neurons (e.g. medaka, European seabass), whereas species that possess only *gnrh3* gene show heavy projection from GnRH3 neurons (e.g. zebrafish, goldfish). Although this apparent switching of the paralogous gene involved in the pituitary regulation has been considered "compensation", no study has revealed either the mechanism or reason for that. Here, we found that piranha possess both, whereas their relatives (neon-tetra, and other tetras) has lost *gnrh1*, and considered them nice model to study the mechanism of the compensation of the *gnrh1* loss. By using double *in situ* hybridization and immunohistochemistry, we demonstrated that *gnrh1* and *gnrh3* mRNA are co-expressed in the same neurons in the preoptic area, and both of GnRH1-immunoractive (ir) and GnRH3-ir fibers were observed in the pituitary. Furthermore, we clarified the reason of the loss of GnRH3 expression in hypophysiotropic neurons in the fishes that possess both *gnrh1* and *gnrh3*. By generating transgenic medaka that express RFP or GFP under the regulation of piranha *gnrh1* or *gnrh3* enhancer, we examined the enhancer activity of piranha *gnrh1* and *gnrh3* in medaka whose *gnrh3* expression is absent in the hypophysiotropic neurons. Interestingly, similar to the *gnrh1* enhancer, piranha *gnrh3* enhancer induced GFP expression in the hypophysiotropic neurons in POA that express intrinsic *gnrh1* mRNA, although medaka intrinsic *gnrh3* mRNA is not expressed in these neurons. These results suggests that medaka hypophysiotropic GnRH neurons possesses the transcription factors that can induce the expression of the ancestor's *gnrh3*. In other words, the *gnrh3* gene of medaka should have lost enhancers that activate in POA hypophysiotropic neurons, which is the reason why the GnRH1 neurons mainly regulate LH release in many acanthopterygian fishes that have both *gnrh1* and *gnrh3* genes. In this study, analyses of enhancers of non-model teleosts by introducing them into model teleosts enabled the prediction of evolutionary process that made the situation of the living fishes. In this presentation, I also introduce our study that elucidated the cellular linage of FSH and LH cells in teleosts. It is known that FSH and LH cells are separate, while FSH and LH are co-expressed in mammals. Analyses of enhancer activity of the *fshb* and *lhb* of ancient fishes gave evidence to presume the process of the differentiation of FSH and LH cells during the actinopterygian linage.

PLENARY VI- BARRINGTON-KOBAYASHI LECTURE
USE OF NON-MAMMALIAN ANIMAL MODELS FOR BIOMEDICAL RESEARCH

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There are two major aims in comparative endocrinology: 1) to study evolution of biological systems and their adaptation to changing environments; 2) to provide models to expand/elucidate an aspect of biological systems. In this review lecture, I will discuss three biological models. First, comparison among phylogenetically key species allows the identification of the biological and biochemical evolution of the renin-angiotensin system. The renal renin activity and granulated renin secretory cells have evolved during the early evolution of vertebrates, whereas the macula densa (MD) evolved at a later stage of vertebrate phylogeny. Likewise, during ontogeny of rodent and fowl kidneys, renin-expressing cells extend along and beyond the afferent arterioles and become more localized to the juxtaglomerular area with maturation. No MD structure is seen during early embryos. Angiotensin peptides and molecular structures of renin were identified in various vertebrates. Furthermore, renin release occurs via a renal arterial baroreceptor in response to reduced renal arterial pressure. In aglomerular fish, a naturally occurring model that lacks the MD and adrenergic innervation to the renin-secretory cells, Ca^{2+} influx and the Ca^{2+}-calmodulin system appear to play an inhibitory role, whereas the stimulatory effect of cAMP is not seen. Second, model for urine concentration: The kidney is an important osmoregulatory organ for maintaining homeostasis in humans and other vertebrate animals. The structure and function of the kidney differ, however, depending on the phylogenetic stages of vertebrates and on their environments. Distal tubules of freshwater trout show a secondary active NaCl cotransport mechanism without accompaniment of water and act as the so-called "diluting segment." Birds can form urine hyperosmotic to plasma and conserve body water utilizing countercurrent mechanism in which the diluting segment now plays a role in urine concentration. Furthermore, the structure and function of adult quail kidneys have similarities to those of developing rodents: Both have a loop of Henle, consisting of descending limb and thick ascending limb, but they lack the thin ascending limb. Moreover, in both adult quail and developing rodent kidneys, aquaporin 2 gene expression and protein are present in the apical membrane of the collecting ducts, but the water conservation effect of neurohypophysial hormones is rather small. Countercurrent urine concentration of adult quail and developing rodents heavily depends on NaCl recycling without participation of urea transport. These comparative models clarify the shift of aquatic to on land vertebrates. Third, model for fetal origin of health and diseases: Recent epidemiologic evidence and experimental studies in animals indicate that challenges in the intrauterine environment, such as hypoxia, hypothermia, and reduced fetal nutrition, increase susceptibility to diseases after birth, including hypertension, diabetes, kidney diseases, and neurological disorders such as autism and communication deficit. Our recent studies using an avian model indicate that partial egg white withdrawal (insufficient protein) impaired growth, decreased birth weight, and reduced the number of glomeruli after maturation, with mesangial inflammatory injury that partly resembled nephrosclerosis. Furthermore, we noted that the stress imposed during early development induced abnormal structure and early death. Disorders with developmental origins will impose significant impacts on child health and welfare, and public economy.

PLENARY VII
SHAPE SHIFTING: THYROID HORMONES AND DEVELOPMENTAL ONTOGENY IN A CHANGING WORLD

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The stability of the "milieu intérieur" or homeostasis is essential for survival as is the ability of an organism to adjust to changing conditions. The pivotal role of the endocrine system in regulating physiological processes to maintain homeostasis in vertebrates is unquestionable and is particularly obvious during lifecycle transitions when habitat, feeding and behaviour may change. The thyroid hormones (THs), thyroxine (T4) and 5,3-triiodothyronine (T3), have a well-recognised and obligate role in metamorphosis of anuran amphibians that undergo a profound shift in form, function, and habitat. The extraordinary transition from a pelagic symmetric larva to an asymmetric benthic juvenile in flatfish (pleuronectiforms) is also a TH dependent process. Omics technologies have revealed that underpinning the dramatic change in external morphology of flatfish profound molecular, cellular and organ specific changes occur in preparation for the transition to the juvenile stage. The same technology has revealed that although morphologically less dramatic the larva to juvenile transition in round fish is TH-mediated and shares conserved molecular features with flatfish. A puzzling aspect of hatchery production in aquaculture is the sometimes-high variability in the post-metamorphosis phenotype within and between production batches. This variability may be explained by the role of THs in environmentally induced developmental plasticity, to improve fitness and species survival.

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STATE-OF-THE-ART COMMUNICATIONS

SOTA 1
DISCOVERING MISSING LINKS IN NEUROPEPTIDE EVOLUTION AND FUNCTION: INSIGHTS FROM ECHINODERMS

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Echinoderms (e.g. starfish, sea urchins) together with hemichordates (Ambulacraria) are a sister clade to the chordates within the deuterostome clade of the Bilateria and as such can provide key missing links for reconstruction of the evolution of neuropeptide signalling systems and comparative analysis of neuropeptide function. Phylogenetic analysis has revealed a bilaterian clade of G-protein coupled receptors (g-rhodopsin-type) that include receptors activated by somatostatin(SS)/allatostatin-C(ASTC)-type and kisspeptin(KP)-type neuropeptides. Here I will discuss our investigations into the occurrence of these signalling systems in echinoderms, using the starfish *Asterias rubens* as an experimental model.SS-type and ASTC-type neuropeptides are structurally and evolutionarily related and act as inhibitory regulators of physiological processes in vertebrates and insects, respectively. Two precursors of SS/ASTC-type neuropeptides are present in *A. rubens*, with one comprising an ASTC-like peptide (ArSS1) and the other comprising an SS-like peptide (ArSS2). There are three *A. rubens* SS/ASTC-type receptors, which are activated by ArSS2 but not by ArSS1. Furthermore, functional characterisation of ArSS1 and ArSS2 revealed that they have opposing myoexcitatory and myoinhibitory effects in *A. rubens*. Hitherto it was thought that SS-type peptides in chordates and ASTC-type peptides are orthologs. Our discovery and functional characterisation of both ASTC-type and SS-type neuropeptides in starfish and other echinoderms indicates that these peptides are paralogous and originated by gene duplication in a common ancestor of the Bilateria, with subsequent loss of one type in chordates and protostomes, respectively, and unique retention of both types in echinoderms. Interestingly, the receptor(s) that mediate the excitatory effects of ArSS1 in *A. rubens* remain to identified. KP-type signalling is an important regulator of reproductive maturation in vertebrates. Our analysis of the genome of *A. rubens* revealed the presence of eleven genes encoding KP-type receptors (ArKPR1-11), which contrasts with the occurrence of a single KP-type receptor in humans and other mammals. Four precursors of neuropeptides that share sequence similarity vertebrate KPs were identified in *A. rubens* and experimental studies revealed that these neuropeptides act as ligands for six of the eleven *A. rubens* KP-type receptors. The Discovery of this remarkable diversity of neuropeptides that act as ligands for KP-type receptors in starfish provides important new insights into the evolution of KP signalling in the Bilateria. On-going studies are investigating the physiological roles of KP-type neuropeptides in starfish.

Acknowledgments: I am very grateful to PhD students and postdocs in my group and our collaborators who obtained results reported in this presentation and to Leverhulme Trust and BBSRC for financial support.

SOTA 2
ADVANCES IN UNDERSTANDING JUVENILE HORMONE BIOLOGY

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Juvenile hormones (JHs) are sesquiterpenoids synthesized by the *corpora allata* (CA). They play critical roles during insect development and reproduction. Several JH homologs have been identified in insects, all acting through a single intracellular JH receptor, the ligand-activated transcription factor Methoprene-tolerant (Met). Recent advances in understanding the functional architecture and molecular interactions of JH with their transporter and receptor proteins provide new insights into the evolution of sesquiterpenoid hormone biology.

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SOTA 3
NOVEL INSIGHTS INTO THE ACTION OF CORTICOSTEROID RECEPTORS FROM KNOCKOUT ZEBRAFISH

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The corticosteroid stress response is highly conserved in vertebrates, and a major role is in maintaining energy homeostasis as part of the adaptive stress response. The corticosteroid action during stress and under resting conditions is primarily mediated by two receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Both these receptors are ligand-activated transcription factors and initiate gene expression changes either by homodimerization or heterodimerization of the receptors. Most work to date has characterized the metabolic role of glucocorticoids via the activation of the GR, which are low-affinity receptors and activated under high hormone levels. However, glucocorticoids also bind to the MR, a high-affinity corticosteroid receptor active even under basal hormone levels. Unlike mammals, teleosts lack aldosterone, the hormone involved in the mineralocorticoid action in tetrapods. Despite the expression of MR in key tissues, neither a mineralocorticoid function nor any physiological role associated with this receptor activation was evident until recently in teleosts. As both these receptors are evolutionarily conserved, a major focus of our lab has been to discover the stress-related functions of cortisol mediated by MR activation either alone or in combination with GR. To this end, we developed viable zebrafish lines with either a ubiquitous MR knockout (MRca402/ca402) or GR knockout (GRca401/ca401), and together with cortisol manipulations, we were able to differentially activate these receptors. Our studies have revealed that GR and MR have specific as well as complementary roles in modulating stress-related behavior and metabolism. A key discovery has been that MR activation promotes anabolic processes, and may also modulate the catabolic actions associated with GR activation. Specifically, MR promoted nutrient storage, and restricted energy substrate mobilization under resting conditions, whereas GR activation resulted in increased nutrient utilization. Interestingly, a loss of MR improved GR-driven metabolic flexibility, suggesting that the activation state of these receptors is a key determinant of skeletal muscle's ability to switch fuel sources. Overall, emerging evidence points to a key role for MR, along with GR, in the metabolic adjustments and energy repartitioning during stress in fish.

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SOTA 4
EVOLUTION OF LIGAND SELECTIVITY FOR THE MELANOCORTIN-2 RECEPTOR: IMPLICATION FOR THE HPA/HPI AXIS OF VERTEBRATES

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The melanocortin 2 receptor (MC2R) is a critical component of the hypothalamus-pituitary-adrenal/interrenal (HPI/HPA) axis in vertebrates. MC2R in the adrenal/interrenal receives neuroendocrine signaling from the anterior pituitary in the form of ACTH and translates this signal into intracellular steroidogenic action of the glucocorticoid-producing cells. In most vertebrates, the functionality of MC2R is dependent on co-expression with the accessory protein, MRAP1. MC2R is one of five melanocortin receptors (MCRs) present in extant gnathostomes. The diversification of the MCR gene family to its five modern receptors (MC[1-5]R) appears to have occurred after the emergence the ancestral gnathostomes and prior to the divergence of the cartilaginous fishes. Recent studies on MC2R orthologs from holocephalan and elasmobranch fishes indicate that the cartilaginous fish MC2R can be activated by either ACTH or MSH. These findings imply that there may be separate hypothalamus/anterior pituitary/interrenal and hypothalamus/intermediate pituitary/interrenal axes in cartilaginous fishes. Numerous studies on neopterygian fishes, amphibians, reptiles, birds, and mammals have shown that the derived MC2R is exclusively selective for ACTH—only ACTH can activate MC2R of these groups. To date, there have been no investigations into the pharmacological properties of MC2R orthologs from basal actinopterygians or basal sarcopterygians. Thus, it remains unknown at what point in the divergence of the gnathostomes that MC2R became exclusively selective for ACTH. In this presentation, we discuss the pharmacological properties of MC2R in basal actinopterygian fishes of the group Chondrostei (acipenseriformes and polypteriformes) and a representative species of lungfish (a basal sarcopterygian) with a focus on ligand selectivity and MRAP1 dependence, filling a gap in knowledge regarding MC2R function in basal bony fishes.

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SOTA 5
REGULATION OF CELL PLASTICITY BY IGF AND CALCIUM SIGNALING: NEW INSIGHTS FROM FISH IONOCYTES

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Many types of differentiated cells are endowed with the ability to reenter the cell cycle. This cell plasticity is critical for tissue renewal and regeneration. The underlying mechanisms, however, are incompletely understood. A major challenge in the field is that our current knowledge is mainly derived from injury models. Whether differentiated cells can de-differentiate and re-enter the cell cycle in a physiological context is still unclear. We have recently developed a fish model, in which a population of Ca^{2+}-transporting ionocytes (NaR cells) are genetically labeled by GFP expression. NaR cells are strucrually and functionally similar to human intestinal and renal epithelial cells. When transferred to a low [Ca^{2+}] embryo medium, these differentiated and polarized cells are reactivated and undergo robust proliferation. Using this model, a combination of genetics, RNA-seq, chemical biology, in vivo imaging, and biochemical studies were carried out and several secreted and cell autonomous factors regulating cell plasticity have been elucidated; they all converge on the nutrient sensitive insulin/IGF (IIS)-PI3 kinase-Akt-Tor signaling pathway. Importantly, these mechanisms are conserved in mammalian cells. Among the autonomous factors is Trpv6, a constitutively open epithelial calcium channel which promotes NaR differentiation by conducting Ca^{2+} and inhibits IGF signaling via a Ca^{2+}-regulated protein phosphatase. One of the secreted factors is Stc1a, a known hypocalcemic hormone. Stc1a inhibits NaR cell proliferation by suppressing IGF1 signaling in a Ca^{2+} state-dependent manner. Genetic deletion of Stc1a led to elevated ionocyte proliferation, leading to higher body Ca^{2+} levels, cardiac edema, body swelling, and premature death. Mechanistically, Stc1a acts by inhibiting the activity of Papp-aa, a zinc metalloproteinase degrading Igf binding protein 5a (igfbp5a). Genetic deletion of *papp-aa* or its substrate *igfbp5a* in the *stc1a*$^{-/-}$ background reduced ionocyte proliferation and rescued the edema and premature death phenotypes. These findings will be presented and discussed in this talk.

Acknowledgments: This work was supported by National Science Foundation grants IOS-1557850 and IOS-1755268.

SOTA 6
MOLECULAR MODULES THAT CREATE EGGS AFTER SEX DETERMINATION OF GERM CELLS

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Gametogenesis, the production of eggs and sperm, is essential for sexually reproducing animals. However, it remains unclear how different gametogenesis processes are initially integrated. With the advantages of the teleost fish medaka (Oryzias latipes), in which germline stem cells continuously produce eggs and sperm in mature gonads and a sexual switch gene in germ cells is identified, we found that distinct pathways initiate gametogenesis cooperatively after commitment to gametogenesis. These pathways are genetically independente and involve an event of female-specific meiosis and folliculogenesis. This evokes the concept of functional modules, in which functionally interlocked genes are grouped to yield distinct gamete characteristics. We would like to discuss the possibility that various combinations of modules explain the evolution of diverse reproductive systems, such as parthenogenesis and hermaphroditism. In addition, artificial production of hermaphrodite by modification of the modules will also be introduced.

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SOTA 7
HOW EARLY ARE T3 EFFECTS UPON CENTRAL NERVOUS SYSTEM MYELINATION?

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During development, the bioactive thyroid hormone T3 is key for central nervous system myelination by promoting the differentiation and maturation of oligodendrocyte (OL) precursor cells (OPCs) as well as by regulating the expression of genes that encode for myelin structural proteins. These processes lead to the myelination of neuronal axons and are fully functional around the perinatal period, at least in mammals. However, the onset of T3 action during myelination has not been fully described, possibly due to the methodological complexity of using *in vivo* models. Interestingly, we and others have shown that the zebrafish oocyte is provided with a maternal load of the pro-hormone T4 and T3, as well as by maternally supplied transcripts encoding proteins that participate in the thyroid hormone signalling pathway (*dio2*, *dio3a*, *b* and thr) as well as in oligodendrogenesis and myelin formation (*olig*2, *sox10*, *mbp*, *plp1b*), suggesting that T3-signalling participates in myelinogenesis earlier in development than previously thought. To further understand T3 action in these processes, we decreased embryonic T3 availability by blocking deiodinase action and observed reduced myelination in the fore- mid and hind brain at 3 days post-fertilization (dpf), observed by Black Gold II staining and in *mbp:egfp* transgenic organisms. This T3 deficiency also produced a significant decrease in OPC population marked with anti-NG2 antibodies as well as in the expression of genes involved in OL specification and myelination, such as *olig2*, *mpz*, and *plp1b*. T3 administration (5 nM) succeeded in restoring brain myelination. Subsequently, we evaluated the effects of exogenous T3 administration on oligodendrogenesis, analysing the development of neural precursor cells (NPCs), OPCs, and OLs during zebrafish development. Interestingly, embryos exposed to a single treatment of T3 (25 pM) at 1 hpf showed a significant decrease in the OPC population and myelin content when analysed at 3 dpf. Given that NPCs emerge and begin their specification processes at around 10 hpf, our results suggest that even a discrete increase of T3 in the developing embryo negatively impacts NPC to OPC progression. Also, these effects were not observed when the embryo was exposed to the same T3 treatment in more advanced developmental periods (24 and 48 hpf), further highlighting the importance of precise timing of T3 action during developmental programs. In this context, the fact that neural tube closure in the zebrafish occurs at around 20 hpf, and that we observed a clear negative effect of T3 in developmental events that take place at around 10 hpf strengthen the idea that T3 indeed plays an earlier role in the determination and/or progression of OL cell lineage than previously thought.

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SOTA 8
ROLE OF THYROID HORMONES IN THE MULTIFACTORIAL CONTROL OF REPRODUCTION AND SPERMATOGENESIS IN FISH

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Gametogenesis in fish is controlled by gonadotropin hormones (LH and FSH) working in concert with a number of peripheral hormones, including thyroid hormones, gonadal peptides and steroids. Our studies demonstrate that the production of LH and FSH are influenced by thyroid hormones working in concert with neurohormones, including gonadotropin-releasing hormones (GnRH) and gonadotropin-inhibitory hormone (GnIH). Circulating LH and FSH interact with thyroid hormones and neuropeptides of gonadal origin to regulate gonadal function and gametogenesis. Goldfish and zebrafish were used to study the effects of thyroid hormones on the pituitary LH and FSH production and spermatogenesis. Isolated adult zebrafish testis was used in an ex-vivo culture system to demonstrate that thyroid hormones exert direct actions on different stages of spermatogenesis, using histomorphometric, gene markers and immunohistochemistry approaches. In zebrafish, spermatogenesis starts with the mitotic division of undifferentiated spermatogonial stem cells and the generation of diploid spermatogonia (Spermatogonia A and B). This is followed by a meiotic phase and the generation of primary and secondary spermatocytes (haploid). The final stage is further differentiation into spermatids and eventually flagellated spermatozoa. In the testis, LH and FSH interact with thyroid hormones to control the production of hormones, which regulate spermatogenesis. The presence of thyroid hormones is essential for the control of early and late stages of spermatogenesis in zebrafish.

Acknowledgments: The findings support the hypothesis that thyroid hormones work in concert with the pituitary gonadotropins and are essential components of the multifactorial control of spermatogenesis in the zebrafish testis.

SOTA 9
ACROSS THE METAMORPHIC DIVIDE: DEVELOPMENTAL MECHANISMS UNDERLYING THE STRESS-INDUCED SUSCEPTIBILITY HYPOTHESIS IN AMPHIBIANS.

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According to the stress-induced susceptibility hypothesis, environmental challenges can increase the severity of infection outcomes, but how an animal responds to these challenges depends on the complex endocrine-immunological responses during critical developmental windows. When infections are incurred during early life stages, these responses intersect with developmental processes that exacerbate the severity of infections, but they can also help animals evade death by stimulating life history transitions. Study of the host-pathogen interactions in wood frogs, an abundant, cold-adapted North American species that is highly sensitive to environmental cues and susceptible to infections during its larval stage, has allowed us to experimentally manipulate environmental conditions, endocrine factors, and infection status to better understand how environmental stressors alter host-pathogen dynamics across multiple scales and life history stages. Our studies of the ranavirus-wood frog system revealed that the stress of elevated salinity associated with road deicing-salt run-off intersects with the stress of infection, resulting in more severe infections through the elevation of glucocorticoids and suppression of immunity. Yet, not all individuals suffer lethal consequences, as some accelerate metamorphosis, which allows them to survive and escape from the pond. This response may be condition-dependent and the nutritionally regulated hormone leptin may be involved. Leptin enhances immune responses and wound healing in amphibians, and potentially through its actions on corticotropin-releasing hormone in the hypothalamus, sensitizes wood frog tadpoles to stress-induced metamorphosis. When looking at the fungal pathogen, *Batrachochytrium dendrobatidis*, which is more lethal after metamorphosis, we have shown that when wood frogs undergo stress-induced metamorphosis from pond drying, their immune system is suppressed into the juvenile stage, as antimicrobial peptide responses are suppressed. These case studies illustrate how endocrine responses to pathogen infections are context specific, and because endocrine responses associated with metamorphosis are conserved across similar life history transitions, these studies have implications for understanding how stress and infectious disease interact across vertebrates.

SOTA 10
THE STRESS OF SUBORDINATION: IMPACTS OF CHRONIC SOCIAL STRESS ON HPI AXIS FUNCTION IN RAINBOW TROUT

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Juvenile rainbow trout form social hierarchies that differentially affect hypothalamic-pituitary-interrenal (HPI) axis activity according to social status. Subordinate trout exhibit chronically elevated cortisol levels and a blunted cortisol response to additional acute stressors. We used juvenile, size-matched rainbow trout that interacted in pairs for 4 d, and a combination of *in vivo* and *in vitro* approaches, to investigate the mechanisms underlying these effects. Prolonged elevation of cortisol in subordinate fish did not reflect impaired cortisol clearance or dysregulation of negative feedback. Rather, it may reflect a change in the set point at which cortisol levels are regulated, mediated by changes in 11ß-hydroxysteroid dehydrogenase 2 (11bhsd2) in the preoptic area of the brain and the pituitary. Chronic social stress also altered steroidogenesis in the interrenal cells of the head kidney. In particular, subordinate trout had higher transcript abundances of several key components of steroidogenesis, including the melanocortin receptor accessory protein (*mrap*), a paralog of steroidogenic factor 1 (*ff1d*), p450 side chain cleavage enzyme (*p450scc*) and steroidogenic acute regulatory protein (*star*). These differences translated into higher cortisol production under baseline conditions from isolated head kidney preparations *in vitro*. However, the preparations showed blunted cortisol production in response to ACTH or the cAMP analogue dibutyryl-cAMP (db-cAMP), corresponding to the blunted cortisol response to ACTH observed *in vivo*. Collectively, the data indicate that chronic social stress modifies cortisol steroidogenesis and that these changes contribute to the effects of social stress on whole-animal cortisol dynamics.

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SOTA 11
EPIGENETICS IN THE DIVERSITY OF SEXUAL SYSTEMS AND *SEX DETERMINING* MECHANISMS IN FISH

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In this talk, first, an overview of the different sexual systems and sex determining mechanisms present in fishes will be offered. Then, recent examples on the involvement of epigenetic regulatory mechanisms of gene expression in sex determination will be shown, focusing first on species with genetic sex determination and, specifically, chromosomal sex-determination, with either male or female heterogamety. Thus, examples of DNA methylation, non-coding RNAs and transposon-mediated silencing of gene expression will be presented. We will then focus on species with polygenic sex determination, in which the environment also plays a prominent role in the control of testicular and ovarian development, and will conclude this part of the talk debating the situation in species with environmental sex-determination. Next, the role of epigenetics during the process of gonadal sex differentiation in gonochoristic species and in the process of sex change in sequential hermaphrodites will be presented as examples of epigenetics underpinning phenotypic plasticity. In the last part of the talk, we will discuss two areas where epigenetics can provide new insights. First, given that instances of sex reversal are increasingly being found in wild populations around the world, we will show how the incorporation of epigenetics can help to investigate and better understand the effects of global warming on sex ratios, contemplating epigenetic traps. Secondly, how the development of epigenetic biomarkers may be of use if integrated into selective breeding programs in fish farms to exploit certain desirable phenotypes. The talk will conclude by identifying knowledge gaps and suggesting avnkenues for future research.

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SOTA 12
SINGLE CELL TRANSCRIPTOMICS IN ZEBRAFISH WITH NATURAL CHROMOSOMAL SEX DETERMINATION

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The genetics underlying sex determination varies across taxa, sometimes even within a species. For zebrafish (*Danio rerio*), the domesticated laboratory strains AB and TU lack a strong genetic sex determining locus, but strains more recently derived from nature (e.g., Nadia (NA)) possess a sex-determining locus on chromosome-4 with a ZZ male/ZW female system. AB fish pass through a juvenile ovary stage, forming oocytes that survive in fish that become females but die in fish that become males. To understand the mechanisms of gonad development in NA zebrafish, we studied histology, bulk RNA-seq, and single cell transcriptomics in ZZ and ZW fish. ZW fish developed oocytes by 22 days post-fertilization (dpf) but ZZ fish directly formed testes, avoiding the juvenile ovary phase. Gonads of some ZW fish, however, developed oocytes that then died as the gonad became a testis, like AB strain gonads. RNA-seq of adult gonads revealed transcriptional silencing on the right arm of chromosome-4 in ZW NA and AB female ovaries that was actively transcribed in ZZ NA and AB testes. Single-cell RNA-seq of 19dpf gonads showed similar cell types in ZZ and ZW fish, including precursors of gonadal support cells and germline stem cells, consistent with a bipotential juvenile gonad. In contrast, scRNA-seq of 30dpf gonads revealed cells with transcriptomes characteristic of testicular Sertoli, Leydig, and germ cells in ZZ fish and ovarian theca and granulosa cells and developing oocytes in ZW fish. These results show that juvenile NA zebrafish initially develop a bipotential gonad, that a factor on the NA W chromosome is essential to initiate oocyte development, and without that factor, NA ZZ gonads develop directly into testes. Gonad development in AB and TU mimics NA ZW and WW zebrafish, suggesting loss of the Z chromosome during domestication. Analysis of the Nadia strain will facilitate our understanding of the evolution of sex determination mechanisms.

SOTA 13
EMERGING QUESTIONS ON THE TELEOST HYPOTHALAMIC-PITUITARY SYSTEM DURING DEVELOPMENTAL AND ADULT STAGES

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The hypothalamic-pituitary (HP) system regulates major physiological functions in vertebrates through a complex network involving different neuropeptides, neurotransmitters, neuromodulators, and pituitary hormones. Among vertebrates, the fish HP system presents unique characteristics such as direct innervation of pituitary cells, a high regionalization of the different cell types, and the presence of two different gonadotrops, among others. These characteristics make this system an excellent model for comparative neuroendocrinological studies. Recently, the classical concept of HP regulation in fish has been modified by the use of different tools including knockdown of specific neuropeptides, transgenic fish, and advanced microscopic technics. So, new and interesting lines of research on this topic are knocking labs' doors. In addition, the HP axis develops early in life, and it is functional from its early establishment. The early appearance of different players of this network during embryonic or larval stages, and the fact, that if they are silenced, developmental alterations beyond the classical function known in adults occur, suggest that these components play specific roles during development. Besides, any developmental alteration on the components of the HP system may have an impact on adult life. In this presentation, historic and new perspectives of the HP system will be presented, focusing on possible new developmental functions of the classical neurohormones. In this frame we will also discuss results obtained in different Neotropical fish species.

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SOTA 14
PITUITARY PLASTICITY IN THE TELEOST FISH

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The pituitary gland, which is a center of command for many physiological functions, is a highly plastic organ. It adapts the hormonal production to the demand that changes throughout the life of an animal. While changes in cell activity (gene expression and secretion) are widely studied, only little is known about the mechanisms allowing changes in cell number. To study the organization and reorganization of the different cell types in the pituitary we developed a 3D atlas mapping the different endocrine cell types. These atlases have allowed us to identify differences in cell numbers between sexes and between different physiological states. Focusing on gonadotropes, we investigated how new endocrine cells are produced in adults. Contrary to mammals and birds, Lh and Fsh are mostly produced by two separate pituitary cell types in teleost. Using transgenic lines of medaka (Oryzias latipes) where DsRed2 and hrGfpII are respectively under the control of the endogenous fish and lhb promotors, we investigated Fsh and Lh cells hyperplasia. We looked at Fish and Lh cell division, migration and phenotypic conversion, and identified some of the regulatory factors.

SOTA 15
EVOLUTION AND FUNCTIONS OF UROTENSIN II-RELATED PEPTIDES

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The urotensin II (UII) family is a multigenic family of neuropeptides, evolutionary related to somatostatin (1), which consists of four paralogous genes called uts2, uts2-related peptide (urp), urp1 and urp2. All peptides of this family act through a canonical family of G protein-coupled receptors called urotensin II receptors (Utr or Uts2r). The occurrence of multiple utr genes (utr1–utr5) has been reported in non-mammalian vertebrate genomes, which is in contrast to the single gene (utr1) present in mammals. The diversification of the UII and UTR families is mainly due to the two rounds of whole genome duplication that arose during vertebrate history. The functions of UII have been extensively studied for more than 40 years and it has been shown that UII is involved in many physiological processes, including osmoregulation, cardiovascular activity, metabolism and reproduction. In contrast, only very little is known about the roles of URPs. We will report on recent studies investigating the functional properties of URPs both in vitro and in vivo, in this latter case by using the zebrafish and xenopus as models.

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SOTA 16
LOOKING FOR THE TRUE LOVE HORMONE: EVIDENCE THAT SECRETONEURIN CONTROLS REPRODUCTION

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The dogma of reproductive endocrinology is that hypothalamic kisspeptin (Kiss) and gonadotropin-release hormone (Gnrh) neurons drive the Lh surge that triggers ovulation. Research in teleosts is now challenging this widely held view. Mutations in genes encoding for Kiss, Gnrh, Oxytocin and numerous other classical neuropeptides have little to no impact on reproduction in zebrafish (ZF) and medaka. In the last decade we have shown that the secretogranin-2 (Scg2) derived peptide secretoneurin (SN) stimulates in vitro Lh release from dispersed goldfish pituitary cells and mouse LbT2 tumour cells independent of Gnrh. Critically missing has been direct in vivo evidence for a role of endogenous Scg2/SN neuroendocrine systems. Most vertebrates have one Scg2 precursor of ~600 amino acids. Selective Scg2 processing produces the bioactive peptide SN which is 31-34 amino acids long. Teleosts have two paralogous scg2 genes: the phylogenetically ancient scg2a and the lineage specific scg2b. Double scg2a-/-;scg2b-/- frameshift mutant females are very poor spawners. This repressed reproduction can be partially rescued by treatment with SNa but not SNb. Behavioural analysis indicates that ZF lacking the entire coding regions for scg2a or scg2b have delayed oviposition. Our data indicate that Scg2/SN stimulate both Gnrh3 neurons and Lh cells. Mass spectroscopy measurements of SNa in brain implies activation of Scg2a neurons prior to ovulation. Injection (i.p.) of SNa increases expression of classical reproductive genes at all levels of the HPG axis and robustly stimulates ovulation in otherwise anovulatory female ZF that have been isolated from males. Our observations indicate that the Scg2/SN system is a critical new player in vertebrate reproduction.

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SOTA 17

GONASCINA: GERMLINE STEM CELL-DERIVED HORMONE WITH GLUCOGENIC, OREXIGENIC, AND GONADAL ACTIVITIES

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Fish are unique in requiring abundant nutrients to generate large number of eggs for spawning. Based on evolutionary conservation of human FBN2 and its C-terminal placensin-like sequences in fish, we identified a peptide hormone gonascin (GONAd germ Stem Cell placensIN) and found its high expression in germline stem cells in ovary and testis of zebrafish. Treatment with recombinant gonascin increased oocyte maturation. Gonascin could also stimulate glucose secretion and promote food intake in vivo whereas neutralizing gonascin antibodies suppressed glucose secretion and appetite. Gonascin null fish showed larval lethality that was rescued by overexpression of gonascin in gonads. In addition, conditional knockout of gonascin in germ cells led to defects in appetite, glucose secretion, and ovarian development in zebrafish. Similar expression pattern and functions of gonascin was also demonstrated in rainbow trout. Thus, gonascin is a novel hormone secreted from gonads with glucogenic, orexigenic and ovarian functions important for fish survival and reproduction.

SOTA 18
SPEXIN AS A SATIETY FACTOR: THE STORY FROM FISH TO MOUSE MODEL

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Spexin (SPX) is a neuropeptide with pleiotropic functions and its biological actions are mediated by the receptors GalR2/GalR3. Recently, SPX has been confirmed to be a novel satiety factor in fish species but whether the peptide has a similar function in mammals is still unclear. Using the mouse as a model, the functional role of SPX in feeding control and the mechanisms involved were investigated. As a first step, the NMR solution structure of mouse SPX and its molecular docking with its cognate receptors GalR2 and GalR3 were established. After characterizing the tissue expression of SPX, functional study was performed to reveal that food intake could up-regulate SPX release in circulation and SPX expression in glandular stomach but not in other tissues examined in mouse model. In glandular stomach, SPX signals induced by food intake were localized in the foveolar cells, parietal cells and chief cells within the gastric glands of the mucosal layer. In mouse model, SPX treatment reduced food intake by inhibiting hypothalamic expression of NPY, AgRP, NPY type 5 receptor (NPY5R) and ghrelin receptor (GHSR) via GalR3 but not GalR2 activation. In parallel studies, glucose treatment was found to elevate SPX transcript level in glandular stomach but the opposite was true with insulin stimulation, and these differential effects on SPX gene expression could be mapped to the gastric mucosa by in situ hybridization. Using a gastric mucosal cell culture prepared from mouse stomach, the opposite effects on SPX gene expression by glucose and insulin could be noted with parallel blockade of glucose-induced SPX gene expression by insulin co-treatment. In this cell model, glucose-induced SPX gene expression was mediated by glucose uptake via GLUT, ATP synthesis by glycolysis/respiratory chain, and subsequent modulation of K_{ATP} channel activity. The corresponding inhibition by insulin, however, was mediated by PI3K/Akt, $MEK_{1/2}/ERK_{1/2}$ and $P_{38}{}^{MAPK}$ cascades coupled to insulin receptor but not IGF-1 receptor. Our findings, as a whole, suggest that (i) the postprandial signal of SPX from glandular stomach can activate GalR3 in the hypothalamus to trigger a feedback inhibition on feeding by modulation of feeding regulators and their receptors involved in the feeding circuitry of the CNS, and (ii) glucose uptake in mouse can induce SPX expression in glandular stomach through glucose metabolism and subsequent modification of K_{ATP} channel activity, which may contribute to SPX release into circulation after feeding. The insulin signal caused by glucose, presumably from the pancreas, may serve as a negative feedback to inhibit the SPX response via activating the MAPK and PI3K/Akt cascades in the stomach.

SOTA 19
SWIMMING ECONOMY OF FISH AND EXERCISE-ENHANCED GROWTH

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Because fish swim almost constantly, exercise is their basic physiological condition. Swimming costs energy but can have important anabolic effects, for instance in reproductive migrants where sexual maturation occurs during a constant state of exercise or when training enhances growth and builds muscle. Exercise plays an important role in mobilizing energy resources for fuel and for developmental processes. Studying the swimming economy, its trade-offs and the regulatory mechanisms behind them can provide not only fundamental insights, but also a toolbox for manipulating energy flows in cultured fish. Athletic swimming performance is heritable (in European sea bass and Nile tilapia). Early life swimming performance has predictive value for growth performance during later life showing a clear relation between swimming and growth. The negative correlation between them in tilapia may reflect strategies towards predator avoidance by either swimming fast or growing fast. Numerous studies have shown exercise-enhanced growth of fish, specifically through hypertrophy of white skeletal muscle and increase of filet mass. For example, cyprinid zebrafish experimentally exercised for 20 days increased their body weight by ~40% compared to resting fish, and carangid yellowtail kingfish exercised for 18 days increased their body weight by 46%. We are currently testing whether these experimental results apply under farming conditions. Also the sparid Gilthead seabream showed exercise-enhanced growth but only at speeds lower than their optimal swimming speed, the speed at which they swim most efficiently. Swimming at 1 BL s-1 enhanced their growth by 15% without inducing lordotic abnormalities. The mechanism(s) behind exercise-enhanced growth are not well understood. Swim training lowers baseline cortisol levels but cortisol acting through the glucocorticoid receptor is not involved in exercise-enhanced growth as elucidated by comparing exercised zebrafish with a mutant receptor vs. wild-type fish. RNAseq revealed that genes involved in transcriptional regulation and protein ubiquitination may play an important role. The GH/IGF axis may play a pivotal role although exercise-enhanced changes observed in this axis lack consistency. The potential role of androgens remains unclear. Currently our research is focussed on the application of sensor technology for studying the swimming physiology of fish. We apply real time acoustic acceleration tags and heart rate-acceleration loggers to obtain a more comprehensive view on activity patterns and energy economy. An integrative approach combining swim devices, sensor technology and hormone measurements should provide mechanistic insights.

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SOTA 20
FROM LAMPREYS TO MULTIPLE SCLEROSIS AND BACK AGAIN

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Lampreys and hagfish (agnathans) are the most primitive extant vertebrates. One way in which they distinguish themselves is by metamorphosis being triggered by a decrease in thyroid hormones (THs), in contrast to other vertebrates where metamorphic developmental changes are triggered by an increase in THs. All vertebrates except agnathans have a myelin sheath that surrounds nerve axons, giving protection and expediting signalling. Myelin is formed by oligodendrocytes wrapping around nerve axons. The development of myelin is (at least in part) regulated by THs. We wondered if the decrease in THs during metamorphosis was in part related to the lack of myelination in lampreys. In the absence of lampreys, we used mice lacking a TH distributor protein: transthyretin (TTR). TTR mice have delayed brain development and delayed development of other TH-regulated features (e.g. long bones). We investigated if TTR null mice have delayed and/or reduced myelin in the corpus callosum. To our surprise, adult TTR null mice had thicker myelin than wildtype mice. TTR null mice developed myelin earlier, with a greater proportion of NSCs maturing into glial than neuronal linages compared with wildtype mice. The absence of TTR enhanced proliferation and migration of oligodendrocyte precursor cells with decreased apoptosis. Significantly, we found mature oligodendrocytes to synthesise TTR, suggesting an alternative function for TTR than TH distribution. Furthermore, following chemically-induced de-myelination, TTR null mice re-myelinated axons faster than wildtype mice. Thus, TTR slows the processes of myelination and re-myelination. We are now identifying small compounds to inhibit the function of TTR in myelination, to develop as a potential therapeutic against diseases such as multiple sclerosis. It is curious that in humans, myelin damage results in debilitating disease and eventually death, yet lampreys have survived for >360 million years without myelin.

SOTA 21
THE GUT-BRAIN AXIS IN FISH: FROM THE DETECTION OF NUTRIENTS IN THE GUT TO THE MODULATION OF CENTRAL APPETITE-REGULATORY SYSTEMS

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In mammals, there is unequivocal evidence that the gastrointestinal tract (GIT) plays a key role in the homeostatic control of food intake and energy balance through the so-called gut-brain axis. Such a role for the GIT is based on the ability of intestinal cells to sense the presence of nutrients (carbohydrates, lipids/fatty acids and proteins/amino acids) in the lumen through several receptors and transporters. Although three intestinal cell types (brush cells, enterocytes, and enteroendocrine cells -EECs-) have been proposed to participate in nutrient sensing, EECs are the primary chemosensory cells within the GIT. EECs respond to the sensing of nutrients with the secretion of different hormones, mainly ghrelin (GHRL), cholecystokinin (CCK), peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1), which, apart from acting locally, send information on energy status to the central nervous system, ultimately modulating brain circuits governing appetite and energy balance. While this pathway is well established in mammals, there is very little information in fish on gut nutrient sensing mechanisms and their implication in the control of food intake. During the last years, our research group has devoted to the characterization of the gut-brain axis in fish, using rainbow trout as a model, and has found promising evidence in favor of the existence of nutrient sensing mechanisms in the trout GIT, putatively involved in food intake regulation. This talk offers a brief overview of our main findings and aims to summarize the current state-of-the-art knowledge on the gut-brain axis in fish.

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SOTA 22
LESSONS FROM AMPHIBIANS: TH ACTION AT METAMORPHOSIS

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Thyroid hormones (TH) and glucocorticoids (GC) signalling cooperate at forming a functional interface that integrate internal signals together with stress responses inferred from biotic and abiotic cues. The action of each hormone alone is well described, but their combined action and dynamic remain poorly known. In previous work, we addressed this in a highly responsive tissue (tailfin) of Xenopus tropicalis pre-metamorphic tadpoles. We showed that TH and GC interactions are more complex than expected and can not be accounted for by the sole action of GC at regulating dio2 and dio3 activity and indirectly modulating TH availability. Functional genomics coupled to system biology offered a qualitative and quantitative description of transcriptional responses. In addition to TH and GC specific responses, there is an additional component, quantitatively modest (only 12 % of all regulated genes) and qualitatively more diverse, corresponding to the set of crosstalks responses. TH and GC response genes are very tissue-specific and very little overlap is usually found between them, but it is of interest to ask whether crosstalk responses share similar properties between tissues. This is the point we question in this work, and we provide additional transcriptomic data from liver, by following the same experimental setup, data analysis and modelisation workflows. Liver, which is left anatomically unchanged after metamorphosis, is of special interest because of its strong metabolic activity and especially urea metabolism, with the transition from ammonotelism to ureotelism during metamorphosis. We found that despite vastly different TH and GC target genes, the liver transcriptional response remains qualitatively similar to that of tailfin: mostly TH-only responses (> 70 % of all regulated genes), around 15 % of crosstalk and very little GC specific response. In biological networks, crosstalks-specific response cluster together and form a subnetwork involved in the control of cell prolieration, suggestive a potential source of cellular imbalance at metamorphosis when exposed to high doses of GC.

SOTA 23
THE LONG AND SHORT OF THE ADIPOKINETIC HORMONE/RED PIGMENT-CONCENTRATING HORMONE PEPTIDE FAMILY IN INSECTS.

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The AKH/RPCH peptide family is well-known since the first structural elucidation in a decapod crustacean 50 years ago and in an insect shortly thereafter. The primary function of these short neuropeptide hormones had already been deduced from even earlier experimentation (100 years ago in the case of RPCH and more than 60 years ago for AKH), and is reflected in the naming of the peptides, viz. blanching of the decapod integument due to translocation of red pigments into the center of chromatophores (RPCH activity) and the mobilization of stored lipids from the fat body cells into circulation (AKH activity). Over the many decades, additional functions have been added to the list, the inventory of peptide sequences have ballooned and through genomic sequencing, the corresponding receptors have been elucidated, and it is now possible to study signal transduction pathways on a molecular level and to predict receptor-ligand interactions through molecular dynamic modeling. In the spirit of the Symposium topic, this lecture will focus on AKHs in insects and reflect on the role that Gerd Gäde has played in shaping the AKH landscape since his first report on AKH signal transduction in the migratory locust 46 years ago as a young postdoc, to our current AKH projects that span physiology, chemistry, molecular biology and phylogenetic interpretations with the goal of providing information for comparative endocrinologists and towards the design of target-specific insecticides.

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SOTA 24

THE ADIPOKINETIC HORMONE - GONADOTROPIN RELEASING HORMONE FAMILY OF PEPTIDES: ROLE DURING POSTEMBRYONIC DEVELOPMENT OF THE DESERT LOCUST

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Adipokinetic hormone (AKH) was one of the very first peptide hormones being identified in insects. At present, it is a highly researched peptide known to induce the mobilization of carbohydrates and lipids from the insect's fat body at times of high physical activity, such as flight. As naturally occurring ligands, the AKH family members have undergone quite a number of amino acid substitutions throughout evolution, and in some insect species, such as locusts, multiple AKHs are present. AKHs usually consist of 8-10 amino acids and have an N-terminus blocked by pyroglutamate and a C-terminus blocked by an amide group. AKHs act by binding to rhodopsin-like G protein-coupled receptors, which are homologous to vertebrate gonadotropin-releasing hormone (GnRH) receptors. In this presentation, we will provide an overview of the state-of-the-art regarding the evolution of GnRH/AKH related peptides, as well as their receptors and functions. In addition, we will report on the molecular evidence we obtained in the desert locust, *Schistocerca gregaria*, a migratory pest species that is continuously being monitored by FAO. Locust AKHs, which are released from the corpora cardiaca during high energy demanding processes, interact with their receptor in the fat body, thereby triggering lipid mobilization. Our research also resulted in the selection of synthetic peptide analogs that display interesting pharmacological characteristics. Moreover, functional knockdown studies revealed the physiological importance of the AKH signaling system in this species. Locust swarms threaten the livelihood of people living in some of the world's poorest countries. In gregarious locusts, energy availability can be a crucial determinant enabling flight and migration. Development of strategies that interfere with energy demanding processes may therefore lead to novel opportunities for controlling devastating locust swarms.

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SOTA 25
PROGESTIN AND METALLOPROTEASES IN ZEBRAFISH GONAD DEVELOPMENT

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Endocrine and paracrine signallings control the development, growth, and maturation of gonads and germ cells, and therefore determining fertilities of the animals. In this lecture, I will discuss our contributions to the field of Endocrinology and animal fertility cetered around on my pioneering studies on characterization and reporting of a novel membrane progestin receptor family in vertebrates. I will emphasize latest results and ongoing efforts concerning 1) The functions and mechanisms of membrane or nuclear progestin receptor and their downstream targets; 2) Steroid signalling and their regulation of metalloprotease activation and coagulation in ovulation, gonadal development, and sex determination in zebrafish model.

SOTA 26
MOLECULAR AND CELLULAR REGULATION OF FISH SPERMATOGENESIS: FOLLICLE-STIMULATING HORMONE AND DOWNSTREAM EFFECTORS

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Spermatogenesis in vertebrates is a developmental process in which a spermatogonial stem cell (SSC) population produces germ cells that go through a series of mitotic and then two meiotic divisions, eventually providing highly differentiated spermatozoa. Early stages of this cellular development are orchestrated by the pituitary follicle-stimulating hormone (FSH), through the production of Sertoli cell-derived factors and by Leydig cell-released androgens. Extrinsic mechanisms, in particular those regulating "strategic" decisions of the SSC population, such as the level (quiescence vs. cell cycling) and quality (self-renewal vs. differentiation) of proliferation, are the basis for a precocious or delayed/limited onset of spermatogenesis that compromise sustainability, animal welfare, and efficiency aspects of finfish aquaculture. To approach these questions, we used zebrafish (*Danio rerio*) and Atlantic salmon (*Salmo salar*) as experimental models. First, we identified Fsh-regulated candidate growth factors relevant for spermatogenesis via gene expression profiling (microarrays, RNA sequencing). Among those, prominently regulated factors by Fsh such as insulin-related peptides Insl3 and Igf3, retinoic adic (RA) and Wnt signaling pathways were selected. Next, we characterized the biological activity of identified candidate factors by gain-of-function and loss-of-function approaches, often using a primary testis tissue culture system (e.g. pharmacological approaches, production of recombinant proteins) or targeted gene knock-out by CRISPR/Cas9. Additionally, we studied the endocrine regulation of expression and/or release of identified candidate factors. We found that Fsh stimulated testicular RA production in zebrafish, a species lacking *stra8* (key gene mediating RA effects in mammalian spermatogenesis), thus linking for the first time in vertebrates the endocrine system to local RA signaling. Genetic ablation of RA signaling in germ cells compromised spermatogenesis, but activated steroidogenesis, leading to an over-compensation of spermatogenesis and testicular hypertrophy. Genetic ablation of *stra8* in Atlantic salmon resulted in increased germ cell apoptosis, but mutants compensated this cell loss by an elevated production of spermatogenic cysts, and were able to produce functional sperm. Surprisingly, the highly Fsh-responsive growth factor Insl3 only moderately promoted germ cell differentiation in zebrafish. The genetic loss of Insl3 is compensated initially, until germ cell apoptosis increased progressively starting at 9 months of age. Fsh also uses canonical (via Sertoli cell-derived Igf3) and non-canonical (via Leydig cell-derived Wnt5a) signalling to achieve a balanced regulation of SSC self-renewal and differentiation. Hence, Fsh makes use of several, locally produced signaling molecules in zebrafish, operating in parallel to regulate spermatogenesis, such that – different from the situation in mammals – failure of one pathway is (partially) compensated by one or more alternative, parallel pathways. Even the loss of Fsh signaling is compensated in zebrafish, however, this is different in salmon, where puberty is blocked in male Fsh receptor mutants.

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SOTA27
USES OF BIOTECHNOLOGY TO CONTROL REPRODUCTION IN ATLANTIC SALMON

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Genetic introgression of escaped farmed Atlantic salmon (Salmo salar) into wild populations is a major environmental concern for the salmon aquaculture industry. Using sterile fish in commercial aquaculture operations is, therefore, a sustainable strategy for bio-containment. So far, triploidization is the only methodology commercially used for producing sterile salmon; however, triploid fish are less robust. Novel approaches to achieve reproductive isolation involve producing germ cell-free salmon, or salmon that cannot enter puberty. One way to accomplish reproductive isolation is to knock out genes encoding proteins involved in either formation of germ cells or initiation of puberty. The inability to form germ cells or to enter puberty, thus, prevents reproduction. However both approaches lead to a reproductive arrest and therefore hinder large scale production of sterile individuals. Searching for alternatives suitable to obtain sterility in production fish led us to combine a method to inherit sterility from a genetically sterile but phenotypically fertile broodstock. Inheriting sterility in broodstock salmon can be achieved by transiently rescuing the function of the lost protein or by producing genetically sterile gametes in wildtype surrogates. Using the first method, we succeded in inducing germ cell production in genetically sterile fish. We have further followed these fish through oogenesis and spermatogenesis and obtained an F1 generation from incrosses and have shown sterility/lack of germ cells in the F1 generation. We are currently investigating whether there may be partial, but not critical, limitations of the method due to potential other functions of the knocked out gene. This approach may solve the problems of both, genetic introgression and precocious maturation in farmed salmon, while ensuring a stable production of 100% sterile fish, and thus represents a significant commercial potential of the technique. The use of this sterility technology may also pave the way for safe genome editing of other traits, such as disease resistance, which would be contained in sterile individuals and hence presented a negligible risk of passing edited alleles on to wild stocks.

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SOTA 28

BIOTECHNOLOGY IN AQUACULTURE - LESSONS FROM RECOMBINANT HORMONES

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Pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play central roles in regulating reproduction and the production of gonadal hormones, in fish as in other vertebrates. With the development of gene cloning techniques and next generation sequencing the availability of Gth coding cDNA's paved the way for the productions of recombinant hormones sidestepping the highly demanding techniques of native protein purification, not only in fish but also in other important species in aquaculture. During the last two decades, recombinant gonadotropins have been used to develop specific antibodies for the development of immunoassays for the quantification of gonadotropins in a variety of aquaculture species allowing us to better understand the hormonal profiles of numerous animals specifically in Tilapia that was the first non-synchronies modal that showed that LH play a role not only during final oocyte maturation, but also during vitellogenesis. specific receptor-based bioassay to assess activity in different species by the production of many different Gth's allowed us to design better hormones with higher potency. In recent years in-vivo administration of recombinant gonadotropins and DNA constructs was used to enhance gonadal development both for spermatogenesis, oogenesis and spawning induction in fish (i.e. Carp) and in other important aquaculture species such as the sea cucumber. Finally, hermaphroditism and the specific roles of gonadal reconstruction in fish are still largely unknown, together with the Evac implant technology and the use of recombinant gonadotropins advances for treatments of sex inversion are made in including the sevenband grouper and Barramundi. The availability of recombinant gonadotropins enabled us to better define their physiological role and pituitary control as well as to further explore the usefulness hormonal treatments for aquaculture.

SOTA 29
THE CHALLENGE OF EVALUATING ENDOCRINE DISRUPTING CHEMICALS FOR MARINE LIFE

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Oceans are harbouring a vast and understudied biodiversity, threatened by climate change and pollution. The ever-increasing human population needs more chemicals and pharmaceuticals to meet sustainable development goals. However, at global level, more than 80% of the domestic and industrial effluents enter the marine environment raw, without any treatment. Although acutely toxic chemicals target biological pathways that present a great degree of conservation amongst phyla, the lack of empirically derived data from key ecological species is becoming a critical need for chemicals that have a special mode of action, such as endocrine disrupting chemicals (EDCs). A key framework for organising toxicological knowledge, the Adverse Outcome Pathway (AOP), is a developing OECD web tool. AOPs, when applied to the context of environmental protection require the construction of a scientifically plausible biochemical pathway that starts from the molecular initiating event and links a series of key events at cellular or tissue level to effects on individual and population trajectory. Generating AOPs for the effect of EDCs on marine species is not an easy task due to the uncertainty generated by the (still) largely undescribed endocrinology of most marine phyla, especially invertebrates. This prevents an adequate understanding of toxicodynamic interactions between pollutants and animals and thus creates uncertainties for chemical management, especially for suspected EDCs.In recent years a large body of literature on ecotoxicology suffered from a superficial interpretation of gene ontology (GO) terminology, assuming functional conservation of endocrine pathways in cross-species extrapolations. Furthermore, the well-documented presence of vertebrate steroids in molluscan tissues was also linked with a putative functional role. There is therefore an urgent need to both advance basic biology knowledge and build targeted resources for chemical risk, including toxicokinetic and toxicodynamic data from multiple species, to be able to protect biodiversity and prevent its loss. Advancements in sequencing and computational tools are becoming vital in studying the diversity of endocrine systems from an evolutionary perspective and can be better used for regulatory decisions. A USEPA web resource, seqAPASS, is such a tool, that following expansion of marine species information, can be used as an *in silico* screen to assess species sensitivities to chemicals. An important future development will require the AOPs to expand their applicability domains to marine species/taxa and phyla. High level phylogenetic analysis can help identify neglected, yet important taxa, which have no or poor representation in chemical testing. Until some critical knowledge gaps are closed along with the misconceptions they caused in the past, we rely on vigilant monitoring programmes and the application of the precautionary principle in regulatory chemical management.

SOTA 30

ENVIRONMENTAL ESTROGENS INTERACT WITH THE GROWTH HORMONE-INSULIN-LIKE GROWTH FACTOR SYSTEM TO REDUCE SEAWATER ADAPTATION AND RETARD GROWTH OF RAINBOW TROUT

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Rainbow trout were used as a model to assess the effects of environmental estrogens (EE) on seawater (SW) adaption and organismal growth. Juvenile trout exposed to 17β-estradiol (E2), β-sitosterol (βS), and 4-n-nonylphenol (NP) during the course of a 48-h SW challenge displayed reduced Cl^- extrusion resulting in elevated plasma Cl^-. EE exposure also attenuated normal SW-associated increases in the hepatic mRNA expression of growth hormone receptors (GHR) and insulin-like growth factors (IGF) as well as increases in mRNA expression of IGF and IGF receptors (IGFR) in gill. Exposure to EE for 28 days resulted in growth retardation, reduced food conversion, decreased mRNA and functional expression of GHRs and IGFRs in liver, muscle, and gill, and decreased IGF mRNA expression in liver and muscle. EE also were found to have direct in vitro effects on the expression of GHRs, IGFs, and IGFRs. The effects of EE on cell signaling processes associated with the GH-IGF system also were examined. Reduced expression of components of the GH-IGF system by EE was linked to deactivation JAK, STAT, ERK, and Akt. Moreover, blockade of GH-stimulated IGF expression was accompanied by deactivation of JAK, STAT, ERK, and Akt. EEs also increased the expression of suppressor of cytokine signaling 2 (SOCS-2), a known inhibitor of JAK-2, which is mediator of GH-stimulated IGF expression. Lastly, the effects of EE on the activation state of JAK, STAT, ERK, and Akt and on increased SOCS-2 expression were estrogen receptor (ER)-dependent.

Acknowledgments: These results indicate that EEs reduce SW adaptation and retard organismal growth by disrupting growth-related cell signaling pathways that result in the repression of elements of the GH-IGF system. Supported by the US Geological Survey/North Dakota Water Commission and the National Science Foundation, USA (IOS 0920116).

REGULAR ORAL COMMUNICATIONS

O1

LOCALISATION OF RELAXIN-LIKE GONAD-STIMULATING PEPTIDE EXPRESSION IN STARFISH: ARE THE GONODUCTS THE PHYSIOLOGICAL SOURCE FOR ITS ROLE AS A REGULATOR OF SPAWNING?

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Oocyte maturation and gamete release (spawning) in starfish are triggered by relaxin-like gonad-stimulating peptide (RGP), a neuropeptide that was first isolated from the radial nerve cords of starfish. Hitherto, it has been assumed that the radial nerve cords are the source of RGP that triggers spawning physiologically. To investigate other potential sources of RGP, here we report the first comprehensive anatomical analysis of its expression, using both mRNA *in situ* hybridisation and immunohistochemistry to map RGP precursor transcripts and RGP, respectively, in the starfish *Asterias rubens*. Cells expressing RGP precursor transcripts were revealed in the ectoneural epithelium of the radial nerve cords and circumoral nerve ring, arm tips, tube feet, cardiac stomach, pyloric stomach and, notably, gonoducts. Accordingly, specific antibodies to *A. rubens* RGP revealed immunostaining in cells and/or fibres in the ectoneural region of the radial nerve cords and circumoral nerve ring, basiepithelial nerve plexus of tube feet, terminal tentacle and other arm tip-associated structures, body wall, cardiac stomach, pyloric stomach and gonoducts. Our discovery that RGP is expressed in the gonoducts of *A. rubens* proximal to its gonadotropic site of action in the gonads is important because it provides a new perspective on how RGP may act as a gonadotropin in starfish. Thus, we hypothesise that it is the release of RGP from the gonoducts that triggers gamete maturation and spawning in starfish, whilst RGP produced in other parts of the body may regulate reproduction-associated or other physiological/behavioural processes.

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O2

AN ANCIENT THYROSTIMULIN-LIKE SIGNALING SYSTEM REGULATES GROWTH IN *C. ELEGANS*

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Glycoprotein hormones are evolutionary ancient and conserved neuroendocrine factors that play an important role in the control of homeostasis. In humans, the glycoprotein hormone thyrostimulin has been most recently discovered as a potent activator of the thyroid stimulating hormone receptor (TSHR). Orthologs of thyrostimulin alpha and beta subunits (GPA2/GPB5) are highly conserved across bilaterian animals. Yet, the functions of thyrostimulin and its plausible interaction with the hypothalamus-pituitary-thyroid (HPT) axis are not well understood in any animal. To investigate this, we have characterized the function and signaling mechanisms of thyrostimulin in the nematode *Caenorhabditis elegans*. Its well-defined nervous system of only 302 neurons and extensive genetic toolkit allow dissecting the role of neuroendocrine factors at single cell resolution. The *C. elegans* genome encodes single orthologs of the two thyrostimulin subunits and its G protein-coupled receptor, called FSHR-1. We found that *C. elegans* thyrostimulin activates FSHR-1 *in vitro*, resulting in increased cAMP signaling. Like in mammals, CRISPR-mediated knockout of thyrostimulin and its receptor impairs growth in *C. elegans*. Expression analysis revealed that the thyrostimulin subunits are primarily expressed in the *C. elegans* pharyngeal and defecation motor circuit, suggesting a role in feeding and intestinal function. We discovered that thyrostimulin regulates *C. elegans* body size by activating its receptor FSHR-1 in the intestine and interacting with the thyrotropin-releasing hormone (TRH) pathway that stimulates growth. Taken together, we have identified a functional thyrostimulin-like signaling system in *C. elegans* that may ancestrally have been involved in the control of postembryonic growth. These results shed light on the function and mode of action of this conserved neuroendocrine pathway in growth regulation.

O3

KNOCKDOWN OF HALLOWEEN GENES SPOOK, SHADOW AND SHADE AFFECTS THE LENGTH/WIDTH RATIO OF OOCYTES AND EGGS IN THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

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Ecdysteroids are widely investigated for their role during the molting cascade in insects. However, they are also involved in the development of the female reproductive system. Ecdysteroids are synthesized from cholesterol, which is further converted into the main molting hormone, 20-hydoxyecdysone. This biosynthetic conversion process heavily relies on the activity of cytochrome P450 hydroxylases, which are encoded by the *Halloween* genes. Five *Halloween* genes, *spook* (*spo*), *phantom* (*phm*), *disembodied* (*dib*), *shadow* (*sad*) and *shade* (*shd*) were identified and their spatiotemporal expression profiles were characterized in the desert locust, *Schistocerca gregaria*. In addition, we investigated the possible role(s) of these genes during oocyte maturation using RNA-interference mediated knockdown experiments. Our results showed that depleting the expression of *SchgrSpo*, *SchgrSad* and *SchgrShd* had a significant impact on oocyte development, oviposition and hatching of the eggs. Moreover, the shape of the growing oocytes, as well as the deposited eggs, was very drastically altered by the experimental treatments. Consequently, it can be proposed that these three enzymes play an important role in the ovary of female locusts.

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O4

MECHANISMS OF NEUROPEPTIDERGIC REGULATION OF REPRODUCTIVE PHYSIOLOGY IN STARFISH

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Neuropeptides are intercellular signaling molecules secreted by neurons that act as neurotransmitters, modulators of synaptic transmission or hormones. One of the most important physiological processes regulated by neuropeptides is reproduction. For example, gonadotropin-releasing hormone (GnRH) regulates reproductive maturation and function in humans. Here we have performed the first comprehensive investigation of neuropeptide expression and function in the reproductive system of an echinoderm – the starfish *Asterias rubens*. This is of interest from an evolutionary perspective because of the phylogenetic position of echinoderms as non-chordate deuterostomes. The reproductive system in *A. rubens* comprises five pairs of gonads that are connected to the exterior by a gonoduct and one or more gonopores. Previous studies have revealed that a relaxin-type neuropeptide (RGP) regulates spawning in starfish. Here, immunohistochemical analysis of the *A. rubens* reproductive system using antibodies to a variety of other starfish neuropeptides revealed spatial, seasonal, and sexual differences in patterns of expression. For example, expression of a corticotropin-releasing hormone-type neuropeptide (ArCRH) was observed only in female gonads at late stages of development. Pedal peptide-type 1 (ArPPLN1b) and a calcitonin-type peptide (ArCT) are strongly expressed in the gonoduct, whereas pedal peptide-type 2 (ArPPLN2h) is strongly expressed in gonadal tissue. Investigation of neuropeptide expression in the starfish reproductive system has provided a basis for on-going pharmacological experiments, where the effects of neuropeptides on the contractile state of gonad tissue are examined *in vitro*. This has revealed that an allatostatin-C-type peptide (ArSS1) triggers gonad contraction and pedal peptide-type 2 (ArPPLN2h) triggers gonad relaxation, indicating that these peptides may be involved in regulating gonadal contractility during spawning in starfish. Discovery of the roles of different neuropeptides as regulators of reproduction in starfish is providing new insights into the evolution of neural mechanisms controlling reproductive physiology and behaviour in the animal kingdom.

O5

THE ROLE OF THE BIVALVE CALCITONIN (CALC) PEPTIDES IN SHELL BUILDING AND RECOVERY

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Calcitonin (CALC) is a single-chain peptide hormone that in humans and other vertebrates regulates plasma calcium levels and controls the function of bone forming cells. Homologues of the vertebrate CALC peptide precursor and its specific G-protein coupled receptors (GPCRs) exist in invertebrates such as Molluscs, raising questions about its potential role in the production of the calcium carbonate shell. In the marine bivalve, the Mediterranean mussel, two peptide precursors (CALCI and CALCII) that encode for two mature peptides (a and b) and six calcitonin receptors (CALCRs) exist and are expressed in the mantle, the shell building organ. The peptide CALCIIa activates CALCRIIc and increases calcium levels in mantle edge cells. We previously reported that gene number of the CALC ligand and receptor family is species-specific and in the present study we revisit the evolution of the invertebrate calcitonin system (peptide precursors and receptors) with the view of providing further evidence for its role in shell biomineralization. Damage-repair assays were established in the mussel by drilling two holes at the edge of both valves and monitoring shell repair. Total shell recovery was achieved around 20 days post damage (dpd), but repair initiated immediately and 5 dpd 64% of the drilled hole was covered by a thin layer of newly regenerated shell. Monitoring of the CALC ligand and receptor gene expression in the mantle under the damaged area revealed the CALC peptide precursors were not significantly changed 36 hours post damage (hpd). By 10 dpd the CALCII precursor was significantly up-regulated ($p < 0.05$) while CALCI remained unchanged. The results suggest that the mussel calcitonin precursors acquired different functions after they emerged from a common ancestral gene by gene duplication. We propose that CALCII is the functional homologue of the vertebrate peptide and regulates calcium transport and shell biomineralization in the mussel and most likely other bivalves.

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O6
EVOLUTIONARILY CONSERVED NEUROPEPTIDERGIC SIGNALLING SYSTEMS IN NEMATODES

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Neuropeptidergic signalling is a modulatory mechanism used by (neuro)endocrine cells and conserved across virtually all metazoans. As signalling molecules, contrarily to classical neurotransmitters, bioactive neuropeptides are not restricted to synapses and can also travel long distances across the body. They regulate a wide array of functions ranging from homeostatic adjustments to complex modelling of neuronal plasticity, and are related to several pathologies, explaining why they belong to an actively researched class of genes. For their broad number of biological functions, these active proteins and their metabotropic receptors are of high interest both in fundamental biology research, as well as appealing targets for pharmaceutical uses. Following a rising pharmaco-resistance rate, one of the novel applications is the use of neuropeptidergic pathways to develop new anthelmintics. To prioritize possible targets for this application a deeper knowledge of intra-phylum conservation of this signalling system is crucial. To date, most of the available phylogenetic comparisons only account for a limited number of species per phylum, making the selection of possible targets more difficult. Furthermore, one limitation of most of the available data is the large use of *C. elegans* as main representative for nematodes, reducing the chances of finding possible targets more specifically expressed in parasitic species. We have therefore built a pipeline to expand and automatize the pan-phylum analysis of neuropeptides-specific receptors (NP-GPCRs). The pipeline has been built to analyse all the available nematodes' proteome databases and enriched with several filters to gather sequences of high quality and with specific topological features. From the analysed datasets we have obtained an exhaustive view of the conservation of the 153 known and predicted *C. elegans* NP-GPCRs across 124 different nematodes. From this extensive analysis we are able to see specific patterns such as intra-clade conservation of sequences, receptor-specific duplication, and the nematodes' potentially fundamental receptors. In addition, by performing a clustering analysis, we have highlighted groups of neuropeptides' receptors that are not sharing orthologs with *C. elegans*, thus remarking the importance of studying other nematodes beyond the well-characterized model organism to expand our understanding of their neuropeptidergic system. This is, to our knowledge, the first automated pipeline for such application on nematodes. This practical tool will render large-scale phylogenetic comparisons and further updates with newly available datasets faster, providing a steady support for both fundamental research and pharmacological applications.

O7

MULTIFACTORIAL HYPOPHYSIOTROPIC REGULATION OF THE HPI AXIS IN ATLANTIC SALMON

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While in vitro studies have shown that multiple neuropeptides can stimulate pituitary ACTH secretion in fish, the physiological conditions under which these hypophysiotropic factors contribute to the regulation of the HPI axis remain unclear. Therefore, taking advantage of the known increase in HPI axis activity associated with smolt development in Atlantic salmon, we evaluated seasonal changes between migratory smolts and pre-migratory parr in the circulating levels of cortisol and ACTH, as well as transcript abundance of known and potential regulators of HPI axis activity in two key hypophysiotropic regions of the brain, the preoptic area (POA) and the hypothalamus (HYP). Smolts had higher plasma cortisol levels than parr throughout the spring and summer and plasma ACTH peaked in May for smolts. While transcript abundance of POA corticotropin-releasing factor b1 (*crfb1*) and arginine vasotocin (*avt*) were upregulated in smolts compared to parr throughout the spring and displayed very similar patterns of expression, the transcriptional changes in *crfa1*, *crfa2*, *crfb2*, urotensin 1a (*uts1a*), and *uts1b* were generally unrelated to, or in opposition of, changes in circulating ACTH and/or cortisol levels. In the hypothalamus, smolts have much higher *uts1a* expression compared to parr in May through July, but all other transcripts generally decreased across the spring and summer. Transcript levels of HYP *uts1a* also increased following seawater transfer in May, with immediate (24 h) and delayed (96 h) upregulation in smolts and parr, respectively. *In situ* hybridization also revealed that *uts1a* is highly abundant in specific hypophysiotropic nuclei of the hypothalamus, consistent with a role in regulating the HPI axis. Overall, our results support a multifactorial hypophysiotropic regulation of the HPI axis during seawater acclimation in Atlantic salmon and suggest functional partitioning of neuroendocrine control between CRFa, CRFb, and UTS1 paralogs in teleosts.

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O8

REGULATION OF FISH NEURAL MELANOCORTIN RECEPTORS BY MELANOCORTIN-2 RECEPTOR ACCESSORY PROTEIN 2

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Melanocortin-3 and -4 receptors (MC3R and MC4R), primarily expressed in the central nervous system, are called neural MCRs. They have essential nonredundant roles in regulation of energy homeostasis. MC3R regulates Neural MCRs interact with melanocortin-2 receptor accessory proteins (MRAPs, MRAP1 and MRAP2), modulating neural MCR expression and signaling. We have studied neural MCRs in several teleosts. Consistent with the lack of gamma-MSH (a selective agonist for MC3R) in many fishes, MC3R is also missing in some fishes, whereas MC4R is identified in al fishes studies so far. Interestingly, fish neural MCRs have very high constitutive activities, when compared with corresponding human receptors in the same experiments. We also showed that ligands developed at mammalian MCRs might have different pharmacology in fish receptors, even differing between different fishes. Some of these ligands bind orthosterically in mammalian receptors but allosterically in fish receptors. Expression of these receptors peripherally is also more wide spread in fishes than in mammals, suggesting potential wider physiological roles. For example, several studies have highlighted the role of MC4R in regulating fish reproduction. In topmouth culter, both MRAP2a and MRAP2b markedly decrease MC3R basal signaling. However, only caMRAP2a significantly decreases cell surface expression, Bmax, and Rmax of caMC3R. At the MC4R, culter MRAP2a significantly increases Bmax and decreases agonist-stimulated cAMP, while MRAP2b increases cell surface and total expression but does not affect Bmax and agonist-stimulated cAMP. These data indicated that MRAP2a and MRAP2b have different effects on expression and signaling of culter MC3R and MC4R. In Nile tilapia, MRAP2b decreases MC4R cell surface expression and constitutive activity, and enhances its sensitivity to ACTH. In orange-spotted grouper and snakehead, MRAP2 significantly decreases basal and agonist-stimulated cAMP signaling. These results suggest that MRAP2 may have different effects on neural MCR signaling. Further studies are needed to define the physiological roles of the neural MCRs in different fishes.

O9
TRENDS IN THE EVOLUTION OF THE MELANOCORTIN-2 ACCESSORY PROTEIN, MRAP1

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One of the novel features of the melanocortin-2 receptor (MC2R) is the interaction with the accessory protein MRAP1 (melanocortin-2 receptor accessory protein 1). Studies on representative neopterygian fishes and tetrapods indicate that the formation of a MC2R/MRAP1 heterodimer is required for trafficking of the receptor to the plasma membrane and activation of the receptor by the pituitary hormone, ACTH. The transmembrane domain of MRAP1 is required for the trafficking, and the four-amino acid motif δDYδ (δ = hydrophobic amino) in the N-terminal of MRAP1 is required for activation. Interestingly, an earlier study on a mammalian MRAP1 ortholog revealed that alanine substitution of the activation motif LDYI completed blocked activation of human (h) MC2R, and within this motif the Y position played a significant role in the activation of hMC2R, whereas single alanine substitution at the other positions had no negative effect on activation of hMC2R. To determine whether the Y position plays a similar role in the activation of other bony vertebrate MRAP1 orthologs, alanine substitution experiments were performed on the activation motif of avian MRAP1 ortholog (*Gallus gallus*) and neopterygian MRAP1 ortholog (*Amia calva*). These studies indicated that in non-mammalian MRAP1 orthologs both the D amino acid position and the Y amino acid position play major roles in the activation of the respective MC2R orthologs. An MRAP1 ortholog is also present in the genomes of two cartilaginous fishes, the holocephalan (elephant shark; es) and the elasmobranch (whale shark; es). Sequence alignment indicates the N-terminla domains of the cartilaginous fish MRAP1 orthologs lack the δDYδ motif, but have a E(L/Y)(D/Y)(I/V) motif. wsMC2R does not require esMRAP1 for trafficking or activation, whereas wsMC2R does require wsMRAP1 for trafficking, and the ELDI motif enhances the activation of wsMC2R. Apparently, there have been two trends in the evolution of MRAP1 which may help to explain the ligand selectivity of cartilaginous fish and bony vertebrate MC2R orthologs.

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O10

OSMOREGULATORY RESPONSE INDUCED BY HYPERSALINE CHALLENGE IS DIFFERENTIALLY MODULATED BY CORTICOSTEROIDS, CORTISOL AND DEXAMETHASONE, IN THE GILTHEAD SEABREAM (*SPARUS AURATA*)

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Hydromineral imbalance promotes the release of corticosteroids that orchestrate the expression, localization, and function of solute transporters to recover homeostasis. In teleosts, cortisol acts as both mineral- and glucocorticoid. The influence of dietary treatments for 30 days with control fish feed (CT), supplemented with cortisol (F; 400 mg/kg fish feed) or with dexamethasone (DEX; 300 mg/kg fish feed), on physiological and molecular biomarkers of the osmoregulatory system before and after hyperosmotic challenge (direct transfer from seawater -SW, 38 ppt- to high salinity water -HSW, 60 ppt- during 3 days), was evaluated. Plasma osmolality was significantly enhanced in CT and DEX groups after HSW challenge, while intestinal fluid osmolality slightly increased in DEX. Hematocrit was reduced in DEX in SW, and after HSW challenge in all groups. Muscle water content remained unaltered in SW, but it decreased significantly after HSW transfer in CT. The *ex vivo* whole intestines of treated fish showed higher water absorption rates in DEX group after HSW challenge. All branchial genes (*atpa1a*, *abcc7*, and *slc12a2*) evaluated in SW fish were not statistically affected by exogenous corticoids. HSW challenge significantly up-regulated *atpa1a* and *slc12a2* expressions in most groups. However, *slc12a2* was not significantly up-regulated in DEX, suggesting already slightly higher expression levels at SW. In anterior intestine, *abcc7* and *slc12a1* expressions were unaltered by hormonal treatment in SW. HSW transfer up-regulated *atp1a1* and *slc12a1* in all groups. Gene expression of *abcc7* and *slc12a1 was* significantly lower in F and DEX groups compared to CT. Regarding Na^+/HCO_3^- symporters, we found significantly lower *slc26a6* expression in F and DEX in SW. After HSW challenge, *slc26a3* and *slc26a6* remained significantly lower in DEX than in CT. The only Na^+/H^+ exchanger we could identify in intestine (*slc9a1*) remained statistically unaltered due to exogenous corticoids. Our results provided information on the osmoregulatory effects of exogenous corticosteroids before and after a hypersaline challenge, from the molecular mechanisms to the physiological response in the organism.

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O11
IMPACT OF OCEAN ACIDIFICATION ON THE NEUROENDOCRINE RESPONSE TO AN ACUTE STRESS IN A TELEOST FISH.

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Ocean acidification is a direct consequence of the anthropogenic climate change and the capacity of the ocean to uptake carbon from the atmosphere. This rapid changes in seawater carbonate system and pH can impact the physiology and behavior of marine organisms. Exposure to acidified water can impact the allostatic load of fish since the acclimation to suboptimal environments requires physiological adaptive responses that costs energy. As a consequence, ocean acidification may impact the stress response of fish and compromise they ability to cope to additional stress which may impact individuals' life traits and ultimately their fitness. In this context, this study aims to evaluate the physiological and behavioral response of juvenile European sea bass acclimated to 2 different pH/pCO2 conditions during recovery from netting and confinement stress. The pH/pCO2 conditions tested correspond to the current condition and the "as business as usual" scenario predicted by the IPCC for 2100 (RCP8.5).Our results show showed slower post stress return to plasma basal concentrations of cortisol and glucose the RCP8.5 scenario condition. No clear regulation in the central and interrenal tissues of the expression levels of gluco- and mineralocorticoid receptors and corticoid releasing factor was observed. The response to acidification was also associated with divergent neurotransmitters' concentrations pattern in the hypothalamus (higher serotonin levels and lower GABA and dopamine levels) and a reduction in motor activity at 120 minutes post-stress.In conclusion, experimental data indicate that ocean acidification alters the physiological response to acute stress in European sea bass via the neuroendocrine regulation of the corticotropic axis, a response associated to an alteration of the motor behavioral profile. Overall, this study suggests that behavioral and physiological adaptive response to climate changes related constraints may impact resilience of marine organisms to further stressful events.

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O12

EFFECT OF CLIMATIC AND ESTROGENIC STRESS ON THE LIFE CYCLE AND PHYSIOLOGY OF AN ESTUARINE FISH

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The climate change leads to the alteration of the physico-chemical parameters of the oceans, with consequences for the physiology and resilience ability of aquatic organisms. In addition, these organisms are also subject to a growing number of chemical contaminants, including endocrine disruptors, which are also likely to impact their fitness. In this multi-stress context, by an experimental approach, this study aims to: 1/ characterize the effects of seawater warming (+3°C) and acidification (-0.4) on the physiological functions of the marine stickleback (*Gasterosteus aculeatus*) during its entire life cycle and 2/ assess whether the exposure to a contaminant with a known estrogenic activity (ethynylestradiol (EE_2)) modulates these effects. Juvenile sticklebacks (F_0) were acclimated to 2 environmental scenarios (Current; RCP8.5 by 2100) as well as their offspring (F_1). F_1 fish were submitted, during the embryo-larval stage until metamorphosis (beginning of the juvenile stage), to an environmentally realistic chemical contamination (15ng $EE_2.L^{-1}$). Based on regular biometric measurements and quantitative and qualitative knowledge of the food, growth rates were evaluated on the two generations of sticklebacks maintained in the laboratory (F_0, F_1) and revealed a significant effect of environmental parameters. The reproductive success was also assessed on both generations through the fertilisation, hatching and survival rates of the larvae for the F_0 reproduction, and by an histological analysis of the gonads and the expression of the brain genes for F_1 generations. These measurements are currently being analysed, but the preliminary results obtained already show an effect of environmental scenarios.These initial analyses need to be continued and expanded upon in order to better understand the effects of the combination of climatic and chemical stress on stickleback physiology and, ultimately, to anticipate the impact on the population.

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O13

EGG THERMAL REGIME MODIFIES THE ENDOCRINE RESPONSE TO FOOD DEPRIVATION IN THE FOREGUT AND LIVER OF THE EUROPEAN SEABASS *(DICENTRARCHUS LABRAX)*

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Fish are ectotherms and thus are highly vulnerable to temperature fluctuations, particularly during early developmental stages, with persistent effects on fish phenotypic traits such as growth. In this study, the effect of egg incubation temperature on the metabolic and endocrine response of adult seabass (*Dicentrarchus labrax*) after food deprivation and refeeding was assessed. Seabass eggs were incubated at 11, 13.5 and 16 ºC until hatching and then reared at a common temperature until 9 months when fish were deprived of food for one week. The recovery from food deprivation was evaluated 10 hours and 2 days post-refeeding. Histology and molecular analysis revealed egg thermal imprinting modified the metabolic response to food deprivation in adult seabass. Fish from eggs incubated at the highest temperature (16 ºC) exhibited the most morphological and molecular changes in the liver and foregut to food deprivation. The liver had a significant reduction in the lipid area and an increased number of hepatocyte nuclei. Foregut atrophy was coupled with a significant up-regulation in transcripts associated with gluconeogenesis (*pck1*) and peptide absorption (*pept1*). Modified metabolism was reflected by a significant decrease in plasma lactate levels and may have come from up-regulation of deiodinase 2 (*dio2*) in the foregut. Fish incubated as eggs at lower temperatures (11 ºC) exhibited less changes following food deprivation and refeeding. Food deprivation did not significantly modify the morphology of the foregut and the liver parenchyma recovered sooner than in fish from the other thermal regimes following refeeding. In these fish *igf-1* and *igf-2* were significantly up-regulated in liver, possibly due to a transient compensatory growth response to fasting. The liver parenchyma of fish from eggs incubated at a control temperature (13.5 ºC) did not recover at the end of the experiment and the antioxidant enzyme *cat* was down-regulated compared to fish from other egg thermal regimes. Our results suggest that egg thermal imprinting modified the adult metabolic and endocrine response to food deprivation and recovery when feeding was resumed.

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O14
ADAMTS9 IS CRITICAL FOR THE DEVELOPMENT OF PRIMARY OVARIAN FOLLICLES

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Adamts9 is an evolutionarily conserved extracellular matrix metalloprotease critical for the development in vertebrates and has been linked to various human disorders including ovarian and uterine diseases. Our previous work has shown that Adamts9 is necessary for ovarian development in zebrafish. Adamts9 knockout (KO) zebrafish had heavily male-biased sex ratio as 6-month-old adults, and female Adamts9 KOs were infertile. Further, we found intersex Adamts9 KOs fish lacked clearly defined ovary or testis structure and only contained a few spermatogenic cysts on the periphery of the gonadal tissue. To further investigate the functions of Adamts9 in zebrafish ovarian development, we investigated Adamts9 expression, role in primordial germ cell (PGC) migration, gonad development, and sexual differentiation in zebrafish. We found *adamts9* was widely expressed during embryonic and larval development and is maternally deposited at the one cell stage. We found strong expression in the developing retina, that moved to the ciliary marginal zone at 72 hours post fertilization (hpf). We also found expression in somites surrounding the PGCs during migration, and in primary follicles in juvenile and adult ovaries. In contrast to invertebrate models, we only observed delay in PGC migration in Adamts9 KOs. Importantly, we saw significant slower development of juvenile gonads in Adamts9 KO, and significantly reduced size and number of primary oocytes (Stage IB) in Adamts9 KO zebrafish. Surprisingly, Adamts9 KO had no effect on primary sex determination, but in female Adamts9 KO the ovary remained dramatically underdeveloped compared to wildtype control siblings. Rescuing general growth defects by overfeeding did increase female percentage but did not rescue the abnormal ovarian phenotype. Further, follicles in the overfeeding rescued Adamts9 KO females remained at Stage IB and only a minority of follicles could continue maturation. We also found morphological evidence for sex reversal in Adamts9 KO at 90 days old, including coexistence of Stage IB oocytes and sperm in the same tissue section. As the fish aging, the male biased sex ratio continued to increase, indicating that female Adamts9 KOs are sex reversing into males. Finally, the underdeveloped ovary phenotype could be rescued with P53 knockout. Taken together, we show that Adamts9 is essential for proper ovarian development and that loss of Adamts9 leads to folliculogenesis deficiency, follicle arrest, and eventual loss of ovarian follicles.

O15

REGENERATIVE ANGIOGENESIS AND NEUROGENESIS AFTER ZEBRAFISH TELENCEPHALON INJURY: DELETERIOUS EFFECTS OF CHRONIC HYPERGLYCEMIA

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After brain injuries such as stroke or traumatic injuries, regenerative angiogenesis and neurogenesis occur almost simultaneously, suggesting intimate links between these two processes. Interestingly, diabetes is known to impair regenerative neurogenesis and to disrupt angiogenesis. However, the mechanisms by which diabetes affects these two processes are not well-understood. In this work, we aimed to determine the effect of diabetes on angiogenesis and neurogenesis after stab wound injury of the telencephalon in normo- and hyperglycemic conditions. To this end, we take benefit of zebrafish as a relevant model to study the impact of such a metabolic disease on brain repair mechanisms. First, using the transgenic *Tg(fli:GFP)* fish line in which endothelial cells express GFP, we analyzed regenerative angiogenesis from 1 to 14 days post-lesion. In parallel, we also followed neurogenesis through immunohistochemistry staining experiments. We reported that after brain damage, the expression of the *fli* transgene, the number of vessels and their width were increased as well as the gene expression of endothelial factors (i.e. *vegfaa*). In parallel, neural stem cell proliferation was also increased peaking from 3 to 5 days post injury in a way similar to angiogenesis. Then, by incubating adult zebrafish in 111 mM of glucose, we studied the effects of hyperglycemia on angiogenesis and neurogenesis. Hyperglycemic zebrafish showed a differential modulation of both processes under homeostatic and regenerative condition. Lastly, as we recently demonstrated that a medicinal plant, *Hypericum lanceolatum*, prevents impaired neurogenesis induced by chronic hyperglycemia, we aimed to investigate its effects on regenerative neurogenesis and angiogenesis. In conclusion, we showed that regenerative angiogenesis and neurogenesis appears associated during brain repair mechanisms in zebrafish and that hyperglycemia disrupts both processes. This study opens the way to use zebrafish as an interesting model to screen molecules to promote brain repair favoring angiogenesis and neurogenesis. Keywords Angiogenesis, Cerebral damage, Diabetes, medicinal plants, Neurogenesis, Stab wound, Zebrafish.

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O16

MOLECULAR MECHANISM OF SEXUAL PLASTICITY IN FISH: A VIEWPOINT FROM A NATURAL SEX CHANGING FISH

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Teleostean sex change is a dramatic example of sexual plasticity, with the phenotypic and transcriptional ontogenies of hormone driven intersex developments being quite similar among vertebrates. Hermaphroditism has been documented in about 2% of all extant fish species. The bamboo leaf wrasse, Pseudolabrus silboldi, is a diandric protogynous labrid fish. The female spawns almost every day during the 2-month spawning period, and has a diurnal rhythm in oocyte growth, maturation, ovulation, and spawning. However, during absence of male, the largest female in the group can transform into functional male. These features make this fish a good model for studying the endocrine control of sexual plasticity. On the other hand, gonadal sex change requires simultaneous changes in germ cells (e.g., sperm and their associated processes) and their surrounding somatic cells. Due to extreme histological and structural similarity between sex reversed gonochoristic fish and hermaphrodite fish, it is suggested that some common progenitor contributes to the sexual transformation. In this regard, it was recently found that, gonad houses a special population of stem cell which are increasingly considered as the precursor of germ cell in the adult gonad. So, in the present study, we investigated the potential of gonadal stem cells in sexual plasticity maintenance in fish using the bamboo leaf wrasse. For the first time, we have found two different types of stem cells, hereafter named as germline stem cells (GSCs) and undifferentiated somatic cells (SSCs) from ovary, testis and intersex fish. Notably, we have found similar cell population of GSCs in medaka (gonochoristic model species), and upon transplantation, these GSCs differentiated into both testicular or ovarian germ cell depending on the surrogate host. Interestingly, our experimental wrasse histology, cell sorting, and -omics analysis suggests that the cellular and molecular characteristics of both cell types are different from differentiated germ and somatic cells, which highlights that, both these cell populations are the starting point and prime candidate for sexual plasticity. Owing to the similarity among vertebrate sexual development, in-depth analysis of sexual re-differentiation associated molecular cues and dynamics of these cells are pertinent to unravel the sexual and plasticity process, thereby ensuring higher reproductive security. Further, we are also examining the molecular connections between pituitary and gonad during sex change.

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O17

CHARACTERIZATION OF EARLY EVENTS OF PUBERTY IN FEMALE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*) AND THE ROLE OF THE TRANSCRIPTIONAL COACTIVATOR NCOA7

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Puberty comprises the developmental period that leads to the first successful reproduction. In female European sea bass *(Dicentrarchus labrax*) puberty occurs at the end of the third year of life, although intensive rearing leads to the appearance of precocious maturation one year earlier. This study is aimed at the characterization of the onset of precocious puberty at the gonad level in sea bass females through a hemigonadectomy approach. Hemigonadectomy allows the study of early events occurring in the ovaries prior to the appearance of any histological changes induced by the onset of puberty. With this purpose, by the time puberty was about to start the left ovary of a group of fish was removed by precise surgery. These ovaries were frozen and kept for further gene expression and ovarian steroid analysis. After the established gonadal maturation period of sea bass, histological observation of the developmental stage achieved by the remaining ovary was used to classify the fish into precocious or non-precocious. Blood samples were taken monthly to analyze plasma levels of sex steroids, follicle-stimulating hormone, luteinizing hormone and vitellogenin using specific ELISAs. Gene expression was analyzed by RT-qPCR.In February, the ovaries of non-precocious fish remained in previtellogenesis, while those of precocious animals attained different stages of secondary growth or oocyte maturation. Plasma hormonal profiles during the experiment showed differences between the two groups, and vitellogenin showed the most divergent pattern. Ovarian steroids and gene expression analyses revealed also differences between non-precocious and precocious fish. RNA samples were subsequently hybridized on a sea bass specific microarray for the identification of differentially expressed genes involved in early events of precocious female puberty. One of these genes is *ncoa7* that encodes a nuclear receptor co-activator that, in mammals, increases the transcriptional activity of the estrogen receptor. We cloned the complete cDNA of sea bass *ncoa7* and studied its tissue expression and its annual expression pattern in gonads of both sexes. Functional analyses of Ncoa7 in HEK293 cells cotransfected with estrogen, androgen or progesterone receptors, and exposed to 17b-estradiol, testosterone, 11-ketotestosterone or 17,20β-P, showed that Ncoa7 is able to increase the transcriptional activity of all the different receptors. Moreover, immunoprecipitation and targeted mutation analysis showed which Ncoa7 domains are involved in direct binding to the nuclear receptors. All together our data indicate that in sea bass ovaries entering secondary growth for the first time the progesterone receptor is the most probable partner of Ncoa7, and therefore progestogens may have a decisive role in gonadal precocious puberty in females sea bass.

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O18

THYROID HORMONE (T_3) IS INVOLVED IN INTESTINAL DEVELOPMENT DURING SW ACCLIMATION OF ATLANTIC SALMON (*SALMO SALAR*)

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Atlantic salmon (*Salmo salar L.*) starts its lifecycle in freshwater (FW) and as juveniles undergo smoltification, a process under endocrine control driving morphological and physiological changes that enable salmon to adapt to seawater (SW). During the transition from FW to SW, the intestine plays a pivotal role in osmoregulation. The major thyroid hormone, T3, has a crucial role in mammalian and anuran post-natal intestinal development. T3 is known to play a role in salmon smoltification but our understanding of the role of T3 in intestinal development during smelting is limited. To test whether T3 is involved in Atlantic salmon intestinal development and SW acclimation, we established a four-week experiment with FW-adapted salmon juveniles (parr) divided into four groups fed with commercial feed supplemented with 1) T3 (20 mg/kg feed), 2) the goitrogen methimazole (MMI; 5000 mg/kg feed), 3) T3+MMI (20 mg/kg feed + 5000 mg/kg feed), and 4) control non-treated (feed supplemented with vehicle 70%EtOH). Fish were sampled at the end of the FW period and transferred to 25 ppt SW for two days. In both time points, the anterior and posterior intestines were sampled. Experimental conditions modulate the transcription of intestinal thyroid hormone transporters, deiodinases, and receptors. Likewise, the Na+/K+ -ATPase (NKA) and the Na+/K+/2Cl- cotransporter (NKCC) transcription and subcellular distribution are also modulated by treatments. Our data indicate that T3 is a key factor in intestinal development and SW acclimation of Atlantic salmon.

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O19

THE THYROID AXIS PARTICIPATES IN TEMPERATURE-INDUCED SEX REVERSAL THROUGH ITS ACTIVATION BY THE STRESS AXIS IN THE MEDAKA

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Biological sex fate in fish may be driven by an enormous number of sex-determining mechanisms. Changes in the environment, such as increased temperature, are sensed by the stress axis, initially in the brain, to increase cortisol synthesis, ultimately promoting androgen synthesis with concomitant development of a testis. The involvement of thyroid hormones (THs) in the response to various types of stress has been reported; however, the role of THs in stress-induced sex reversal remains unexplored. In this study, using medaka fish, we first analyzed the role of THs in testicular development by quantifying thyroid-related genes during ontogeny and a T3 exposure experiment. Both dio1 and dio2, involved in the T3 activation, and TH receptors were up-regulated in XY males at st. 39 and in XX females in T3 treatment. Next, we analyzed the transcript abundance of thyroid axis-related genes during the gonadal development in embryos reared at normal (NT) and heat-stress temperature (HT). An up-regulation of tshba, dio2 and thra was observed at HT, while dio3 was negatively regulated. Furthermore, when total T3 was quantified, a higher amount of this TH was observed in both XX and XY reared at HT. To evaluate the interaction between THs and HT-induced stress axes, we analyzed tshba expression in a crhrs (crhr1 and crhr2) double mutant strain, observing a lack of tshba up-regulation responses in HT and when the number of thyroid follicles was analyzed, a decrease in number was observed. Finally, our last experiment consisted of analyzing the additive effect of cortisol and THs and their blockade during heat stress masculinization. We observed that both hormone treatments did not show an additive effect. Furthermore, when we incubated embryos at HT with methimazole, an inhibitor of thyroid hormones, RU-486, Gr antagonist, and both, we observed that both treatments reduced female-male reversal and the combination completely blocked it. Summarizing, we provide clear evidence for the involvement of the thyroid axis in environmentally induced masculinization through an interaction with the stress axis in the medaka

O20
MATERNAL THYROID HORMONES AFFECT ZEBRAFISH EMBRYO DEVELOPMENT

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In vertebrates, thyroid hormones (THs; thyroxine (T4) and triiodothyronine (T3)) play regulatory roles in different biological processes, such as embryo and larva development. The initial stages of embryo and larva development are dependent of maternal supply of THs. In fish, maternal THs are transferred to eggs and embryos and play regulatory roles in development which are poorly investigated. The aim of this study was to investigate the effects of maternal TH in zebrafish embryo and larva development focusing on the somatotropic axis. For this purpose, zebrafish females were fed with commercial food containing low or high concentration of T4 (12,5 or 25 µg T4) (g body mass) for 5 days. One group was fed with commercial food containing the vehicle used to dissolve the hormone (100% ethanol). On day 6, females were transferred to breeding tank with untreated males. Then, embryos/larvae (F1) from each group were evaluated from 0 h post-fertilization (hpf) to 144 hpf according to following parameters: survival rate, rate of heartbeat and presence of morphological abnormalities. Furthermore, embryos/larvae were collected from 0 h to 72 hpf for gene expression. The relative mRNA levels of following genes were evaluated: crf, tsh, thrα, thrβ, dio2, dio3, mct8, gh, igf1, mtor, foxO3a, myoG and myf5. Our results demonstrated that the progeny obtained from females fed with low concentration of T4 exhibited accelerated growth, but normal parameters as compared to control group. However, the progeny obtained from females fed with high concentration of T4 showed reduced growth, morphological and physiological alterations such as yolk sac and pericardial edema, uninflated swin bladder, spine deformation, increasing heartbeat hate, and increase mortality. Gene expression analysis showed alterations in the transcripts related to thyroid hormones axis in the progeny obtained from females fed with high concentration of T4. In addition, expression of genes related to growth and development were responsive to changes in maternal TH concentrations. In the high concentration of T4, the mRNA levels of gh and igf1 tend to decrease. In the same group, mtor levels down- and up-regulated in the progeny with 48 and 72 hpf, respectively. However, these levels tend to increase in the progeny obtained from females fed with low concentration of T4. This study shows that maternal THs are crucial to coordinate embryo and larva developmental programmes related to somatotropic axis, and alterations in maternal THs can affect severely the development and the offspring phenotype.

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O21

IDENTIFICATION OF BIOMARKERS OF THYROID DISRUPTION IN *XENOPUS LAEVIS* BY TRANSCRIPTOMIC ANALYSIS

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Pollutants are a major concern in households and ecosystems, many of which interfere with the homeostatic balance of living systems. Endocrine disruptors affect many biological pathways including reproduction and thyroid axis. To this regard, and because of their high sensitivity to thyroid signaling and environmental changes, *Xenopus laevis*, the African clawed frog, is a well-established in vivo model to evaluate the impact of thyroid axis perturbations. XETA (for Xenopus Eleutheroembryonic Thyroid Assay) is an official OECD guideline test designed to estimate the thyroid disruption potential of chemicals. Experimentally, this is carried out by measuring expression changes of a thyroid hormone (TH) responsive GFP reporter transgenic tadpole. Our objective is to complement this test and provide novel molecular endpoints by using a combination of functional genomics and system biology. We thus addressed the impact of several compounds acting at different levels of thyroid signaling in xenopus tadpoles: sodium perchlorate (PCL) and propylthiouracil (PTU) prevent iodine uptake in the gland and incorporation, respectively. Iopanoic acid (IOP) inactivating deiodinases and tetrabromobisphenol A (TBBPA) binds to the thyroid hormone receptor. Tadpoles were exposed to compounds in the lower range of concentrations detected by the XETA test. Although thyroid signaling disruptors act at different level of the axis, we found very similar transcriptional response profiles, where we could discriminate between nonspecific responses versus thyroid disruption activity. There are only little degrees of thyroid disruption activity and the vast majority of TH responsive genes is unaffected by treatments: PCL and PTU only affect ~6 % of TH responsive genes whereas TBBPA en IOP display a much stronger effect (between 16 % and 25 %). Given that the PCL concentrations used delays metamorphosis significantly, this cannot be disregarded as a small effect, and it is clear that altering the expression of only a few percent of TH responsive genes is enough to initiate strong physiological and phenotypic outcomes.

O22

T2 EFFECTS UPON GILL REMODELING DURING AXOLOTL METAMORPHOSIS. WHAT DO TRANSCRIPTOMIC ANALYSIS REVEAL?

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Vertebrate metamorphosis is a fascinating phenomenon in which a post-embryonic and dramatic shift from larva to juvenile occurs; this life transition involves a change in physiology, morphology, ecology, and behavior as beautifully exemplified in amphibians. Interestingly, some salamanders like the axolotl (Ambystoma mexicanum) have lost the natural capacity to initiate metamorphosis. The axolotl forgoes metamorphosis and retains an aquatic lifestyle accompanied by external gills and a high tail fin. Thyroid hormones (THs) trigger and regulate vertebrate metamorphosis; these hormones act through their binding to specific nuclear receptors (TR) that function as transcription factors, regulating a cascade of target genes. 3,5-diiodo-L-thyronine (T2) is an endogenous alternative TR ligand with demonstrated biological actions in mammals and fish, mimicking the actions of the most bioactive TH, (3,5,3'-triiodo-L-thyronine or T3). With the aim to analyze if T2 could induced metamorphosis, we designed an experimental paradigm in which we treated juvenile axolotls with T2 or T3. While T3 treatment (500 nM) induced a full metamorphosis, only the length of the secondary gills decreased (~50%) after 6 days of equimolar concentrations of T2 treatment. Interestingly, after T2 withdrawal, the length of secondary gills were restored to their original length. Notably, a higher concentration of T2 (2 μM) was able to induce full metamorphosis. With the aim of analyzing the molecular mechanisms involved in differential gill gene regulation elicited by T2 or T3, we performed a high-throughput transcriptome sequencing with a differential analysis. We found a total of 1075 and 1241 genes regulated by T2 and T3, respectively out of which 222 were T2- and 388 T3-exclusively regulated, and sharing the regulation of 851 genes. The gene ontology analysis revealed that T2 and T3 are regulating specific biological pathways that could explain the differences observed in the gill of metamorphic axolotls. These analysis shed light in to the thyroid hormone gene regulation and its pleiotropic system; and that T2 was capable of induce the tissue remodeling of the gill, and metamorphosis with a higher dose supports the notion of its bioactivity in another class of vertebrate.

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O23

DECIPHERING THE DIALOG BETWEEN THE ENVIRONMENT AND THE BRAIN DURING THE METAMORPHOSIS OF THE CLOWNFISH *AMPHIPRION OCELLARIS*

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The clownfish *Amphiprion ocellaris* exhibits a life cycle divided in two distinct phases: a dispersal larval phase in the ocean, followed by a reef sedentary phase during which juveniles permanently settle within a giant sea anemone. During this transition from the ocean to the reef, called recruitment, larvae undergo drastic behavioural, ecological, morphological and physiological changes. This transformation corresponds to a thyroid-hormone (TH)-controlled metamorphosis, triggered by the activation of the HPT (Hypothalamus Pituitary Thyroid) axis. Additionally, it has also been demonstrated that larvae are attracted to the chemical signature of sea anemones and/or that of conspecifics during their recruitment. Given the importance of chemical cues during metamorphosis, we questioned if chemical signals could impact the metamorphosis and how these environmental signals are translated into neuroendocrine signals. For this purpose, we exposed clownfish larvae (aged 2, 5, 8 days post hatching) to seawaters containing either the chemical signature of clownfish conspecifics or of their natural host anemone *Heteractis magnifica*. Our results revealed that the expression of several genes belonging to the HPT axis is stimulated in larvae exposed to each chemical signature tested (congeners and anemone) compared to control larvae. Moreover, larvae exposed to these waters undergo metamorphosis more rapidly than control individuals. Together, these results suggest that chemical signals contained in bathing waters from clownfish congeners or from the host anemone can stimulate metamorphosis in clownfish larvae.

O24
ITCH AND ITS MOLECULAR/FUNCTIONAL EVOLUTION

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"Itch" is stress and the essential warning signals. Gastrin-releasing peptide (GRP) system was discovered as an itch-specific mediator in the rodent's spinal somatosensory system, and we showed these neural circuits in the trigeminal and spinal somatosensory systems are conserved in mammals. Here, we analyzed the effects of chronic stress on the itch sensitivity using rat model of chronic corticosterone administration, because chronic psychological stress is known to exacerbate chronic itch diseases such as atopic dermatitis. The stress decreased the body weight and markedly increased itch-specific scratching behavior without changes in the tactile/pain sensitivity. Stress group showed an increase in Grp expression in the sensory system. Intrathecal administration of GRP receptor (GRPR) blocker attenuated the increase in itch behavior. Taken together, these results suggested that the chronic stress via HPA axis increased the itch sensitivity through sensory GRP system. We further extended our research on the sensory GRP system to *Xenopus* and zebrafish to analyzed the molecular/functional evolution of itch. GRP/GRPR distributions in the sensory systems were similar. Then, we tried behavioral pharmacology analyses to determine whether fish also feel pain and itch. We found that zebrafish treated with a human itch substance rubbed their skin against the bottom of the experimental tank, but did not with a human pain substance, suggesting that fish distinguish itch and pain stimuli. Then, we produce GRPR KO zebrafish to understand the involvement of GRPR in the fish sensory system. Swimming speed decreased with pain substance but not change with itch substance in KO fish. These results indicate that the GRP system may be involved in pain transmission in fish, whereas the GRP system is involved in itch transmission in mammals. Currently, we performed Ca^{2+} imaging by using G-CaMP in zebrafish larvae to analyze whether sensory neurons distinguish between the itch and pain stimuli at the neural circuit level. In summary, our study revealed a mechanism by which stress increases the itch sensitivity through sensory GRP system in mammals and GRP system may have different sensory functions in mammals and fish.

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O25

CRHR1 INTEGRATES THE TEMPERATURE-INDUCED HORMONAL AND METABOLIC RESPONSE IN ZEBRAFISH

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Corticotropin-releasing hormone (CRH) is the primary step in the activation of the hypothalamus-pituitary-adrenal (interrenal tissue in teleosts) (HPA/HPI) axis in response to stress in vertebrates. CRH activation of the CRH receptor 1 (CRHR1) stimulates the release of adrenocorticotropic hormone (ACTH), leading to the secretion of cortisol from the interrenal tissue in teleosts. We recently showed that CRH-CRHR1 signalling mediates acute stress-related hyperactivity in zebrafish, while the longer-term maintenance of this stress-related behaviour also requires the contribution of cortisol. Here we tested the hypothesis that CRHR1 is a key integrator of the stress-related metabolic response in zebrafish. This was tested using a thermal stress paradigm, where zebrafish were acutely subjected to a +5°C temperature (33°C) increase above the ambient water temperature (28°C). The direct role of CRHR1 in mediating the behavioural and metabolic stress response was assessed by utilizing a ubiquitous CRHR1 knockout (CRHR1KO) zebrafish. Larval wildtype zebrafish subjected to the temperature stressor showed increased activity and this response was completely abolished in the CRHR1KO larvae. This lack of temperature-mediated hyperactivity in the CRHR1KO fish was also evident in the adult zebrafish. Furthermore, the temperature-induced increase in metabolic rate seen in the adult wildtype was also absent in the CRHR1KO zebrafish. As the CRHRIKO showed a reduced cortisol response to temperature, we tested whether the lower cortisol levels may play a role in the lower metabolic rate. To this end, wildtype fish were treated with metyrapone to reduce cortisol levels, but this did not affect the temperature-induced elevation in metabolic rate, suggesting that CRHR1 action may be independent of HPI axis activation. To confirm if the mutants showed a conserved heat shock protein response, we measured the transcript abundance of heat shock protein genes in the brain. Both genotypes responded to the temperature stress by upregulating a set of heat shock protein genes. Taken together, our results suggest that CRHR1 may be a key integrator of the temperature-related acute metabolic activity, but not the heat shock protein response in zebrafish.

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O26

STRESS RESPONSE IN THE SILVER CATFISH RHAMDIA QUELEN TO THE INTERACTION STOCKING DENSITY – FEEDING REGIMEN

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The aim of this study was to verify the effect of both stocking density and food deprivation on the gene expression of several hormones and enzymes related to the stress axis, as well as on metabolic parameters in *Rhamdia quelen* under different feeding and stocking conditions: 1) fed at low stocking density (LSD-F); 2) fed at high stocking density (HSD-F); 3) food-deprived at LSD (LSD-FD); and 4) food-deprived at HSD (HSD-FD). Fish from LSD-F and HSD-F groups were fed once a day with commercial dry pellets, whereas animals from food-deprived groups (LSD-FD and HSD-FD) were not fed during the 14 days of the experimental time. Plasma metabolic parameters (glucose, lactate, triglycerides, and proteins) and hepatosomatic index were evaluated. In addition, expression of genes related to stress axis (crh, pomca, pomcb, nr3c2, star, hsd11b2 and hsd20b), heat shock protein family (hsp90 and hspa12a), neurotransmitter transporter noradrenalin (slc6a2) and growth axis (gh and igf1) were assessed. Specific growth rate and HSI were lower in food deprived fish regardless of stocking density. The HSD-FD group showed lower weight compared to the other groups. Plasma glucose and triglycerides were lower in food-deprived groups, while lactate and protein levels did not change. The expression of key players of the stress response (crh, pomca, pomcb, hsd11b2, nr3c2, hsp90b) and growth (gh and igf1) pathways were differently regulated depending on the experimental condition, whereas no statistical differences between treatments were found for hsd20b, scl6a2, hsp12a, and star gene expression. This study suggested that LSD acted as a stressor affecting negatively physiological status of feed-fish, as demonstrated by lower growth rates, altered metabolic organization, and higher crh mRNA expression. In addition, food deprivation also showed higher expression of other assessed genes (nr3c2, hsp90b, pomca and pomcb) in fish from HSD groups, indicating higher responsiveness to stress in this stocking density when combined with food deprivation.

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O27

SEROTONIN PLAYS A KEY ROLE IN THE ACTIVATION OF THE HYPOTHALAMIC PITUITARY INTERRENAL AXIS DURING HIGH ENVIRONMENTAL AMMONIA EXPOSURE IN THE TELEOST MODEL RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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In fish, the endocrine stress response is primarily mediated by the release of cortisol from interrenal cells into the bloodstream, stimulating several mechanisms to cope with the stressor. The release of this glucocorticoid is centrally controlled by neurons located mainly in the preoptic area (POA) that act as upstream elements of the hypothalamic-pituitary-interrenal (HPI) axis. Corticotropin-releasing factor (CRF), located in the POA, has been postulated as a central player in the activation of the HPI axis, integrating the activity of a complex neural network that encodes the modality, severity, and duration of the stressor. Several studies postulate that serotonin may play a role in stressor integration and response initiation, gradually activating the HPI axis in a stressor-dependent manner. However, the conditions under which serotonin regulates the HPI axis and the serotonin receptors involved are poorly understood. With this in mind, we conducted a set of experiments to evaluate the impact of a common aquaculture stressor, high environmental ammonia, on the serotonergic system and the HPI axis. In particular, we evaluated the role of serotonin 5HT2C receptors in the activation of the HPI axis. Our results show that the serotonergic system and the HPI axis are activated following ammonia exposure. Furthermore, our data indicate that serotonin 5HT2C receptors mediate the activation of the HPI axis, suggesting an important role of this indolamine in the central intregation of information related to stress.

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O28

THE ROLE OF CORTISOL IN THE INTESTINAL BICARBONATE SECRETION OF ATLANTIC SALMON

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Intestinal bicarbonate secretion (BCS) is a central process in the hypo-osmoregulatory mechanisms of seawater fish. The study of this process is important in the Atlantic salmon (*Salmo salar*) since they undergo preparatory changes for seawater entry during smoltification. However, much remains unknown about BCS and its hormonal control in this species. This study aimed to investigate the regulatory role of cortisol in intestinal BCS and other osmoregulatory responses in Atlantic salmon. Atlantic salmon parr and post-smolts in freshwater (FW) were given intraperitoneal implants with 0 μg g^{-1} or 40 μg g^{-1} cortisol (control and cortisol group, respectively). Twelve days after injection, half of the fish were exposed to 25 ppt (parr) or 30 ppt (post-smolt) seawater (SW) for 48 h. We analyzed plasma and intestinal ion content and characterized the anterior and posterior intestines at the molecular level by qPCR. During SW exposure, mortality was observed only for the parr control group (31.5%). Cortisol treatment resulted in significantly lower plasma osmolality, and chloride and magnesium concentrations in parr after 48 h in SW compared to controls. In post-smolt, only plasma magnesium levels decreased following cortisol treatment. Cortisol treated fish also had a significantly lower mass of intestinal carbonate aggregates in SW fish. However, the mRNA levels of genes involved in HCO_3^- transport across the enterocytes, i.e., *Slc26a6*, *Slc4a4*, and *Atp6v1a*, were upregulated by cortisol. Our preliminary results confirm that cortisol improves salinity tolerance in parr and post-smolt and suggest that cortisol affects BCS, possibly acting on the regulation of carbonate and bicarbonate turnover in the intestine of Atlantic salmon.

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O29

CART AND CRH MEDIATED RESPONSES IN THE BRAIN OF *EUPHLYCTIS CYANOPHLYCTIS* TADPOLES SUBJECTED TO NATURAL POND DRYING

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Cocaine and amphetamine regulated transcript peptide (CARTp) is extensively distributed in the brain of vertebrates except reptiles. Its role along with corticotropin releasing factor (CRF) in antagonizing the physiological consequences of stress in mammals is well established, however in anurans, the neuroendocrine mechanisms and roles of these peptides are less understood. In the mammalian hypothalamus, CRF is known to mediate CART in the paraventricular nucleus (PVN) thereby initiating a stress response, whereas in anurans the mediator of CART is still unknown. Anuran tadpoles during the post monsoon season are exposed to wide range of environmental factors such as increased temperature, hypoxia and salinity as a result of intermittent dry spells that cause pond drying thereby disrupting homeostasis. Therefore, the present study was an attempt to check whether CRF mediates CART in maintaining the stress physiology of anuran larvae to adverse environmental factors? In this study, tadpoles of *Euphlyctis cyanophlyctis* were collected from a drying puddle and the expression of CARTp and CRF was examined in the tadpole brain using immunohistochemistry. Tadpoles of overcrowding group showed higher immunoreactivity in various neuroanatomical regions that had previously been linked to stress responses in anurans. Cells with strong CART-immunoreactivity were observed in the preoptic area (POA) and posterior entopeduncular nucleus (PEN) of the diencephalon of tadpoles exposed to chronic overcrowding as compared to normal. In the suprachiasmatic nucleus, the dorsal (dHy) and ventral hypothalamus (vHy), moderate CART-ir cells were observed in the control groups while number of cells and intensity of immunoreactivity was increased in tadpoles of the overcrowding group. The strongest immunoreactivity was observed in the Edinger Westphal nucleus (EW) of the overcrowding group as compared to the normal group. Additionally, increased levels of CRF were noted in the median eminence and EW nucleus. These regions constitute the stress and anxiety regulating centres of the vertebrate brain. The upregulation of CART along with CRF in the POA, PEN, SCN, EW and the hypothalamic areas suggests its possible role to reinstate homeostasis in the adverse environmental conditions of pond drying. Also, the varying levels of CRF in the POA which is a homologous region of PVN along with the CART suggest that it could be a mediator of CART release in the brain as reported in the mammalian system. The study underscores the possibility that endogenous CART system might play a major role in reinstate homeostasis in the adverse environmental conditions during anuran development.

O30

GDNF ACTS AS A GERM CELL GROWTH FACTOR AND REGULATES ZEBRAFISH GERM STEM CELL NICHE IN AUTOCRINE AND PARACRINE-DEPENDENT MANNERS

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Glial cell line-derived neurotrophic factor (GDNF) and its receptor GFRα1 (GDNF Family Receptor α1) are well known to mediate spermatogonial stem cell (SSC) proliferation and survival in mammalian testes. In nonmammalian species, Gdnf and Gfrα1 orthologs have been found but their functions in the testis remain poorly investigated. Considering this background, this study aimed to understand the roles of the Gdnf-Gfrα1 signaling pathway in zebrafish testes by combining *in vivo*, *in silico* and *ex vivo* approaches. Our analysis showed that zebrafish have two paralogs for Gndf (*gdnfa* and *gdnfb*) and its receptor, Gfrα1 (*gfrα1a* and *gfrα1b*), in accordance with a teleost-specific third round of whole genome duplication. Expression analysis further revealed that both ligands and receptors were expressed in zebrafish adult testes. Subsequently, we demonstrated that *gdnfa* is expressed in germ cells, while Gfrα1a/Gfrα1b was detected in most undifferentiated spermatogonia and Sertoli cells. Functional *ex vivo* analysis showed that Gdnf increased spermatogonial niches by stimulating the proliferation of both type A undifferentiated spermatogonia and their surrounding Sertoli cells but without changing *pou5f3* mRNA levels. Strikingly, Gdnf also inhibited late spermatogonial differentiation, as shown by the decrease in proportion area occupied by type B spermatogonia and down-regulation of *dazl* in a co-treatment with Fsh. Altogether, our data revealed that Gdnf acts as a germ cell-derived factor involved in maintaining germ cell stemness through creation of new available niches, supporting the development of spermatogonial cysts and inhibiting late spermatogonial differentiation in autocrine- and paracrine-dependent manners.

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O31

STEROIDOGENIC ACTIVITY OF ANTI-MÜLLERIAN HORMONE IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*) MALES

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The Anti-Müllerian hormone (AMH) belongs to the TGFβ superfamily, promotes the regression of Müllerian ducts in tetrapods, and signals through a heterodimeric receptor complex, consisting of type 1 and type 2 receptors. Despite the absence of Müllerian ducts in teleosts, their genomes contain amh orthologs, and the encoded hormone plays a role in sex and gonad differentiation in males. However, in adult teleost gonads, the exact mechanism of Amh signaling and its implication in gametogenesis are poorly investigated. As tool for studying Amh actions in European seabass, we previously produced recombinant sea bass Amh using Pichia pastoris and Chinese hamster ovary (CHO) expression systems. These Amhs were used in Amh type 2 receptor (Amhr2) transactivation experiments in COS-7 cells and in vitro gonadal tissue cultures, in order to study the Amh mechanism of action and the intracellular signaling pathways of its receptor. Human AMH and AMHR2 were also tested for comparative purposes in combination with the sea bass hormone or receptor. Recombinant sea bass Amh proteins acting through sea bass Amhr2 were able to activate the consensus Smad intracellular pathway, in the same way that human AMH/AMHR2 did. Interspecific activation was obtained when sea bass and human Amh were used to stimulate the human and sea bass receptors respectively. Surprisingly sea bass Amh/Amhr2 pair could also signal through the cAMP pathway, while hAMH/hAMHR2 had no direct effects on this pathway, neither when hAMH was interacting with sbAmhr2. The BMP-signaling inhibitor LDN-193189 and the cAMP antagonist Rp-cAMPS were used to investigate intracellular cross-talk between these pathways. In pre-meiotic testis explants from adult sea bass, addition of Amh increased steroidogenic enzyme gene expression levels and steroid production, which was blocked by addition of the Rp-cAMPS inhibitor. In the same vein, in vivo intramuscular injection of an amh expression plasmid to pre-meiotic male seabass resulted in an increase of circulating 11-KT. In previous studies, Amh was immunodetected in Sertoli cells surrounding early germ-cell generations. Now, we have detected the Amhr2 in spermatogonia of pre-meiotic testis, suggesting a role in the early stage of spermatogenesis. It could be finally concluded that, contrary to what is shown by other teleost species, in sea bass Amh is able to promote steroid production in immature testis and it is clearly involved in the gametogenic process.

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O32
MOLECULAR CHARACTERIZATION OF SIBERIAN STURGEON OVARIAN SEX DIFFERENTIATION

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Factors controlling sex differentiation in sturgeons are not yet well understood. The recent possibility to sex the fish at genomic DNA level allows to study the molecular basis of the ovarian program during the molecular sex differentiation period (3,5 months, n=18; 5 months, n=19; 6 months, n=13). Sixteen candidate genes were selected from the literature for qPCR studies: 1.- oestrogen-related genes (star, cyp11a, hsd3b1, cyp17a1, hsd17b3, cyp19a1, hsd17b1); 2.- transcription factor genes activated in gonads of future females (foxl2, foxl3, irx3, irx5, lhx2); 3.- the female gene fst; 4.- genes involved in the canonical pathway (rspo1-wnt4-bcat). The in vivo effect of oestradiol (5 µg/kg body weight) was studied after 12 h of treatment on the genes up-regulated in future females. Among all the genes studied only those related to regulation of oestrogen and its production resulted sex dimorphic with significant major levels in future female gonads of fish at 3,5 months of age (cyp19a1, $p<0.038$; foxl2 $p<0.003$, hsd17b1 $p<0.002$). At 5 and 6 months of age cyp19a1 loses its sex dimorphic expression, while foxl2 and hsd17b1 sustained their higher levels in future female gonads (foxl2, 5 months $p<0.02$; 6 months $p<0.01$; hsd17b1, 5 months $p<0.0059$; 6 months $p<0.01$). All the other genes studied were not sex dimorphic except irx3 that showed higher expression levels in future ovaries at 5 months ($p<0.003$). Interestingly, as qPCR values shows, the hsd17b1 (a gene coding for the enzyme that converts estrone in estradiol-17b) is not expressed or it is expressed at very low levels in gonads of future males at 3,5 to 6 months. This suggests a key role of this gene in the regulation of estradiol-17beta levels at very early stages of gonad differentiation of future females. Treatments with estradiol-17beta were studied on cyp19a1, foxl2, hsd17b1 and irx3, but only foxl2 was significantly activated ($p<0.05$) after the in vivo treatment. In conclusion, basal fish as sturgeons share with teleost fish only three genes involved in ovarian sex differentiation, and hsd17b1 appears to be a key gene controlling the oestrogen production levels at early stages of gonad development as it was proposed for Seriola genus.

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O33

TWO GENES CODING FOR GONADAL SOMA-DERIVED FACTORS ACT IN EARLY GAMETOGENESIS IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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Puberty is the developmental period during which an individual becomes sexually mature for the first time and its regulation is not completely known in teleosts. The gonadal soma-derived factor (Gsdf) gene was found to be downregulated in precocious testis of male European sea bass and proposed as an early gonadal marker of puberty. Gsdf is a member of the TGFbeta superfamily, it is found in tetrapods, excluding mammals, and it is apparently involved in the proliferation of type A spermatogonia. The genome of sea bass contains two gsdf duplicates, gsdf1 and gsdf2, whose encoded proteins share 87% identity and have gonad-specific expression. In the present study we aimed at characterizing the Gsdf genes of European sea bass. First, we have confirmed that expression of gsdf1 and gsdf2 decreases in testis of sea bass males that enter precocious puberty compared with their siblings that remain immature. To achieve this, prepuberal one-year-old male European sea bass of two different sizes (Small or Large) were sampled during the months corresponding to onset of gametogenesis in adults (August to November). The males from the Small group did not arrive to full spermiation and showed higher gsdf1 expression than the ones of the Large group during all the experiment, with significant differences in August and October. Most of the animals of the Large group spermiated the next winter. In adult males, gsdf1 showed maximum expression levels in premeiotic (immature) testis, that decreased as spermatogenesis progressed. The expression of gsdf2 followed the same trend but was 5-fold lower. This expression profile matched with the presence of Gsdf protein in testis extracts, and with its location in Sertoli cells surrounding type A spermatogonia, as revealed by IHC with a species-specific antibody. In adult female ovaries, mRNAs from gsdf1 and gsdf2 are present to a much lower level than in testis, and the highest levels correspond to gsdf2 in post-ovulatory ovaries and also in isolated follicular cells. In fact, Gsdf1/2 was located in follicular cells surrounding previtellogenic oocytes. The sea bass gsdf1 and gsdf2 genes are located in the same chromosome, and their coding sequences are placed in different strands and transcription directions. The functional data obtained so far point to common regulatory elements for both genes, but also to a sex-specific regulation for gsdf1 and gsdf2 connected to males and females respectively.

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O34

TRANSCRIPTOME ANALYSIS OF A PROTANDRIC HERMAPHRODITE, THE COMMON SNOOK (*CENTROPOMUS UNDECIMALIS),* DURING GONADAL DIFFERENTIATION UNDER SEX STEROID TREATMENTS.

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The common snook (*C. undecimalis*) is a protandric hermaphrodite fish which has high commercial value and potential for farming. However, one of the main bottlenecks for its culture is obtaining captive females for breeding and maintaining broodstock. As an alternative to capture wild females, males are induced to sex change by steroid hormone treatments. Based on this, we investigated the effects of sex steroids on gonadal differentiation using transcriptome analysis. Sexually undifferentiated common snook were divided into three groups (3 replicas per group), which received a commercial diet supplemented with 17 α-methyltestosterone (MT, 60mg kg-1) (n=150) (group 1), 17 β-estradiol (E2, 100mg kg-1) (n=150) (group 2), or only food without sex steroid (n=150) (control group) for 45 days. The animals of each replicate were kept in 12000 l seawater tanks and fed ad libitum 3x per day. The gonads were sampled after the treatment period and then every 4 months over 1 year. One gonad was collected for histological analysis, while the other one was submitted to total RNA extraction, subsequent library construction and Illumina sequencing. Results show that MT treatment accelerated gonadal development and that the gonad exhibited morphological features of a testis in relation to the control group. The group that received E2 also had more developed gonads, but with morphological characteristics of an ovary. De novo transcriptome assembly generated 45,579 transcripts and a mapping rate of 67%. Differentially expressed genes (DEGs) were identified between treatments; some related to sex differentiation, including pro-testis genes (nr5a2, dmrt3, ptch3, ptch2, shh, etc.) in the MT group, while pro-ovarian DEGs (cyp19a1, foxl2, wnt4a, rspo1, fst, etc) were upregulated in the E2 group. Our results suggest that treatment with MT stimulated testicular development, while E2 seems to have reversed the fate of sex differentiation of the gonads, resulting in an ovary at an early stage of development. Finally, the de novo assembled gonadal transcriptome resulted in a valuable genomic resource for screening of potential pathways and genes involved in sexual differentiation of *C. undecimalis* for further aquaculture applications.

O35

INCREASED BIOMAKER DISCOVERY THROUGH A TWIST ON THE ANALYSIS OF TRANSCRIPTOMIC DATA DURING SEXUAL DEVELOPMENT IN THE EUROPEAN SEA BASS, MOUSE, AND HUMANS

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The transcriptomic analysis is crucial to understanding the functional elements of the genome. The classic method is screening for differentially expressed genes (DEGs). Since 2005 weighted gene co-expression network analysis (WGCNA) has emerged as a powerful method to explore the relationships between genes. However, a method combining the two, i.e., filtering the transcriptome dataset by DEGs or other criteria and then applying WGCNA (DEGs+WGCNA), has become common. This is of concern because it can the resulting underlying architecture of the network and lead to wrong conclusions. Here, we explore a plot twist to transcriptome data analysis: combining WGCNA, exploiting the whole dataset and not affecting the topology of the network, with the strength and relative simplicity of DEGs analysis (WGCNA+DEGs). We tested WGCNA+DEGs against DEGs+WGCNA to publicly available transcriptomic data in one of the most transcriptomically complex tissues and delicate process: vertebrate gonads undergoing sex differentiation. We generalized our results in three systems: sea bass, mouse, and human. Despite the diversity of sex-determining systems in vertebrates, a common feature is that a complex network of multiple regulatory interactions of genes associated with male and female pathways are expressed simultaneously as opposing forces. Although the gene networks involved in converting the gonads into ovaries or testis are relatively conserved during sex differentiation, the temporal and relative expression, as well as the relative position within the network, are different across vertebrates. The use of network properties and tools could contribute to better resolve such complex networks. As a result, WGCNA+DEGs clearly outperformed DEGs+WGCNA. First, the networks model fit, node connectivity measures as well as other network statistics improved, the number of modules and key genes retained increased and, importantly, the GO terms of biological processes provided a more meaningful representation of the biological question under consideration. Lastly, WGCNA+DEGs facilitated the discovery of a relevant biomarker. We propose that building a co-expression network from the entire dataset, and only thereafter filtering by DEGs, should be the method to use in transcriptomic studies, regardless of biological system, species or question being considered.

O36

THE EFFECT OF DAY LENGTH ON THE REGULATION OF PITUITARY GONADOTROPE CELLS IN THE TELEOST FISH, MEDAKA

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Synchronization of gametogenesis with favorable environmental conditions is essential to ensure perfect timing for reproduction. In vertebrates, gametogenesis is regulated by pituitary gonadotropes which produce the two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Photoperiod, as the primary environmental cue in seasonal reproduction, controls pituitary gonadotropes via TSH cells located in the pituitary pars tuberalis (PT), in both mammals and birds. However, limited knowledge is available about seasonal regulation of gonadotropes in species lacking this PT region, such as in teleost fishes. Using medaka as the model, we investigated the effect of photoperiod on the seasonal differential regulation of LH and FSH cells. We first demonstrate that medaka is a good model to investigate seasonal regulation of gonadotropes. Fish raised in long photoperiod (LP) reproduce, unlike fish raised in short photoperiod (SP). This is in line with the higher gonadosomatic index, gonadotropin mRNA levels, and gonadotrope cell number observed in LP fish. We then show that a shift from SP to LP in adult fish increases not only gonadotropin expression but also gonadotrope proliferation. Following a change from SP to LP, we found that gonadotrope proliferation increases after 5 days, fshb mRNA and sex steroid levels increase after 7 days, and lhb mRNA levels increase after 14 days when reproduction is first observed. Interestingly, pituitary melatonin receptors show lower transcript levels in LP fish, and tshb mRNA levels also show photoperiod dependency, suggesting that the inhibition of gonadotrope activity in SP probably occurs through melatonin signal and TSH cells as previously reported in mammals. We also demonstrate that folliculostelate cells (FS) are TSH sensitive and send extensions towards gonadotropes, suggesting that TSH might regulate gonadotropes via FS cells.To conclude, this study demonstrates that increasing photoperiod stimulates both gonadotropin production and gonadotrope proliferation in the pituitary, maybe via pituitary melatonin receptors, TSH, and FS cells.

O37

NEUROENDOCRINE EFFECTS OF 17A-ETHYNILESTRADIOL DURING THE EARLY DEVELOPMENTAL STAGES OF SEA BASS (*DICENTRARCHUS LABRAX*)

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Endocrine disruptors are a major concern because of their ability to interfere with key physiological functions. Among them, xenoestrogens (XEs) which are molecules mimicking endogenous estrogens, have widely studied for their effects on reproduction. However, XEs can also affect development or behavior, particularly when exposure occurs during the early stages of development of organisms and cause deleterious effects on them. In this study, we aimed to assess the effects of XEs exposure on sea bass larvae and compare outcomes across developmental stages. To do so, larvae were exposed to 17α-ethynilestradiol (EE2) (0.5 nM and 50 nM) for 8 days during different period of larval development: 30-38 days post hatching (dph), 40-48 dph and 50-58 dph. For each experiment, we evaluated the effects of EE2 exposure on larval behavior by performing behavioral analyses. In addition, the development of the neuroendocrine brain-pituitary-gonadal (BPG) axis was investigated through the measurement of the expression of the brain aromatase (cyp19a1b), the two kiss isoforms (kiss1, kiss2), the three gnrh isoforms (gnrh1, gnrh2, gnrh3), the gonadotropin beta subunits (lhβ, fshβ) and the estrogen receptors (esr1, esr2a esr2b, gpera, gperb) in the head of larvae by quantitative real time PCR. EE2 exposure during 30-38 dph resulted in a strong upregulation of cyp19a1b, and to a minor extent of gnrh2 and kiss1. During the period 40-48 dph, EE2 exposure resulted in an upregulation of cyp19a1b and gnrh1, a transient upregulation of kiss1, kiss2, and gnrh2 and a downregulation of fshβ. In the 50-58 dph period, EE2 exposure affected the expression of cyp19a1b resulting in an increased expression, and lhβ, fshβ resulting in a decreased expression. Regarding the estrogen receptors, no significant variations were observed in the different experiments. Concerning behaviors, EE2 exposure during 30-38 dph induced an increase of larval activity and anxiety-like behaviors. In contrast, neither anxiety nor locomotion were affected by EE2 exposure in older larvae (40-48 dph and 50-58 dph). Our results demonstrated that early life stages of sea bass are rather sensitive to xenoestrogens exposure

O38

EXPLORING CENTRAL AND PERIPHERAL APPETITE-REGULATING PEPTIDES IN THE BRAIN AND GASTROINTESTINAL TRACT IN GROW-OUT ATLANTIC HALIBUT E.

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The crosstalk in the gut-brain axis is crucial in appetite and feed intake control. Thus, a greater understanding of the signalling pathways involved can enable the aquaculture industry to optimise feeding regimes that improve feed intake, feed conversion and consequently growth. Atlantic halibut (*Hippoglossus hippoglossus*) is a marine species native to North-Atlantic waters and regarded as a delicacy. Due to low wild stocks, it has been an emerging aquaculture species candidate since the 80s. However, slow growth in the grow-out phase (>1 kg), which is believed to be caused by a low feed-intake, is still a major bottleneck, hindering the industry growth. Thus, we have performed an experimental trial to identify an optimised feeding regime that promotes higher feed intake, while decreasing feed waste, for grow-out halibut. Tagged Atlantic halibut (mean size 2 kg) were fed a single large meal every third day during a period of 14 weeks. At the end of the trial, fish were sampled 0.5 h before feeding, and 2 and 6 hours after feeding. The whole brain and tissue samples from stomach, pyloric ceca, and midgut were collected for gene expression analyses. Inner content of the stomach, pyloric caeca, midgut and hindgut were also collected. Biometry data was registered for each fish. In this study, we will provide an overview of the expression of key appetite-related genes among five brain regions (olfactory lobe, optic lobe, cerebellum, telencephalon and hypothalamus), stomach, pyloric ceca and midgut (anterior and posterior) using qPCR. To investigate if signals from the gastrointestinal tract, such as sense of fullness, are important for appetite control and contribute to regulate food intake we investigate if appetite related genes expression is correlated with the gastrointestinal tract compartments inner content of grow-out halibut fed a large meal every 72 hours.

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O39

INVESTIGATIONS ON THE ROLE OF BRAIN AROMATASE IN MEDIATING BEHAVIORAL DEFECTS FOLLOWING EXPOSURE TO ESTROGENIC CHEMICALS IN ZEBRAFISH.

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Disruption of various behavioral traits is an important consequence of exposure to endocrine disrupting chemicals (EDCs). Indeed, beyond reproductive behaviour, other traits (e.g. aggressivity, anxiety, cognition) were shown to be disrupted upon exposure to EDCs. Early-life development is very sensitive to chemicals and there is a risk that organisms will express neuronal defects that can translate into behavioral alterations in adults. However, underlying mechanisms driving late manifestation of early exposure are not well understood. Brain aromatase (aroB) locally converts androgens into estrogens and is the target of some EDCs. In this work, we hypothesised that aroB may be one of the actors involved in delayed effects of early exposure to EDCs. For this purpose we looked at behavioral changes in larval and adult zebrafish exposed for 5 days at the embryo-larval stage to various estrogenic chemicals inducing aroB expression. The results show that behavioral changes observed in larvae were highly substance-specific and therefore suggest no key-role for the expression of aroB in the photomotor response of zebrafish larvae. Further, in adults, exposure to ethinylestradiol (reference chemical) significantly altered traits related to exploration/boldness of zebrafish males and may suggest a role for aroB in mediating delayed hyperactivity-like behavior at adulthood. Overall, the present data show that brain aromatase may be implicated in the sex-specific regulation of complex behavior by estrogenic chemicals, a finding that is currently under validation using exposure to additional EDCs and a knock-out line that does not express aroB.

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O40

GENOTYPE-DEPENDENT ACTIVATION OF CRH-FAMILY GENES DURING HEAT-INDUCED MASCULINIZATION IN PEJERREY *ODONTESTHES BONARIENSIS*

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In recent years, a connection between stress, stress-related CHR-family genes, and sex determination in fish has emerged. Previous studies from our research group in pejerrey *Odontesthes bonariensis*, a species with both, genetic and environmental-sex determination, demonstrated the involvement of cortisol in stress (so far thermal and crowding)-induced masculinization during the critical period of sex determination (CPSD: 2-6 weeks after hatching). However, the involvement of the brain via the hypothalamus-pituitary-interrenal axis (HPI) in the regulation of this process in pejerrey has not been investigated. There are also no studies on the possibility of genotypic-specific stress responses of larvae to the masculinizing conditions. Therefore, in this study we analyzed the expression patterns of several members of the CRH family (a group of genes regulating stress and anxiety) including corticotropin-releasing hormone a and b (crha and crhb), urotensin 1 (ust1), urocortins 2 and 3 (ucn2 and ucn3), and corticotropin-releasing binding protein (crhbp) as well as their receptors crhr1 and crhr2 in the heads of XX and XY pejerrey larva during the CPSD at a masculinizing temperature (29°C). Genes found to be upregulated were further analyzed by in situ hybridization (ISH). The deduced amino acid sequences of crhb, uts1, ucn2, and ucn3 exhibited all the structural elements of functional mature peptides, despite ucn2 been nonfunctional in some vertebrates. Notwithstanding our multiple attempts to clone crha, we failed to identify this gene in pejerrey. Quantification by RT-qPCR showed that crhb, ucn2, ucn3 and their receptor crhr2 were upregulated only in the heads of XX larvae but not in XY during heat-induced masculinization. uts1, crhbp, and crhr1, on the other hand, did not show any statistical difference between genotypes and therefore may not be involved in this process. ISH showed that ligands and the receptor of CRH-family genes were expressed in the tuberal hypothalamus, in areas of great neuroendocrine relevance. The fact that all the three overexpressed genes in XX larvae signal through crhr2 (based on affinity studies conducted in other species) and had close spatiotemporal relation during the CPSD suggests that these genes might be operating in a synergic manner to induce the activation of the HPI axis or perhaps the organization of stress-mediated sexual dimorphism in the neural architecture. Overall, upregulation of crhb and urocortins might be necessary to induce a developmental reprograming of the brain of XX larvae during heat-induced masculinization, which is not necessary in XY due that it is already programmed.

O41
IMPACT OF NIGHT SHIFT WORK ON BRAIN FUNCTION AND MORPHOLGY

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Neuropsychological studies reported that shift workers show reduced cognitive performance and circadian dysfunctions which may impact structural and functional brain networks. Here we tested the hypothesis whether night shift work is associated with resting-state functional connectivity (RSFC), cortical thickness and gray matter volume in 13 PRESENT and 89 FORMER night shift workers as well as 430 control participants who had never worked in shift (NEVER). No associations between night shift work, three graph-theoretical measures of RSFC of 7 functional brain networks and brain morphology were found after multiple comparison correction. Preceding multiple comparison correction, our results hinted at an association between more years of shift work and higher segregation of the visual network in PRESENT shift workers and between shift work experience and lower gray matter volume of the left thalamus. Extensive neuropsychological investigations supplementing objective imaging methodology did not reveal an association between night shift work and cognition after multiple comparison correction. Our pilot study suggests that night shift work does not elicit general alterations in brain networks and affects the brain only to a limited extent.

O42

EFLA-TYPE NEUROPEPTIDE IS PRODUCED BY ALTERNATIVE TRANS-SPLICING IN SOME INSECTS

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EFLa-type neuropeptides are present in many arthropods, including several insect orders. Besides EFLa elusive function, the way how the mature EFLa-encoding transcripts are formed is also enigmatic, as EFLa exons spliced to exons of Proh-4 gene were found in *Pyrrhocoris apterus* (Heteroptera), *Bemisia tabaci* (Sternorrhyncha) and *Locusta migratoria* (Orthoptera). The presence of intergenic Proh-4/EFLa fused transcripts can be theoretically explained by canonical cis-splicing, cis-splicing of adjacent genes (cis-SAG), or even by trans-splicing, a rare molecular mechanism in insects. We focused on P. apterus, where Proh-4/EFLa transcripts represent about 1/10 of Proh-4 expression, EFLa pre-mRNAs lack in-frame translation start site, and where gene-linkage and genome analyses excluded cis-splicing and cis-SAG mechanism of Proh-4/EFLa generation. Trans-spliced Proh-4/EFLa transcripts were detected by full-length mRNA sequencing using Oxford Nanopore technology with isoform-specific resolution. In-silico analysis of 3' splice site (3'ss) strength predicted weak 3'ss of Proh-4 intron 2, permitting trans-splicing with 10-fold stronger EFLa outron 3'ss. Analogous analysis in L. migratoria found a less profound Proh-4 to EFLa 3'ss strength difference, reflected in 10-fold lower Proh-4/EFLa trans-splicing, determined by quantitative real-time PCR. We conclude that although the exact trans-splicing mechanism generating Proh-4/EFLa transcripts stays elusive, it can represent a conserved and sound gene expression mechanism.

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O43

NEUROPEPTIDE PRECURSORS AND G-PROTEIN COUPLED RECEPTORS IN THE BIVALVE MANTLE

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The bivalve mantle is the main tissue involved in the secretion of the calcified biomineralized shell. To understand shell production, mantle transcriptomes and proteomes were generated and matrix proteins, enzymes and ion transporters of the bivalve biomineralization toolbox were identified. Mantle transcriptomes of the Mediterranean mussel (*Mytillus galloprovincialis*) and hard-shelled mussel (*Mytillus coruscus*), two marine bivalve species of economic importance for aquaculture, were analysed and multiple neuropeptide signalling molecules and G-protein coupled receptors (GPCRs) were identified, raising questions about the neuroendocrine regulation of the mantle and its functions. In the mussel mantle edge, the most active shell building region, we identified at least 40 different partial and full-length neuropeptide precursors that share similar organization and sequence with those described in neural ganglia transcriptomes available for other bivalves. Gene transcript precursors encoded multiple small peptides of highly identical sequence such as the FMRF-amide, APGW-amide and MIP precursors, as well as homologues of vertebrate neuropeptide Y, gonadotropin releasing hormone and the calcitonin precursor. Of the 494 predicted GPCR genes in the Mediterranean mussel genome assembly, at least 200 were found in the mussel mantle edge transcriptomes and they are representatives of the different neuropeptide GPCR superfamilies and putative neuropeptide-GPCR ligand-receptor pairs were assigned. Our data reveal that the bivalve mantle is the target of a large number of biologically active neuropeptides which are likely to play important roles in controlling mantle function including the regulation of shell production.

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O44

SOMATOSTATIN SIGNALLING REGULATES GERM CELL NUMBER, FECUNDITY, PANCREATIC CELL PROLIFERATION AND METABOLISM IN ZEBRAFISH

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Energy allocation between growth and reproduction determines puberty onset and fertility. However, the regulatory networks involved in the trade-off are not evolutionary conserved and in fish is still an enigma. Based on experimental and transcriptomic information we identified somatostatins (ss), of which there are 6 genes in zebrafish (*Danio rerio*), as potential candidates for that role. We report the phenotypes of CRISPR/Cas9 zebrafish mutants of ss1 and ss3. Both of mutant lines showed over-proliferation of primordial germ cells via their receptors sstr2b and sstr5. However, only adult ss3, not ss1, were hyper fecund compared to their wild type controls. The ss1 mutant line were hyperglycemic, with improved glucose tolerance and higher triglyceride levels compared to the wild type due to an increase in pancreatic α-cell proliferation. In contrast, ss3 mutants showed hypoglycemia, glucose intolerance, lower triglyceride and total cholesterol compared to the wild type due to increased pancreatic β-cell proliferation. Our results demonstrated for the first time that somatostatin signalling tightly controls energy and fecundity through its inhibitory role on pancreatic cells and PGCs proliferation. This is likely to be a general function of somatostatin in vertebrates.

O45
RELAXIN-LIKE GONAD-STIMULATING PEPTIDE FAMILY IN ASTEROIDEA

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In 2009 a gonadotropic substance was purified from radial nerve cords of the blue bat star *Patiria pectinifera*. The purified substance was a heterodimeric peptide, comprising A- and B-chains, with disulfide cross-linkages of one intra-chain and two inter-chain disulfide bonds. The A-chain contained a cysteine motif [CCxxxCxxxxxxxxC], which is a signature sequence of the insulin/insulin-like growth factor/relaxin superfamily. More specifically, phylogenetic sequence analysis revealed that the starfish peptide is a member of the relaxin-type peptide family. From this, the gonadotropic peptide in starfish was designated as relaxin-like gonad-stimulating peptide (RGP). Thus, *P. pectinifera* RGP (Ppe-RGP) is the first identified gonadotropic hormone to trigger final gamete maturation and ovulation among invertebrates. The cDNA of Ppe-RGP encodes not only the A- and B-chains, but also the signal and C-peptides. After Ppe-RGP gene is translated as a precursor, mature Ppe-RGP is produced by elimination of the signal and C-peptides. Hitherto, eighteen RGP orthologs have been identified or predicted from starfish in the orders Valvatida, Forciplatida, Paxillosida, Platyasteroidea, Spinulosida, and Valatida. It is interesting that the C-terminal residue of RGP identified from Astropecten scoparius (Asc-RGP) of the order Paxillosida was amidated. Asc-RGP is the first finding of a C-terminally amidated functional RGP. RGP generally acts non-species-specifically, with some exceptions. The structural basis of the interaction of RGP with its receptor using chimeric derivatives suggests that the B-chain of RGP is important for receptor binding and bioactivity. It also seems likely that species specificity is caused by the three-dimensional position of an amino acid residue in A-chain with respect to the receptor. The molecular evolution of the RGP family is in accordance with the phylogenetic taxonomy of the Asteroidea.

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O46

RETHINKING THE REGULATION OF PRE-OVULATORY LH SURGE IN FEMALE ZEBRAFISH: IS THE HYPOPHYSIOTROPIC GNRH (GNRH3) DISPENSABLE?

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The hypophysiotropic gonadotropin-releasing hormone (GnRH) is essential for controlling luteinizing hormone (LH) secretion, especially for ovulation, in all studied vertebrates. However, recent studies in zebrafish, a fish with two Gnrhs (Gnrh2 & Gnrh3), in which the hypophysiotropic Gnrh3 is considered to function like GnRH1 in the pre-ovulatory LH surge, have shown that the lack of Gnrh3 does not affect fertility. These findings may be explained by functional compensation or redundancy hypotheses. Here, we present evidence pointing to Gnrh3 dispensability in modulating the Lh surge. First, to determine the role of the hypophysiotropic Gnrh and its neurons in controlling ovulation in zebrafish, we performed chemogenetic Gnrh3 neuronal ablation, which resulted in depletion of Gnrh3 neurons and Gnrh3 but normal reproductive capacity. Next, to examine whether other neuropeptides can regulate LH surge. We demonstrated that vasoactive intestinal peptide A (VipA, homologue of mammalian Vip) induces Lh secretion in zebrafish females. Despite the observation that VipA axons are found in close vicinity of the pituitary gonadotropes, in vitro pituitary incubation with VipA and intraperitoneal injection of VipA, did not induce Lh secretion and lhb mRNA expression, respectively. On the other hand, intracerebroventricular administration of VipA augmented plasma LH levels in both wild-type (WT) and gnrh3–/– females at 1 hour post-treatment, with no observed changes in pituitary Gnrh2 and Gnrh3 contents and gnrh3 mRNA levels in the brains. The stimulation of Lh by VipA seems to occur via a different pathway than Gnrh3, dopamine, and 17b-estradiol. The results indicate that VipA induces Lh release, possibly by acting with or through a non-GnRH factor(s). Finally, to determine whether the absence of Gnrh3 affects the pituitary, and to identify potential factors pertinent to the regulation of Lh secretion, we performed a single-cell RNA sequence. We then analyzed gene expression profiles for individual pituitary cells of WT and gnrh3–/– sexually mature females. While there were no significant differences in Lh secretion pathway-related gene expression profiles of Lh gonadotropes between the genotypes, some non-reproductive hormone gene expressions were significantly increased in Lh gonadotropes of gnrh3–/–. We also identified a potential novel cell type, which significantly (highly) expresses fshb and lhb but no other typical gonadotrope genes (e.g., cga & nr5a1b). This cluster was only faintly detectable in the gnrh3–/–, indicating that gnrh3 possibly affects pituitary cell specialization. Hence, Gnrh3 may not work alone or as an essential factor in controlling the pre-ovulatory Lh surge and ovulation in female zebrafish. Our results support the idea that Gnrh3 function is different from GnRH1 in controlling reproduction in other vertebrates.

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O47

IMPACT OF CRISPR/CAS9 COMPLETE SECRETOGRANIN-II GENE KNOCKOUT ON NEUROPEPTIDES AND REPRODUCTION IN ZEBRAFISH (*DANIO RERIO*)

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Gonadotropin-releasing hormone (Gnrh) neurons in the hypothalamus represent the dogmatic primary stimulatory system for reproduction. Yet, when both forms of Gnrh are mutated in zebrafish (ZF) they remain fully fertile. This forces revaluation of what constitutes the main control system of the HPG axis in teleost compared to mammals. The lack of reproductive disruption has also been observed for most other ZF neuropeptide mutants except for secretogranin-II (Scg2a and Scg2b). Proteolytic processing of the Scg2 precursors produce the secretoneurin a (SNa) and SNb neuropeptides. Our lab demonstrated that exogenous SNa stimulates the release of luteinizing hormone (Lh) in goldfish, while single and double mutant ZF exhibit reduced spawning activity associated with suppressed expression of classic reproductive genes including gnrh3, lhb and fshb. The reductions in the critical SNa and SNb are hypothesized to be responsible, but this must be tested rigorously. Therefore, we created Scg2a and Scg2b complete gene knockout (KO) zebrafish using CRISPR-Cas9 to excise the entire coding regions. Large-scale PCR screens and gene sequencing uncovered 8 possible KO lines, but targeted mass spectroscopy confirmed complete absence of the peptides in only 5 lines. Using a specific polyclonal antibody against SNa, we report the complete absence of immunoreactivity in the pituitary of true Scg2a KO fish. Behavioural analysis indicates that both KO lines have delayed oviposition while only scg2b-KO has a significantly reduced spawning success. The double scg2a/scg2b KO animals obtained are rare yet viable. We are now testing the bioactivities of various SNa/b peptides by injection into wild type and full KO lines to determine effects on gonadal function and the underlying basis for SN-regulated sexual behaviours. The scg2a/scg2b KO model provides a unique system to search for new modulators of vertebrate reproduction.

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O48
INHIBIN IN FISH REPRODUCTION– A MOLECULE THAT SHOULD NOT BE FORGOTTEN

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Inhibin was first characterized in mammals as a gonadal dimeric protein that inhibited pituitary follicle-stimulating hormone (FSH) secretion. The discovery of inhibin and its related molecules activin and follistatin has been considered one of the most important breakthroughs in reproductive endocrinology in 1980s. As in mammals, inhibin has also been characterized in teleosts; however, its functions in fish reproduction remain largely unknown. Using zebrafish as the model, we demonstrated that inhibin-specific alpha subunit (INHA/Inha/inha) was exclusively expressed in the gonads (ovary and testis). In the ovary, its expression was restricted to the somatic follicle cells, together with all three b subunits (inhbaa, inhbab and inhbb). During folliculogenesis, inha expression in the follicles increased slightly but steadily from primary growth (PG) to the mid-vitellogenic (MV) stage; however, its expression surged dramatically at the full-grown (FG) stage, suggesting an important role for inhibin in oocyte maturation and ovulation. Both pituitary extract (goldfish) and forskolin significantly stimulated inha expression in vitro. Further experiments showed that recombinant zebrafish FSH but not LH significantly increased inha expression in the same assay system. The stimulation of inha expression by FSH and the potential inhibition of FSH by inhibin suggest a negative feedback loop between the pituitary and ovary in the zebrafish, similar to the situation in mammals. Using CRISPR/Cas9 method, we recently generated an inha-deficient zebrafish line (umo19) and analyzed its reproductive performance. As expected, pituitary expression of fshb increased significantly in both young and adult inha mutant. The juvenile female mutant showed clear signs of early follicle activation or precocious puberty onset. However, the adult female mutant was infertile with follicles arrested at the FG stage without final oocyte maturation and ovulation. In summary, our studies so far have demonstrated clearly that inhibin plays a critical role in controlling ovarian folliculogenesis. It inhibits follicle activation in early stage probably through regulating activin activity but promotes final oocyte maturation and ovulation probably by regulating the upper levels of the hypothalamic-pituitary-gonadal (HPG) axis.

O49
TILAPIA AS A GOOD MODEL FOR STUDYING COMPARATIVE ENDOCRINOLOGY

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The Nile tilapia (*Oreochromis niloticus*) is a worldwide cultured fish. With a moderate body size, it is convenient for taking blood for hormone level measurement. Its sexual maturation time is shorter than that of most cultured fish. Tilapia can be artificially propagated in the laboratory all year round to obtain genetically all female and all male fry. Its genome sequence has been opened, and a perfect gene editing platform has been established in our laboratory. Therefore, it is a good model for studying fish endocrinology and comparative endocrinology. In collaboration with Prof. Christopher H K Cheng (The Chinese University of Hongkong), we had identified a gonadal specific insulin-like growth hormone 3 (igf3) and demonstrated the existence and functional significance of two isoforms for growth hormone receptors and prolactin receptors in tilapia. In recent years, we demonstrated that steroidogenic enzymes can be classified into two categories based on their expression, enzyme activities and function in tilapia. Mutation of star2, cyp17a1 and cyp19a1a, which are dominantly expressed in the gonads and involved in estrogen production, results in up-regulation of male pathway genes and female to male sex reversal. In contrast, mutation of star1, cyp11a1, cyp17a2 and cyp19a1b, as well as cyp11c1, which are expressed both in gonads and extra-gonadal tissues, alters the steroids (androgen, DHP and cortisol) production and spermatogenesis, fertility, secondary sexual characteristics and sexual behavior, but usually does not affect the sex differentiation. Consistently, mutation of estrogen receptors resulted in female to male sex reversal, while mutation of androgen receptors resulted in no sex reversal. In addition, the differentiated ovary could be transdifferentiated into functional testis by down-regulation of estrogen level, and the differentiated testis could be transdifferentiated into ovary by simultaneous addition of exogenous estrogen and androgen synthetase inhibitor, demonstrating the maintenance of sexual plasticity even after sex differentiation in gonochoristic fish. Interestingly, germ cells lost sexual plasticity in dmrt1 XY and foxl3 XX single mutants, as aromatase inhibitor (AI) and estrogen treatment failed to rescue the respective phenotypes. However, recovery of germ cell sexual plasticity was observed in dmrt1/foxl3 double mutants. Overall, these studies demonstrate that tilapia is a good animal model for studying fish growth endocrinology and reproductive endocrinology.

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O50
NEUROPEPTIDE Y IN TILAPIA: EMPHASIS ON THE ROLE OF FOOD INTAKE

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Food intake is a must to the survival of vertebrates. The regulation of food intake needs delicate coordination of brain and peripheral signals. Among the many endocrine factors involved in the regulation of feeding that have been studied, neuropeptide Y (NPY) system has attracted much attention due to its involvement in the regulation of feeding by both brain and peripheral signals. At present, the research on the role of the NPY system in teleost fish feeding regulation is still relatively lacking. Whether NPYb and PYYb in teleost fish are involved in feeding regulation and the types of NPY receptors involved in feeding regulation in teleost fish and their specific roles are still unclear. In the present study, we determined the composition of the NPY system in Nile tilapia (*Oreochromis niloticus*) by gene cloning, chromosomal location and homology analysis, and verified the biological activity of the cloned NPY receptor. Based on it, the role of NPY family polypeptides in tilapia in feeding regulation was further studied, and a pilot test was carried out. The findings suggest that NPY has a promising prospects for regulating fish feeding and growth.

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O51
THE ROLES OF NEUROPEPTIDES IN FISH REPRODUCTION

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Reproduction is driven by the hypothalamic peptide gonadotropin-releasing hormone (GnRH), which is secreted into the hypophysial portal blood in a pulsatile manner to cause both synthesis and secretion of the gonadotropins (FSH and LH). Gonadotropins act on the gonads to maintain their functional and structural integrity. Sex steroids released from the gonads in turn feed back on the hypothalamus to increase (positive feed back) or to decrease (negative feed back) gonadotropins release. Neuropeptides are short chain peptides produced in neuronal or endocrine tissues. They are signaling molecules by binding and activating G-protein coupled receptors. More and more studies have demonstrated that Neuropeptides play important roles in the regulation of reproductive axis. In the vertebrates, Kisspeptin and GnIH have been now demonstrated as the important positive and negative factors in reproductive axis in vertebrates, respectively. With more teleost fish model genomes sequenced, many neuropeptides have been identified and characterized in teleost fish. Here, we discuss their significance in the reproductive axis in teleosts.

O52

DENDRIMER-SMALL RNA DRUGS TARGETING RENIN-ANGIOTENSIN SYSTEM FOR CANCER THERAPY

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Activation of angiotensin receptor type I (AGTR1) promotes ovarian cancer spheroid formation and peritoneal metastasis by increase lipid desaturation to reduce endoplasmic reticulum stress. Since Mas receptor (MAS1) have been shown to counteract the AGTR1 pathway in cardiovascular system, therefore activation of MAS1 or silencing of AGTR1 may suppress cancer progression and metastasis. However, small-molecule agonists/antagonists targeting those receptors have the problems with potent side-effects and lack of specificity. As an alternative, small nucleic acid molecules offer a unique opportunity to address the "undruggable" targets, thus providing precision medicine. Here, we report for the first time that small activating RNAs (saRNAs) effectively modulate a G protein-coupled receptor (GPCR) for cancer treatment. Specifically, we identified saRNAs promoting the expression of MAS1. These saRNAs, delivered by an amphiphilic dendrimer vector, lead to significant suppression of tumorigenesis and the inhibition of tumor progression of multiple cancers. Our study provides not only a new strategy for cancer therapy by targeting the renin-angiotensin system, but also a new avenue to address GPCRs or any other undruggable target.

O53

EARLY GONADAL DEVELOPMENT, EXPRESSION PROFILE AND REGULATION OF SEX-RELATED GENES AND HORMONAL INDUCTION OF SEX REVERSAL MECHANISM IN *SCATOPHAGUS ARGUS*

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In vertebrates the development of the gonad into the testis or ovary requires the combined effect of genes and hormones to trigger differentiation from a bipotential gonadal primordium. Knowledge of the gonad development process is vital in fish sex determination and differentiation studies. The spotted scat (*Scatophagus argus*) is an economically important marine fish with an XX/XY sex-determining system and exhibits sexual growth dimorphism favoring females. Meanwhile, information on gonad development and gene expression patterns in the early gonads is unknown. It is also unclear whether external hormones could reverse the sex of the spotted scat. This study explores the pattern of spotted scat early gonad development by morphological and molecular means; studies the incidence of transient intersex associated with testis-ova; analyze the gene expression pattern by RNA-seq and qPCR in the differentiating gonads; establishment of sex reversal via hormonal treatment.

O54

IN VIVO DRUG DISCOVERY FOR INCREASING INCRETIN-EXPRESSING CELLS IDENTIFIES DYRK INHIBITORS THAT REINFORCE THE ENTEROENDOCRINE SYSTEM

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Incretin hormones Gip and Glp-1 play important roles in modulating inslulin secretion and plasma glucose levels. In this study, a high-throughput in vivo chemical screen in zebrafish was established by using the gip promoter to drive the expression of luciferase to identify small molecules that increase the number of incretin-expressing cells. All hits identified promote neogenesis of Gip-expressing K-cells, and Glp-1-expressing L-cells originating from neurogenin 3-expressing enteroendocrine progenitors. Among them, a dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) inhibitor regulated incretin cell number in zebrafish and reduced glucose levels in both larval and juvenile fish. DYRK inhibition also increased the number of incretin-expressing cells in diabetic mice, suggesting a conserved reinforcement of the enteroendocrine system and a possible novel therapeutic approach.

O55

MULTIFACETED BRAIN-DERIVED NEUROTROPHIC FACTOR – A GROWTH FACTOR IN BRAIN, A MYOKINE, OR AN ENDOCRINE HORMONE?

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Switching between glucose and fatty acid as the dominant energy source is essential to achieve energy homeostasis during various metabolic stresses. However, the underlying mechanism that regulates this metabolic flexibility in the muscle remains elusive. In addition to its major role in locomotion, skeletal muscle is now recognized as an endocrine organ via the production of secretory factors called myokines. Our recent study shows that brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family that plays an essential role in synaptic plasticity and food intake, is an energy stress-responsive myokine to modulate systemic energy metabolism. Using metabolomics, gene expression, and indirect calorimetric analyses, we found that skeletal muscle-specific Bdnf knockout (MBKO) mice were incapable of shifting the fuel source from fatty acid to carbohydrates during nutrient stress, resulting in the accumulation of acylcarnitines and reduced energy production in glycolytic muscle. Moreover, lipid-induced mitophagy was impaired in the Bdnf-ablated muscle. MBKO mice thus displayed myofiber necrosis, reduced muscle strength, decreased physical activity, metabolic inflexibility, and muscle-specific insulin resistance. These findings demonstrate that BDNF is not only an anorexic factor in the central nervous system but also an essential metabolic regulator to link mitophagy and metabolic flexibility in skeletal muscle.

O56

THE ABSENCE OF LIGHT AND FEEDING-RELATED SYNCHRONIZERS DIFFERENTLY AFFECTS ENERGY BALANCE IN GOLDFISH.

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The light/dark and feeding cycles are the main environmental signals synchronizing animal physiology and behavior. In mammals it is well known that misalignment of these signals disrupts the circadian system, causing adverse neuroendocrine and metabolic effects. In fish, this relationship and the mechanisms beyond are less studied. The goal of this work was to investigate the effects of chronic chronodisruption on energy balance in goldfish. For this purpose, 60 fish were divided in three groups under different conditions. Control (i.e., standardized conditions of photoperiod and feeding; 12L:12D and feeding once daily at ZT1) and two chronodisruption models: random feeding (named RF; 12L:12D photoperiod and food supplied at random times) and constant light (named LL; 24L lighting conditions, feeding at ZT1). Growth, food intake, metabolic rate, and locomotor activity were quantified during the course of the experiment. After 53 days feeding-regulating genes expression (npy, orexin, ghrelin, cart, pomc) in hypothalamus and intestine, and plasma cortisol were studied. Metabolic rate elevated in both RF and LL groups in parallel with a raise in plasma cortisol, confirming the altered conditions produce stress in these fish. A higher food intake was also found in both chronodisrupted groups, associated with a dysregulated expression of feeding neuroendocrine regulators, raising the orexigenic/anorexigenic balance. Despite this higher intake in both experimental groups, only RF animals showed an increase in length, weight gain and a higher hepatosomatic index, suggesting the LL fish had higher energy demands, probably related with the observed higher locomotor activity. Overall, the loss of feeding-fasting or light-dark cycles increases stress and metabolic rate suggesting an increase in energy expenditure. However, non-predictable feeding times increase energy inputs more than requirements, causing a positive energy balance. Present results support that disruptive circadian conditions significantly alter energy homeostasis in fish.

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O57

DIABETES IMPAIRS REACTIVE GLIOSIS AND INCREASES EXTRACELLULAR MATRIX SYNTHESIS AFTER ISCHEMIC STROKE IN MICE

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Diabetes affects 10 % of people in the world and this metabolic disorder causes many physiological complications. Among them, diabetes increases by two the risk of ischemic stroke that is a leading cause of disabilities in the world. After stroke, microglia and astrocytes become reactive in the infarct hemisphere (microgliosis and astrogliosis) and these cells lead to the formation of "the glial scar". At the acute phase, this process protects the brain from increased inflammation and cell death. Later, the glial scar produces an extracellular matrix (ECM) that inhibits axonal growth and brain repair. Until now, the impact of diabetes on the glial scar formation is not well understood. We hypothesized that the diabetic condition may impair this process and consequently worsen stroke outcomes. In this study, we used diabetic mice (db/db) and normoglycemic mice (db/+) to investigate reactive gliosis 3 days after brain ischemia, using the middle cerebral artery occlusion method (MCAO). We first showed that the infarct size is bigger in db/db mice than in control mice, and correlated with higher hemorrhagic transformation and worsened neurological scores. By performing immunostaining against GFAP and Iba1 to label astrocytes and microglia respectively, we studied their activation in two different peri-ischemic brain regions. For each region, we compared the increase in GFAP and Iba1-positive area in the ipsilateral hemisphere versus the contralateral hemisphere. We consequently demonstrated that astrogliosis is significantly higher in db/db than in db/+ mice, in both brain regions. Regarding microglia, their recruitment were significantly lower in db/db than in db/+ mice in the regions analyzed. However, in db/db mice microglia displayed an "ameboid" shape while they displayed a more ramified shape in db/+ mice, suggesting a stronger microglia activation in db/db. Interestingly, a higher increase in ECM proteins (collagen-4, CSPG and tenascin-C) was observed in the ischemic core of db/db mice compared to db/+. This study demonstrates for the first time that, in our experimental conditions, the metabolic status of db/db mice impairs reactive gliosis and the glial scar formation increasing the extracellular matrix production in vivo.

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O58

A MULTIDISCIPLINARY APPROACH TO INVESTIGATE PROBIOTIC MITIGATION AGAINST CHRONIC BISPHENOL A EXPOSURE EFFECTS AT HEPATIC AND GUT LEVELS IN *DANIO RERIO*.

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Numerous studies investigated the negative effects of Bisphenol A (BPA), one of the most widespread endocrine disruptors (EDCs). This contaminant interacting with the endocrine system of organisms affects numerous physiological activities. On the other hand, probiotic administration was demonstrated to exert beneficial effects to the host health. Since EDCs exposure represent a global concern for both wildlife and human health, new strategy to reduce this environmental toxicity is needed and probiotics administration could be a possible tool to achieve this goal. Thus, the aim of this study was to investigate if SLAb51 probiotic formulation (109 CFU/g) is able to mitigate negative effects induced by chronic exposure to BPA (10 µg/L for 28 days) in zebrafish adults after. Liver analysis suggests the ability of SLAb51 to mitigate hepatic BPA toxicity. The metabolomic analysis on liver of co-treated group (BPA+P) clustered with the probiotic group (P) sharing similar metabolite changes profile. Differently, retinoic acid was only decreased in the BPA+P group, associated with enhancement of molecules involved in the elimination of the contaminant. Furthermore, transcript level of genes involved in lipid metabolism, inflammation and appetite regulation (pparα, pparγ, ptgs2a and mboat4) was restored to control level in the co-treated group. Liver injury, apoptosis level and CK-18 immunoreaction were also mitigated by the co-treatment with probiotic. In the intestinal tract the microbiota analysis evidenced the absence of Chlamidiae and the reduction of Bacilli colonization in BPA+P group fish, both present in the gut of BPA group. Increase of Pseudomonadales was found in both BPA and BPA+P groups, while Cetobacterium, a cobalamin producing bacteria, was slightly increased by SLAb51 treatment. Our results evidenced the ability of SLAb51 probiotic formulation, to mitigate the BPA toxicity in zebrafish liver and ameliorate the gut microbiota.

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O59

THERAPEUTIC POTENTIAL OF THE MEDICINAL PLANT HYPERICUM LANCEOLATUM LAM. ON METABOLIC DISORDERS IN ZEBRAFISH (*DANIO RERIO*) MODELS

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The use of traditional medicine in health care is a widespread practice. In Reunion Island, a French oversea department belonging to the Mascarene archipelago in the Indian Ocean, some plants are widely used to treat many disorders including fever, minor infections and chronic diseases thanks to their antioxidant, anti-inflammatory, antiviral and "anti-diabetic" properties. In the present work, we investigated the potentially beneficial properties of *Hypericum lanceolatum* Lam. (*H. lanceolatum*) recommended through traditional knowledge as a good anti-inflammatory plant. In a first step, we studied the toxicity of an aqueous preparation of *H. lanceolatum* in zebrafish (*Danio rerio*) eleutheroembryos according to a reliable toxicity assay validated by the Organization for Economic Cooperation and Development (OECD). We therefore determined a maximum non-toxic concentration that was further used to study the properties of our plant aqueous extract in metabolic disorder models. In a high-fat model in zebrafish larvae, we reported that the treatment with the aqueous extract of *H. lanceolatum* decreased the accumulation of neutral lipids (triglycerides and cholesterol esters) in the digestive tract and vessels of larvae overfed with egg yolk powder. In parallel, by performing a diet-induced obesity in adult fish, we observed a significant body weight gain in overfed fish compared to control ones and in our experimental conditions, the therapeutic treatment with *H. lanceolatum* aqueous extract for 1 week was sufficient to significantly reduce the body mass index. Lastly, we analyzed the effects of the aqueous extract of *H. lanceolatum* on brain plasticity that is impaired in hyperglycemic conditions. We showed that the therapeutic treatment with the plant extract prevented the decreased neurogenesis observed in hyperglycemic fish. In conclusion, our results highlighted the potential of the *H. lanceolatum* aqueous extract to counteract some diabetes- and obesity-related complications through its lipid-lowering and pro-neurogenic properties.

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METABOLIC DISORDERS IMPAIR BRAIN HOMEOSTASIS AND NEUROGENESIS

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Diabetes and obesity are global health problems that lead to numerous physiological disorders. Recently, it has been shown that these metabolic diseases can alter brain function, leading to disruption of homeostasis such as increased cognitive impairment, and increased risk of Alzheimer's disease and dementia. However, the mechanisms supporting the impact of diabetes and obesity on brain homeostasis are not well understood. In this work, we aimed to investigate the effect of these two metabolic diseases on brain homeostasis and in particular on brain plasticity. To this end, we took advantage of the zebrafish which shares with mammals many signaling pathways of metabolic and neurogenic mechanisms. We therefore developed (1) hyperglycemic zebrafish by immersing them in 111 mM glucose for 14 days, and (2) obese zebrafish by overfeeding them for 4 weeks using a diet-induced obesity protocol. Using the first experimental approach, we observed that glucose-treated zebrafish had higher fasting blood glucose levels than controls. Interestingly, the brain homeostasis of hyperglycemic zebrafish is altered as evidenced by the misexpression of many genes notably involved in the immune response, oxidative stress and blood-brain barrier function. Furthermore, by performing immunohistochemistry against the proliferative marker PCNA, we demonstrated that brain cell proliferation in the main neurogenic niches of the adult brain was impaired under homeostatic and regenerative conditions. Similarly, in our diet-induced obesity protocol, we observed that treated fish had higher body weight, body mass index, and fasting blood glucose than their respective normally fed controls. In the brain, we also observed increased oxidative stress and neuroinflammation, associated with the leakage of the blood-brain barrier (BBB). In addition, transcriptomic analysis revealed the misexpression of many neuroinflammatory, oxidative stress and neurogenic genes. Furthermore, we demonstrated that obese zebrafish exhibit reduced cerebral plasticity and blunted neurogenesis in the main niches studied. Overall, our transcriptomic and immunohistostaining data highlighted that both metabolic conditions (hyperglycemia and obesity) are associated with common disruptions in brain homeostasis (i.e., impaired BBB function, increased brain oxidative stress, neuroinflammation, and decreased brain plasticity). This study paves the way for further experiments to understand the mechanisms and disrupted signaling pathways impacting neural stem cell activity in metabolic diseases.

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MUSCLE AND BONE INTERACTION AFTER AN INJURY IN GILTHEAD SEA BREAM: IMPLICATIONS OF ENDOCRINE AND REGULATORY FACTORS IN MUSCLE REGENERATION

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The skeletal muscle is a locomotor and endocrine tissue, representing up to 60% of the body weight in fish, formed by multinucleated myofibers originated by episodes of hyperplasia and hypertrophy during myogenesis. Those processes of muscle growth are also produced in response to challenging conditions like an injury. Bones represent the attachment structure for skeletal muscle, and together, the musculoskeletal system has an essential endocrine and metabolic role. The mechanical and biochemical interactions between both tissues are crucial for its growth regulation and response to challenging conditions. After a muscle injury, a process of regeneration that involves inflammation, removal of damaged myofibers, cell migration, myogenesis, and remodeling is triggered. Furthermore, bone can activate different molecular pathways that can support the muscle to carry out this process correctly. In fish, muscle regeneration is still poorly unknown and in the present study, an injury was done into the left anterior epaxial muscle of seventy 15 g gilthead sea bream *(Sparus aurata*) juveniles to evaluate at days 0, 1, 2, 4, 8, 16 and 30 post-wound, the expression of several muscle and bone genes. Results showed an early upregulation of igf-2 and a downregulation of pax7 and igf-1, suggesting a possible IGF-2 induced satellite cell activation and proliferation. Other molecules from the GH-IGF axis did not appear to be involved in early regeneration. The inflammatory cytokines IL-6 and IL-15 were also upregulated the first day's post-injury validating an early inflammation of the tissue before myogenesis activation. However, the main proinflammatory cytokine IL-1β seemed not to be involved in this situation. Myogenic regulatory factors (MRFs) presented a pattern of expression that fitted well within myogenesis progression 16 days after the injury and followed the recovery of the igf-1 expression. Moreover, tnf-α was expressed together with the MRFs suggesting a possible role as a myokine. Also, caveolin-3, as well as myomaker and myomixer, followed MRFs expression as they are implicated in the later myotube fusion stage. Results from bone showed an upregulation of the expression of some genes within the first 8 days post-injury. Firstly, bmp2 and ctsk increased at 1 and 2 days respectively; then ogn1 and ostc peaked at day 4 while lately, runx2 and ogn2 showed a peak after 8 days, before the increase of MRFs expression. Finally, while the myogenic program occurs, ogn2 and bmp2 increased significantly, all indicating possible implications of these molecules in the regenerative process. Overall, the present model allows to study the sequential involvement of regulatory molecules, proposing their role during muscle regeneration, emphasizing the early function of IGF-2 related to proliferation activation and the bone genes bmp2 and ctsk, probably sending modulatory signals to muscle tissue, suggesting how bone could react to stimuli originated in the injured muscle to control myogenesis.

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LEPTIN SIGNALING PROMOTES EPIMORPHIC REGENERATION IN XENOPUS LAEVIS

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Leptin is a pro-Inflammatory cytokine hormone which is highly upregulated after injury in mammals and is one of the most highly upregulated mRNAs in *Xenopus* tadpole tail regeneration. We tested the hypothesis that leptin signaling mediates nutrition-dependent regeneration in *Xenopus laevis*, which are competent to regenerate entire appendages during the larval stage and slow regeneration with food restriction. In both limbs and tails, we showed leptin receptor is locally upregulated at the site of amputation and injection of leptin protein at the time of amputation increases the rate and quality of regeneration in food-restricted larvae. In limbs, leptin administration stimulates cell proliferation in the epidermis and blastema, and immunoneutralization of leptin slows regeneration. We used fluorescent in situ hybridization with immunohistochemistry to show leptin and leptin receptor mRNAs as well as leptin protein are expressed in and adjacent to the blastema of regenerating tails and limbs of *Xenopus laevis* tadpoles. We also show co-localization of leptin receptor mRNA with markers of developing blood vessels and nerves in the regenerating appendage tip. Using the *X. laevis* Flk1:GFP transgenic line, leptin treatment increases both the speed and total number of blood vessels in the regenerating tail. This work supports a role for leptin as a modulatory hormone that regulates appendage regeneration according to nutritive state through paracrine, endocrine, and neuroendocrine pathways, as well as a neurosecretory hormone in the context of limb development. This work also suggests that leptin has pleiotropic actions in epimorphic regeneration, including epidermal proliferation and wound healing, angiogenesis and neurogenesis, making it a strong candidate for therapeutics designed for regenerative medicine.

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THE SNAIL, THE SHARK AND THE WHALE": FROM EVOLUTION TO ENDOCRINE DISRUPTION

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Animal genomes encompass a network of gene families and pathways central for endocrine homeostasis. Importantly, an orchestrated set of these molecular components takes part in the response against toxicants – the so-called Chemical Defensome (CD). Yet, why some animal species are more sensitive than others to man-made chemicals remains elusive. In effect, animals exhibit conserved and divergent sensitivities to external stimuli. The contribution of evolutionary processes in the configuration of critical endocrine components underlying such dissimilar responses is far from fully assessed. Here, we investigate the evolution of two central molecular nodes of the CD: the Nuclear Receptors and the Flavin-containing Monooxygenase (FMOs) in Metazoa lineages. We combine extensive comparative genomics, phylogenetics, and functional assays to decipher the evolution of endocrine systems and their impact by man-made chemicals. We show the vital influence of mutation, gene duplication and gene loss at key gene families of the Chemical Defensome as powerful drivers of evolutionary change, with impacts at the endocrine disruption level. Our approach puts into context the role of evolution in endocrine disruption processes, which would be impossible to reveal with model species.

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DISCOVERY OF A PROLACTIN-LIKE IN LAMPREY REVEALS THE DIVERGENCE OF PROLACTIN AND GROWTH HORMONE IN JAWLESS VERTEBRATES

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Jawless fish (agnathans), one of the oldest groups of living vertebrates, provide a unique opportunity to study the origin and divergence of genes as well as the evolution of hormone function. We discovered for the first time in an agnathan a second member of the growth hormone (GH)-prolactin (PRL) family, which we characterized as prolactin-like (PRL-L) and propose a new model for early evolution of the GH-PRL family. At least two ancestral genes were present in ancestral vertebrates, with GH and PRL-L genes emerging in lampreys. A series of gene duplications, losses, and chromosomal rearrangements account for the various GH-PRL-family members in jawed vertebrates. Sea lamprey GH and PRL-L bind to distinct receptors with high affinity. A basal role of PRL-L was evidenced in osmoregulation of sea lamprey, suggesting the osmoregulatory role of PRL conserved throughout vertebrates.

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O65
CART DYNAMICS DURING CROAKING IN THE BRAIN OF ANURANS

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Croaking is a unique reproductive behaviour in amphibians that plays a major role in intra-species communication and mate evaluation. The mating call is advertisement of sexual maturity and mating readiness by the male frogs to attract receptive females. Coordination be-tween endocrine hormones, gonadal steroids and neuropeptides is essential to complete this process. Although, the role of hormones and gonadal steroids in inducing croaking behaviour is reported previously, the central mechanism of neuropeptides regulating it, is not studied. The role of cocaine- and amphetamine-regulated transcript (CART) peptide in repro-duction is specified in mammals, however, the co-relation of CART with the complex reproductive behaviour of anurans is not known. Therefore, this study is an attempt to unwind CART dynamics during croaking in the brain of two seasonally breeding frogs: *Microhyla nilphamariensis* and *Minerverya sahyadrensis*. The adult croaking and non-croaking male frogs were fixed on field at night in breeding season. We have observed a significant differ-ences in CART expression in anuran brain during croaking as compared to non-croaking. CART immuoreactivity in key reproductive areas of the brain such as Ventral Hypothalamus (NIV) and Anteroventral tegmentum (AV) increased during croaking in both the species of frogs. Whereas, in *M. nilphamariensis*, CART expression was higher in the Preoptic area (POA), Pineal (E) as well as Pituitary gland and in *M. sahyadrensis*, CART immunoreactive cells are more in the olfactory epithelium (OE). On the contrary, CART immunoreactivity in Raphe nucleus decreased in croaking males as compared to non-croaking males in both the species. Moreover, in *M. sahyadrensis*, CART immuoreactivity decreased in Medial septum (MS), Nucleus Accumbens (NAC), Amygdala (Am) and Torus semicircularis (TS) of croaking males than non-croaking males. Upregulation of CART expression in POA, NIV, AV, pineal and pituitary gland is ob-served during croaking, these regions are known to orchestrate the reproductive behavior in anurans. This indicates a probable involvement of CARTirgic system in reproductive behaviour of anurans.

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MATERNAL TRANSFER OF MICROPLASTICS IN THE YOLK OF LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) EMBRYOS AND THEIR CORRELATION WITH DEVELOPMENTAL IMPAIRMENT: EVIDENCE FROM AN ITALIAN PILOT STUDY.

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Microplastics (MPs) are ubiquitous anthropogenic contaminants that are a growing concern for marine environment and wildlife. Loggerhead turtle *C. caretta* is a worldwide symbol of awareness campaigns on marine litter contamination. Considering its long life, trophic position and mobility, this turtle can accumulate pollutants along the trophic chain and overlarge areas indifferent aquatic compartments. MPs accidentally ingested by loggerhead turtles have been usually associated with health status impairment and gastrointestinal damages in addition to impaired reproduction. However, a possible maternal transfer of MPs to the egg yolk and in turn to the embryo has not yet been proven. In this light, the aim of the present study was to investigate the occurrence of MPs in yolk and liver of unhatched embryos and to correlate their presence with embryo development impairment. To this purpose, 80 embryos that failed to hatch from two loggerhead sea turtle (*Caretta caretta*) nests (Rimigliano and Baratti) collected along the coast of the Tuscany region in August 2020, were analyzed. For each embryo biometric parameters including total and carapace length, total, yolk and liver weight were recorded in order to classify the embryonic developmental stages. Livers were divided in two portions: one (0,5 cm3) for histological analysis and the rest for MPs analysis. Histological analysis of liver was performed to quantify melanomacrophages (MMs) and lipids. Chemical digestion (KOH 10%), filtration and Raman spectroscopy analysis were performed separately on yolk and liver of each embryo to investigate the abundance, size, colour and polymer type of MPs. Results evidenced that: 1) different embryos growth rate and yolk consumption was found in the two nests here analyzed; 2) all yolks analyzed showed MPs (mean value=1,2 MPs/yolk for Baratti nest and 1,6 MPs/yolk for Rimigliano nest); 3) in the liver the abundance was found lower than yolk (mean value=1 MPs/yolk for Baratti nest and 0,2 MPs/yolk for Rimigliano nest); 4) the most abundant MPs polymers were Acrylonitrile butadiene styrene-ABS for Baratti and PVC for Rimigliano 5) MMs density correlate with the number of MPs retrieved in the liver. This pilot study evidenced for the first time in sea turtles, maternal transfer of MPs into the yolk, the absorption of MPs from yolk to liver during embryonic development, and the possible effects of MPs on yolk adsorption and in turn embryonic development.

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EVOLUTIONARY ANALYSIS OF TEMPERATURE RECEPTOR TRPV (TRANSIENT RECEPTOR POTENTIAL VANILLOID) FAMILY WITH A SPECIAL FOCUS ON "FISH"

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The IPCC reports that ocean surface temperature will increase by 1 to 3 °C over the next 80 years. Fish, as ectothermic organisms, are particularly vulnerable to temperature changes. From a phylogenetic point of view, "fish" are paraphyletic, with representatives in all major groups of vertebrates (cyclostomes, chondrichthyans, actinopterygians and sarcopterygians). The TRPV family of ion channels is involved in multiple sensory and physiological functions including thermosensing and temperature-dependent regulation. The objective of this study was to investigate the number, origin and evolution of TRPV among vertebrates, with a special focus on the impact of whole genome duplications (WGD), gene-specific duplications and gene losses in "fish".Gene searches, phylogenetic and syntenic analyses revealed a larger number of TRPV genes in vertebrates than currently assumed, with three additional TRPV types in the ancestor of gnathostomes, TRPV7, 8 and 9. Evolutionary scenario suggests that five TRPV types (TRPV1, 4, 5, 7, 8) existed in the vertebrate ancestor after the two rounds of WGD (1R and 2R) and one local gene duplication before the divergence of cyclostomes and gnathostomes. TRPV7 and 8 have been lost independently in various lineages but are still retained in some sarcopterygians including coelacanth, some tetrapods, and basal actinopterygians (Polypteridae). TRPV3 and 9 originated from local duplications of TRPV1 and are present in extant elasmobranchs, while TRPV9 was lost in the osteichthyan ancestor and TRPV3 in the actinopterygian ancestor. TRPV2 arose by local duplication of TRPV1 in the tetrapod ancestor. The coelacanth has retained the ancestral osteichthyan repertoire (TRPV1, 3, 4, 5, 7 and 8), and gained two additional duplicates of TRPV3. Duplications of TRPV5 occurred independently in various lineages such as chondrichthyans, anurans, sauropsids, mammals, Polypteridae and Esocidae. After the teleost-specific WGD (3R), TRPV1a was retained in all teleosts and TRPV1b in some, whereas a single paralog was retained for TRPV4 and 5. TRPV1a and b were further duplicated in Gadidae. The salmonid-specific WGD (4R) duplicated TRPV1a, 4, and 5 leading to a total of six TRPV genes. This study provides a comprehensive evolutionary scenario for the vertebrate TRPV family, revealing additional TRPV types and proposing a classification of TRPV across vertebrates including "fish".

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THE UNIQUE C-MANNOSYLATED GLYCOPEPTIDE ADIPOKINETIC HORMONE OF THE INDIAN STICK INSECT

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This is a review of a long, personal, scientific journey of 43 years that I began in 1979 with a simple report that a corpus cardiacum (CC) extract of *Carausius morosus* has a hyperlipaemic effect in locusts and a hyperglycaemic effect in cockroaches. In the following years we found that the extract was also active in the stick insect itself but caused mild hyperglycaemia best at the end of the 6th instar and only when these larvae were ligated behind the head. Via HPLC two fractions were separated with biological activity. The more hydrophobic one was easily identified as a decapeptide. In 1982 mass spectrometry of the less hydrophobic fraction pointed to a molecule with the same amino acid sequence as the more hydrophobic one but in addition with a unique bond between trypophan and a sugar that was not the usual and only known O- or N-glycosylation. After mass rearing and collection of a few thousand CCs nuclear magnetic resonance experiments in 2008 clearly showed a mannose attached to tryptophan forming a unique C bond. It still took another 13 years until the C-mannosylated glycopeptide could be synthesized via an automated process and mg amounts were available for further experimentation. We could then compare by HPLC the natural with the synthetic compound and show that the correct anomer had been synthesized, and proceed with using this synthetic authentic peptide for physico-chemical and biological experiments.

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O69

DISTRIBUTION AND FUNCTIONAL INSIGHT INTO ADIPOKINETIC HORMONE/CORAZONIN-RELATED PEPTIDE IN THE HUMAN DISEASE VECTOR, *AEDES AEGYPTI*

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The adipokinetic hormone/corazonin-related peptide (ACP) is an insect neuropeptide structurally intermediate between corazonin (CRZ) and adipokinetic hormone (AKH). While the AKH and CRZ signaling systems are widely known for their respective roles in the mobilization of energy substrates and stress responses, the main role of ACP and its receptor (ACPR) remains unclear in most arthropods. The current study aimed to localize the distribution of ACP in the nervous system and provide insight into its physiological roles in the disease vector mosquito, *Aedes aegypti*. Immunohistochemical analysis and fluorescence in situ hybridization revealed expression of the ACP peptide and transcript within a number of cells in the central nervous system, including two pairs of laterally positioned neurons in the protocerebrum of the brain and a small number of ventrally localized neurons within the pro- and mesothoracic regions of the fused thoracic ganglia. Further, extensive ACP-immunoreactive axonal projections with prominent blebs and varicosities were observed traversing the abdominal ganglia. Considering the significant enrichment of ACPR expression within the abdominal ganglia of adult *A. aegypti* mosquitoes as determined in our previous work, the current results indicate that ACP may function as a neurotransmitter and/or neuromodulator facilitating communication between the brain and posterior regions of the nervous system. In an effort to elucidate a functional role for ACP signaling, biochemical measurement of energy nutrients in female mosquitoes revealed a reduction in abdominal glycogen stores in response to ACP that matched the actions of AKH, but interestingly, a corresponding hypertrehalosaemic effect was only found in response to AKH since ACP did not influence circulating carbohydrate levels. Comparatively, in male mosquitoes, both ACP and AKH led to a significant increase in haemolymph carbohydrate levels while both peptides had no influence on glycogen stores. Neither ACP nor AKH influenced circulating or stored lipid levels in both male and female mosquitoes. These results reveal ACP signaling in mosquitoes may have complex sex-specific actions and ongoing research aims to better understand the functional role of this understudied neuropeptide.

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O70

CHROMACTIVATING NEUROPEPTIDES IN CRABS: NEUROARCHITECTURES, RECEPTORS, ESTABLISHED AND NOVEL FUNCTIONS.

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The chromactivating (colour change) hormones, red pigment concentrating hormone (RPCH) and pigment dispersing hormones (PDH) were amongst the first to be structurally identified in invertebrates, over 40 years ago. Recently, there has been a renewed interest in these neuropeptides, with the realization that RPCH belongs to evolutionarily ancient neuropeptide group encompassing the adipokinetic hormones (AKHs), corazonins (CRZs) and adipokinetic hormone/corazonin-related peptides (ACPs) and these have arisen by gene duplication from a GnRH-like ancestor, at the dawn of bilaterian evolution. Similarly, PDH-like molecules (the pigment dispersing factors, PDFs) have a wide distribution in the ecdysozoa, where they play (for example) important roles as outputs in the circadian clockwork. Here we review our recent work in reappraising the architecture of RPCH, CRZ and PDH neurons in our crab model, *Carcinus maenas*, together with functional deorphaning of several GPCRs. These findings, taken together with data on expression of relevant transcripts derived from several tissue specific transcriptomes and from bioassays, suggest novel and rather unexpected functions for several of these neuropeptides, for which preliminary results are presented.

O71

CHARACTERIZATION OF INVERTEBRATE GONADOTROPIN-RELEASING HORMONE/CORAZONIN IN THE WELL-ESTABLISHED MOLLUSCAN MODEL SPECIES *LYMNAEA STAGNALIS*: EVOLUTIONARY AND FUNCTIONAL IMPLICATIONS

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In the last years, our interpretation of the origin and function of the gonadotropin-releasing hormone (GnRH) neuropeptide superfamily has changed substantially. In vertebrates, GnRH peptide is the central mediator of reproduction. Homologous peptides have previously also been identified in molluscan species. However, emerging evidence suggests that these molecules might serve diverse regulatory functions and proposes to consider them as corazonin (CRZ). Clearly, a more global understanding requires further exploration of species-specific functions and structure of invGnRH/CRZ peptides. Towards this goal, we first identified the full-length cDNA of the invGnRH/CRZ preprohormone in the well-established invertebrate model species, the great pond snail *Lymnaea stagnalis* and characterized the transcript and active peptide (termed ly-GnRH/CRZ) distribution in the central nervous system (CNS) and some peripheral organs. Based on the anatomical distribution, we predicted that ly-GnRH/CRZ might regulate feeding, locomotion, heart control, and reproduction. Next, we confirmed the presence of the deduced active peptide from the CNS with mass spectometry. Injection of sexually mature specimens with synthetic active peptide had an inhibitory effect on locomotion and an acceleratory effect on egg-laying, but had no effect on feeding. The predicted modulatory effect of ly-GnRH/CRZ was supported by its identified co-localization with serotonin on the surface of the heart atria. Lastly, we demonstrated not only the presence of ly-GnRH/CRZ in the penial complex but also that ly-GnRH/CRZ-containing neurons project to the efferent penis nerve, suggesting ly-GnRH/CRZ may directly modulate the motor output of this peripheral tissue. Overall, our findings strongly support that peptides originally termed molluscan GnRH serve diverse functions and are more related to CRZs. These results contribute to the understanding of the GnRH superfamily and, more broadly, disciplines such as comparative endocrinology and neurobiology.

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O72

AKH SIGNALING IN THE PROTHORACIC GLAND ALTERS DEVELOPMENT IN RESPONSE TO LARVAL NUTRITIONAL STRESS.

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Metabolism, growth and development are intrinsically linked, and their coordination is dependent upon inter-organ communication mediated by hormones and neuropeptides. In *Drosophila melanogaster*, the corpora cardiaca (CC) influences metabolic homeostasis through the catabolic activity of the glucagon-like adipokinetic hormone (AKH), but its role – if any – in development is unknown. Here we demonstrate that AKH abundance in the CC is negatively regulated by a cGMP-dependent protein kinase (PKG) encoded by dg2. Transcriptional silencing of dg2 during discrete periods of life produces alternative developmental outcomes for adult metabolism and behaviour. Larval malnutrition strongly influences these adult traits through AKH-dependent developmental delays and lethality. These developmental effects are phenocopied when AKH signaling is disrupted in the prothoracic gland (PG) when AkhR expression is reduced. Ecdysteroid secretion is actively regulated by calcium-mediated vesicle exocytosis in the PG, and we demonstrate that AKH signaling stimulates intracellular calcium activity within the PG. Our work identified a novel CC-PG paracrine signaling pathway through which the CC communicates metabolic information to the PG through dg2-regulated AKH signaling. This pathway provides a means whereby juvenile nutritional stress can alter developmental trajectories into adulthood.

O73
(ANTIVIRAL) RNAI PATHWAYS IN INSECTS

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RNA interference (RNAi) is considered the most efficient and broadly-acting antiviral immune mechanism in insects. It is a post-transcriptional gene silencing mechanism triggered by double-stranded RNA (dsRNA) molecules, produced during the replicative cycle of viruses. In short, (viral) dsRNA is recognized within the cytoplasm and processed into sRNAs. These sRNAs are incorporated into an RNA-induced silencing complex (RISC), which is then directed to the RNA target via Watson-Crick base pairing. Subsequently, the effector protein of RISC, namely an Argonaute protein, acts to inhibit or degrade the target (viral) RNA. While RNAi is naturally activated as an antiviral defense mechanism, it can also be triggered by artificial delivery of gene-specific long dsRNA, resulting in specific endogenous gene silencing. This is called RNAi technology, a diverse technique with many applications both for research and commercial purposes. For example, RNAi-based approaches have been proposed to protect beneficial insects from harmful viral infections as well as to contribute to novel strategies of selective control of agricultural insect pests. In addition, RNAi technology is widely used as a loss-of-function research tool, which is particularly important for reverse genetic studies in non-model organisms. In this work we investigated the nature of the RNAi signal in insects upon viral infection and upon exposure to artificially delivered double stranded RNA, an important pathogen associated molecular pattern for viral infection. By focusing on insect species belonging to different orders (mainly Lepidoptera, Coleoptera and Orthoptera), we highlight species-specific differences. Moreover, we set to uncover mechanisms involved in the intercellular spread of the RNAi signal. Our results unravel interspecies variability of (antiviral) RNAi mechanisms in the class Insecta, as well as contribute to the understanding of insect systemic RNAi. Besides contributing to answer important fundamental questions, understanding RNAi mechanisms will stimulate its use as a research tool and as a biotechnological tool to protect beneficials and control pest species.

O74
IMPACT OF LIGHT POLLUTION ON MALE REPRODUCTIVE SUCCESS IN JAPANESE MEDAKA

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In many fish species, males form a dominance hierarchy that influences their reproductive success. This male reproductive competition can also be observed in the model fish, Japanese medaka (*Oryzias latipes*). However, the environmental factors and physiological mechanisms that determine the reproductive success of dominant males remain largely unexplored. Exposure to artificial light at night is of particular interest as light pollution disrupts wildlife that have optimized their reproduction according to diurnal and seasonal light rhythms. This study aims to investigate the effect of light regime on the fitness of male fish within a dominance hierarchy, and the underlying physiological mechanisms involved. When two male medaka are paired with a female, one male establishes dominance and guards the female, limiting access of the subordinate male to the female. We observed that dominant males are significantly more aggressive, remain closer to the female, and spend ten times longer spawning than subordinates. By using males with different genotypes, we determined the paternity of the progeny by genotyping or screening embryos. We found that dominants and subordinates are equally successful at fertilizing eggs in normal light conditions (14h light/10h dark). However, when exposed to light at night (14h light/10h dim light), dominant males fertilize more eggs. We then investigated whether this change was due to behavioral or physiological modifications. We measured behavioral parameters, and collected brains and pituitaries for qPCR analysis, blood samples for sex steroid ELISAs, semen for sperm quality analysis, and testes to determine gonadosomatic index. We found evidence that light pollution influences the brain- pituitary-gonad axis and fish fitness.

O75

PUTATIVE ROLE OF THE CEREBELLUM AND THE VAGAL LOBE AS OSCILLATORS IN GOLDFISH

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The circadian system adjusts many physiological and behavioral processes to the daily environmental changes. A non-hierarchical organization of this circadian system has been suggested in fish, with a network of functional oscillators widely distributed throughout the body. In fact, functional oscillators have been described in periphery (interrenal tissue, liver) and brain (hypothalamus, optic tectum) in some teleost, but some brain areas remain unexplored. The aim of this work was to investigate the cerebellum and the vagal lobe as putative oscillators in goldfish (*Carassius auratus*). For this purpose, firstly we study the influence of the environmental synchronizers light/dark cycle and feeding time on the daily variations on clock genes expression in these brain areas. The expression of the studied clock genes (per1a, per2a, per3, bmal1a, clock1a and rev-erb-a) show significant daily rhythms (ANOVA, cosinor) with similar profiles in both tissues, and amplitudes and acrophases depending on both light/dark conditions and feeding schedule zeitgebers. On the one hand, in the absence of light/dark cycle (24h-darkness), the rhythmic expression of per1a in the cerebellum and per1a and per2a in the vagal lobe is lost. On the other hand, in fish kept at random feeding (without feeding time zeitgeber), most clock genes remained unaltered except per2a which lost the daily rhythmicity in vagal lobe. Secondly, the effect of the temperature as zeitgeber and its interaction with feeding/fasting conditions on clock genes expression was investigated. We found a differential and tissue specific response of each clock gene to the different tested temperatures, but no significant effect was induced by fasting. Overall, our results support the role of the cerebellum and the vagal lobe as new brain oscillators in goldfish.

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O76

IMPAIRED LEPTIN SIGNALING DISRUPTS OOCYTE MATURATION AND OVULATION IN FEMALE ZEBRAFISH

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Reproduction is an energetically costly event across vertebrates and tightly linked to the nutritional status and the amount of energy reserves. In mammals, the adipocyte-derived hormone leptin is considered as a link between energy homeostasis and reproduction. However, its role in teleosts is still unclear. The aim of the present study was to investigate the possible role of leptin in the regulation of reproduction in zebrafish, using a loss of function leptin receptor strain (lepr sa12953). Adult wild-type and homozygote lepr mutant zebrafish were mated pairwise: wild-type males with wild-type females, mutant males with wild-type females and wild-type males with mutant females. The fish were mated frequently and the spawning events were recorded; the laid eggs were counted and the fertilization rate was calculated. Tissues of interest (brain, pituitary, testis/ovary and liver) were sampled for gene expression analysis. Gonadal samples were taken for histological analysis. Fully-grown follicles were isolated from wild-type and lepr mutant ovaries for transcriptomic analysis. Male lepr mutant zebrafish were equally fertile to wild-types. However, lepr mutant females had severe reproductive deficiencies. Follicles from all developmental stages were identified in the ovaries from both genotypes, without significant differences in their ratios. However, oocyte maturation and ovulation were disrupted in lepr mutants, resulting in fewer ovulated eggs with low fertilization rate. Pituitary transcripts of luteinizing hormone beta (lhb) were significantly lower in mutant females compared to wild-types. Analysis of candidate genes involved in steroidogenesis, oocyte maturation and ovulation, which are known to be regulated by LH signaling, were also differentially expressed in the mutant ovaries. Interestingly, the subfertility in the mutant females could be partially restored by administration of human chorionic gonadotropin. Lastly, transcriptomic analysis of isolated fully-grown follicles linked the reproductive deficiencies in the mutants to the suppression of essential metabolic pathways for oocyte maturation and ovulation, such as estrogen regulation, ribosome biogenesis, mRNA translation and lipid metabolism. In conclusion, impaired leptin signaling had strong effect in the reproductive physiology of female zebrafish, but not in males. Overall, our results suggest that the early stages of folliculogenesis are not affected by the lepr deficiency, but leptin might be essential in later steps, such as during oocyte maturation and ovulation.

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O77

SEARCHING FOR THE RELATIONSHIP BETWEEN BODY SIZE AND MATURITY IN FEMALE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*

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The European sea bass is a teleost fish with high interest in aquaculture. A female phenotype exhibiting advanced vitellogenesis in prepubertal fish has been characterized in this species. Although precocious females usually show higher weight than that of the immature ones, a large variation in average body size of advanced (32%) and immature (20%) prepubertal females has been observed. Fish body size variations restricts the aquaculture production as it results in both size dispersion and reduced biomass, a situation that farmers wish to avoid. Also, maturity is often considered a problem, as it negatively affects growth, thus reducing the final yield. Therefore, while the selection of rapid-growth fish is highly attractive for the aquaculture sector, it is also common to select against early-maturing fish. In this work, four female phenotypes were identified at 23 and 34 months of age including small-size females (S) and big-size females (B) which might have both very reduced gonadal growth (S) and significant increase of gonadal growth (B). Thus, the morphological characteristics including body weight and gonadosomatic index determined those phenotypes as follow: SS, BS, SB and BB. Growth history of these four female phenotypes was evaluated over their second and third year of life. Changes in the plasma levels of follicle-stimulating hormone (Fsh), 17b-estradiol (E2) and vitellogenin (Vtg) were also individually determined. Preliminary results demonstrated that BS and BB females were heavier and larger than SS and SB over the second and third year of age, while SB and BB fish had significantly higher GSI than those of SS and BS. On the other hand, SB and BB females had higher levels of Fsh, E2 and Vtg than those of the other groups. Currently, circulating levels of insulin-like growth factor-1 (Igf-1) are being analysed and its pivotal role in puberty-related events is being explored. A comparison of the liver and muscle transcriptome profiles of these four female phenotypes at 23 and 34 months old will be performed in order to identify those major genes regulating sea bass body size at various ages and according to their ovary growth. These findings may provide important information for a better accommodation and benefit in aquaculture production and fish breeding.

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O78

EXPRESSION ANALYSIS OF RECEPTORS FOR GLYCOPROTEIN HORMONES AND OF THE THYROSTIMULIN DURING SPERMATOGENESIS IN THE CATSHARK, *SCYLIORHINUS CANICULA*.

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Pituitary glycoprotein hormones (GpHs), made of a common α subunit (GPA) and of a specific β subunit (GPB), regulate peripheral endocrine glands through specific receptors (GpHRs). Five different functional GpHs have been identified in Gnathostomes including follicle-stimuling hormone (FSH), luteinizing hormone (LH), chorionic hormone (CG), thyroid-stimuling hormone (TSH) and thyrostimulin (A2B5). Classically, LH and FSH modulate spermatogenesis through regulation of germ, leydig and Sertoli cells proliferations and steroidogenesis. More recently, a potential paracrine function of A2B5 in gonads has emerged. Our aim is to explore the evolution of the endocrine versus paracrine regulation of spermatogenesis by considering Chondrichthyans. They have diverged from bony vertebrates around 421-462 million years ago and they would be the first Gnathostomes with differentiated GpH and GpHR since in Cyclostomes only one GpH and one GpHR have been functionally characterized. Thanks to the S.canicula genome databank access, sequences of hormones and receptors have been retrieved and structural and phylogenetic analyses completed. The diametrical testicular structure and cystic spermatogenesis of catshark allow to dissociate spermatogonial, spermatocytes, early spermatid and late spermatid zones where both mRNA and protein expressions have been explored by RT-PCR and heterologous immunohistochemistry, respectively. Results shown that 3D-structures involved in receptor/ligand interactions and signal transduction are preserved between catshark, elephant shark and human for FSHR and LHR but for TSHR, they are more similar between catshark and human than for elephant shark. FSHR and LHR were immunolocalized in somatic-precursors and Sertoli cells, respectively. Fshr and lhr expression decreased during spermatogenesis with an increase of lhr in the late spermatid zone. Tshr and gpb5 expressions strongly increased during spermatogenesis with a sertolian TSHR localisation. These results agree with major FSH implication at early stages of spermatogenesis and LH in spermiogenesis and spermiation as observed in teleost fishes. Our study illustrated, for the first time in a Chondrichthyan, GpHRs expressions during spermatogenesis and highlight the thyrostimulin as a potential paracrine regulator of the testis. Study will be completed by in situ hybridization and proteome characterization of testicular cell cultures in response to GpHs.

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O79

A COMPARISON OF GROWTH PERFORMANCE AND HORMONE PRODUCTION OF PRECOCIOUS AND IMMATURE PREPUBERTAL FEMALE SEA BASS, *DICENTRARCHUS LABRAX*

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In aquaculture production, fish are overfed being able to achieve greater growth in comparison to those in the wild. This increased growth is sometimes counterproductive because some animals reach early maturation. Although precocity in male sea bass has been widely reported, the case in females is poorly documented. In this work, the incidence of precocious females was evaluated in a total of seven batches. To this aim, fish were individually tagged and females histologically identified when animals were terminally sampled over their second year of age. Females could be divided into four distinct groups: females with perinucleolar oocytes, cortical alveoli, oocytes at early vitellogenesis and late vitellogenesis (VTGL). Those females that reached a high gonadosomatic index (4.45%) and showed VTGL at 2 years of age were designed as precocious fish. These findings showed that 18.1% of female fish were precocious, although its incidence varied among sea bass batches from 0 to 43.6%. Body size of precocious females was usually higher than that of immature ones from early stages in development, evidencing that precocious females grew up to 15% more in weight and 2% more in fork length that their counterparts that remained immature during their second annual cycle of life. Precocious female sea bass showed a general increase of circulating plasma levels of insulin-like growth factor-1 (Igf-1) during the late spring/early summer season (May-September), while those of 17-beta estradiol (E2) and vitellogenin (Vtg) increased throughout the tentative ovary growth period (November-February) and with higher plasma levels of follicle-stimulating hormone (Fsh). A principal component analysis (PCA) of all the factors measured showed that > 80% of total variance was explained by the two first components. Accordingly, the percentage of variation explained by each factor considered in the PCA for the principal components 1 and 2, were 23% for weight and plasma levels of E2, 21% for Vtg levels and 19% and 14% for Igf-1 and Fsh levels, respectively. These results provide evidence for the possible interplay among body weight and these hormonal factors during oocyte growth in this species and demonstrate that dynamics of growth in females has some interesting features to be investigated in order to face its profitability in aquaculture production.

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O80
CAN FISH BE ARTIFICIALLY EQUIPPED WITH A SECONDARY FUNCTIONAL GONAD?

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Controlled aquaculture is a breakthrough in aquaculture sustainability of any aquatic species. Apparently, precarious, and costly parent fish maintenance, inferior gamete quality and dwindling reproductive success are still the major challenges for complete aquaculture. Therefore, producing superior gametes from a fewer number of fish will reduce the seed-associate risks and cost. Notably, chub mackerel (*Scomber japonicus*) is a commercially important species, and its artificial seed production and aquaculture has been established by our research group. So, in this study, we try to create allogenic ectopic gonad in the host by intramuscular transplantation of gonadal tissues and test the possibility of producing functional gamete and fertilized embryos from the implanted gonad. For this research, we used Q-saba, a cultured strain of chub mackerel, developed and marketed by our research group. Adult fish were anesthetized and partial gonadectomy were performed and the incision was sutured, and ligated. The collected gonads were shaped into 1.5 mm squares and transplanted into the left body dorsal or ventral muscle. Sampling was performed at different time points, and analysis in histological. Artificial fertilization followed by hatchability measurement were used to test the functionality of the gametes at 6 months. Hereafter, the actual gonad and implanted gonadal tissues will be considered as primary and secondary gonad, respectively. We observed a sex-biased effect of transplantation on gonad development and maturation, especially from 1 month. Specifically, 1 week post transplantation, we could observe regressing gonadal tissue in the implantation site in both sex groups. Though primary gonad showed a normal course of development, but at later stages, gonadal reconstruction was not observed in the implanted areas in female. Contrastingly, in males at secondary gonad site, progressive gonadal reconstitution and size increment was observed at later stages. Histological analysis confirmed at par spermatogenesis in the secondary gonad. Finally, to test the functionality of the sperm, we chopped the secondary gonad, collected the sperm, artificially fertilized freshly collected mature female gametes and calculated hatchability. Interestingly, we could obtain viable hatchling from secondary gonads. Both non-transplanted male and primary gonads of transplanted male were used as experimental control. Cumulatively, our data suggests that secondary gonad has the possibility to produce functional gametes in chub mackerel. However, further studies are necessary to confirm the cellular and molecular differences in gamete development between primary and secondary gonads.

O81

UNVEILING THE POTENTIAL OF PROBIOTICS TO MITIGATE THE TOXIC EFFECTS OF PERFLUOROOCTANOIC ACID ON ZEBRAFISH DEVELOPMENT

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Perfluorooctanoic acid (PFOA) is a synthetic chemical compound, extremely resistant towards thermal, chemical, and biological degradation. This fact, together with the widespread use of PFOA in both industrial and household products, which are usually discharged into the sewer system, makes the aquatic living organisms more vulnerable to its toxic effects. In fact, it has been demonstrated that exposure of zebrafish to PFOA during embryo development leads to alterations of the innate immune system, heartbeat rate, and locomotor behaviour. Despite the well-known potential of probiotics to improve the health status of living organisms, the information about their capacity to counteract the toxic effects of emerging contaminants is still scarce. Therefore, this work aims to determine the role of probiotics in the mitigation of the effects derived from PFOA exposure during early development. For this purpose, zebrafish larvae have been exposed to two different doses of PFOA (50 and 100 mg/L) from hatching to 21 dpf , and half of the batches dietary has been supplemented with Bacillus subtilis natto at a final concentration of 107 CFU/larvae/day. The morphometric results show that the exposure to both doses of PFOA decreases the standard length of the 21 dpf larva but leads to an increase in the head length instead. The probiotic supplementation improves the total growth of non-treated larva and returns the body and head length of PFOA-exposed larvae back to the control values. Regarding the development, exposure to PFOA, especially to 50 mg/L, dramatically increases the rate of skeletal malformations and eyes size of the 21 dpf larva. The probiotic treatment is able to counteract the effects of the toxicant, although to a lower extent for those caused by the lowest and most harmful dose. As shown by the results, the probiotic treatment seems to partially antagonize the deleterious effects of PFOA; however, the molecular mechanisms underlying such activity have yet to be elucidated.

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O82

REANALYSIS OF GONADAL TRANSCRIPTOME USING SEX MARKERS REVEALS NEW GENES INVOLVED IN MALE SEX DIFFERENTIATION OF SIBERIAN STURGEONS

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Genes that control sex differentiation in Siberian sturgeons are under study. Previous works allowed to identify several genes activated in future female gonads but failed to identify genes activated in future male gonads. The report of a sex marker at DNAg level allowed to clarify that cyp19a1, foxl2 and hsd17b1 are undoubtedly activated in gonads of future females (see abstract of Lasalle et al), and recently we discovered that gsdf was activated in gonads of future males (see abstract of Benech-Correa et al). Using these sex markers, we selected and studied the transcriptome of gonads of two future females versus four future males during the molecular sex differentiation period of the species (5-6 months). The number of contigs up-regulated in gonads of future males were 441 while a huge number of contigs (n= 90661) do not show significant differences between sexes. Many genes associated in the literature to the testis sex differentiation in fish (amh, dmrt1, gsdf, sox9, amhr2, sox3, sox13) and vertebrates (dax1, dhh, fgf9, fog2, igfr1, ir, irr, pdrfra, pdgfrb, pdgfb, pgd2r) has been searched but only the gsdf resulted activated in male gonads. Androgen related genes (star, sf1, cyp11a1, hsd3b, cyp17a1, cyp17a2, hsd17b3, hsd11b, cyp11c, srd5a1, srd5a2, srd5a3, ar) were not activated in males. Germ cell markers (nanos1, nanos2, notch1, notch2, piwil2, ddx4) were also not activated. Among the genes up-regulated and annotated in NCBI we found genes described as related with or essential for testis, spermatogenesis and sperm development that were not previously described for sturgeons during sex differentiation period. These genes were spata17 (spermatogenesis-associated protein 17), spata18 (spermatogenesis-associated protein 18), tex43 (testis-expressed sequence 43 protein), theg (theg spermatid protein), tepp (testis, prostate and placenta-expressed protein), cfap91 (cilia and flagella associated protein 91), cfap44 (cilia and flagella associated protein 44), dlec1 (cilia and flagella associated protein 81). The Gene Ontology of genes up-regulated in males shows that 10 of 14 GO representing the biological process (p-adjusted < 0.05) are related with the cytoskeletal microtubule organization, a process known to be key for male fertility, Sertoli cell modulation, and male germ cell division and differentiation. The main results obtained for future male gonads of Siberian sturgeons at the stage studied were: 1.- gsdf is the only gene described as a male promoter that resulted activated; 2.- there are no signs of specific androgen synthesis; 3.-new genes probably important for testis differentiation of the species were identified; 4.- the up-regulation of genes involved in the organization of cytoskeletal microtubules seems to be essential for testis development. In summary, sexual differentiation at the molecular level appears to be fully developed in future males at 5-6 months and independent of a specific androgen synthesis. One candidate for testis control could be the gsdf but several other genes could be essential at this stage according to the o the literature.

O83

TRANSCRIPTOME ANALYSIS OF ENDOCRINE GENES DURING SEA BREAM (*S. AURATA*) AND SEA BASS (*D. LABRAX*) LARVAL DEVELOPMENT IN HATCHERY CONDITION

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The larval stage in fish, especially the transition to the juvenile known as metamorphosis, is a critical period during which many morphophysiological characteristics develop. Overall, the detailed molecular events and endocrine factors that orchestrate fish metamorphosis are still unclear, although the importance of thyroid hormones (TH) is recognised especially in the asymmetric flatfish. In the present study, symmetric larvae of sea bream and sea bass were sampled from commercial hatcheries with different geographical locations and significant changes in endocrine-associated genes during metamorphosis were analysed. Sea bream transcriptomes were generated from larvae at flexion (avg. of 4.6 mg) and larvae at mid-metamorphosis with different weights (avg. of 16.5 mg and 41.8 mg), and sea bass larvae transcriptomes were created from larvae at mid-metamorphosis with different weight (25.1 mg and 44.2 mg). Endocrine-associated genes were isolated from sea bream (n = 258) and sea bass (n= 289) genomes and were used to screen the transcriptomes generated for each species. Three differential gene expression (DGE) comparisons were performed in *limma*. In DGE I, the transcriptome of sea bream larvae at flexion versus larvae at mid-metamorphosis was analysed, in DGEII lower average weight/age were compared to higher average weight/age sea bream larvae at mid-metamorphosis, and in DGE III lower average weight/age were compared to higher average weight/age sea bass larvae at mid-metamorphosis. Principal Components Analysis of 32 endocrine-associated genes suggested a separation of endocrine gene expression based on developmental stage in both species. Among the most expressed endocrine genes 32 were common in metamorphosing sea bream and sea bass. DGE I identified *igfbp1*, *insm1a*, *insm1b*, *ghrb*, *sstr-l*, *dio3* as up-regulated and *dio2*, *dio1*, *sst2-l* as down-regulated in sea bream larvae at flexion compared to mid-metamorphosis. In small versus large sea bream larvae at mid-metamorphosis insm1a and *ghr−l*, were upregulated and in small versus large sea bass, insm1a, *igfbp1a*, *tg*, *trhde*, *insm2* were up-regulated. Overall, the expression of the few endocrine-associated genes changed *significantly* during metamorphosis that could be assigned to three gene clusters. One was a thyroid axis gene cluster that corroborates what is already known about endocrine regulation of fish metamorphosis. The other two gene clusters were IGF binding proteins and neuroendocrine cell differentiation, highlighting other endocrine processes potentially with an important role during metamorphosis.

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O84

TRANSCRIPTOMICS POINTS TO AN ASSOCIATION BETWEEN THE HPT-AXIS AND IMMUNE SYSTEM MATURATION BEFORE AND DURING *SENEGALENSIS SOLE* LARVAE METAMORPHOSIS

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Free-living fish larvae are continuously challenged by environmental conditions and a rich microbiota, for this reason, studies of the ontogeny and maturation of the immune system are of high interest. In the present study flatfish, which have easy-to-identify larvae to juvenile metamorphosis were used to characterize immune system maturation. Since metamorphosis is a thyroid hormone (TH) regulated process the potential involvement of these hormones in the acquisition of the immune repertoire was studied. Gene expression profiles were investigated using RNA-seq libraries of larvae in premetamorphosis (7 days post-hatch (dph) and 12 dph, S0), metamorphosis (14 dph, S1, 15 dph, S2 and 16 dph, S3) and post metamorphosis (20 dph, S4). Differentially expressed genes (DEGs) were identified using DEgenesHunter. Data were processed and grouped by expression pattern using a time-series approach as defined in MasigPro. A total of 8 583 DEGs were identified between larvae at 7 dph and 12 dph (S0) indicating a massive gene-oriented developmental process even before the onset of eye migration. As metamorphosis progressed, the number of DEGs between S0 and S2 or S3 was 192 and 327, respectively. On completion of eye migration (S4), 919 DEGs were identified when compared with S0. To investigate the co-expression of immune and thyroid axis-related genes nine clusters with rsq = 0.7 were analysed. Cluster 1, 4, 6 contained genes with a similar expression pattern namely, a gradual decline in gene expression from 7 dph towards metamorphosis. Clusters 2, 5 and 9 increased exponentially in expression from 7dph to metamorphosis and cluster 3 contained genes with a gradual increase in expression during larvae development. Genes of the hypothalamic-pituitary-thyroid (HPT) axis belonged to cluster 4 (hypothalamic-pituitary regulation) and cluster 5 contained genes associated with TH production, activation, and activity. Immune-related genes were grouped in these two clusters and cluster 6. The results showed concomitant expression of thyroid and hemopoiesis, lymphoid organs development and immune mechanisms like chemotaxis and phagocytosis-related genes. The results indicate that immune system maturation was linked to metamorphosis and supports a role for thyroid hormones in this process.

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O85

SYSTEMATIC EVIDENCE MAP OF THE EFFECT OF ENDOCRINE DISRUPTING CHEMICALS ON THYROID HORMONE MEASUREMENTS IN MAMMALS

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The Hypothalamic Pituitary Thyroid (HPT) axis is responsible for maintaining correct circulating levels of thyroid hormone (TH) in the body. The HPT axis is thought of as a classic endocrine negative feedback loop: when there is an increase of thyroxine (T4) in the blood, there is a decrease of thyroid stimulating hormone (TSH) production in the hypothalamus and vice versa. The TH system is sensitive to perturbation by endocrine disrupting chemicals (EDCs), which can cause alteration of the TH levels inducing hypo- or hyperthyroidism. However, some EDCs leading to decreased T4 levels do not produce the successive increase of TSH. The reasons for these "unusual" patterns are unclear. The aim of the present work was to obtain a comprehensive picture of patterns in the TH-axis, triggered by different kinds of EDCs. To meet this, we conducted a systematic evidence mapping of the literature with experimental exposures and measurements of T4/T3 and TSH in rodent models. After the systematic search of scientific literature, we obtained a total of 25,106 studies, of which 238 were analysed. Data regarding TH levels were retrieved for rodent offspring exposed to EDCs during prenatal or/and postnatal periods. Our results show that the EDCs analysed here decrease T4 levels in most of the cases, however, this depletion of T4 was followed by different TSH patterns: increased, decreased or unchanged levels. In addition, the studies outlined thyroidal histopathological lesions, deregulated mRNA abundances and protein markers for the TH-axis and neurological adverse outcomes in rodent offspring exposed to the EDCs. In conclusion, our systematic approach shows that the idealized view of the HPT axis (increase T4 and decrease TSH) is not supported by the evidence and therefore, how these divergent patterns observed relate to the endocrine modes of action (MoA) need to be fully deciphered for TH System Disrupting Chemicals (THSDC). Future work will try to align the mechanisms underlying these dissimilar MoA and the TH-system disruption and whether or not this leads to developmental adverse outcomes in exposed offspring.

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O86
NEW ENDPOINTS FOR THYROID HORMONE SYSTEM DISRUPTOR TESTING WITH FISH

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Major parts of early vertebrate development are regulated by thyroid hormones (THs) and can therefore be disrupted by environmental pollutants that interact with the TH system (TH system disrupting chemicals, THSDCs). Currently established test systems for environmental assessment of THSDCs use amphibian metamorphosis as a sensitive apical endpoint. For any other endocrine modality, test systems with fish are preferably used. The lack of thyroid-sensitive endpoints in fish tests has been identified as serious gap in the currently available test battery for endocrine disruptor testing. We aim to close this gap by establishing thyroid-sensitive endpoints in fish for implementation into existing fish TGs like the TGs 210, 234 or 236. We performed an extensive literature research to evaluate the impact of THSDCs on fish and identify developmental processes that could be disrupted by THSDCs. Based on the results, combined with data from amphibian tests, we selected a set of different model THSDCs to perform exposure experiments with fish. Experiments were performed according to the established OECD TGs 210, 229, 234, 236 or combinations thereof. Zebrafish (Danio rerio) wildtype and a thyroid transgenic line were used. Swim bladder inflation and eye development were selected as most promising apical endpoints and were combined with analyses of TH levels and thyroid follicle morphology. We observed strong alterations of thyroid follicle and eye morphology in THSDC-exposed fish, as well as impaired swim bladder inflation. Behavioral analyses demonstrate that these changes directly translate into altered swimming behavior and visual function of the larvae. An AOP network describing the sequence of events from molecular impact on the TH system of fish up to population-relevant behavioral changes is already established for swim bladder inflation and currently being developed for eye development. Based on this evidence, we suggest that these new endpoints can and should be implemented as thyroid-specific endpoints into existing TGs for endocrine disruptor testing with fish. All investigated apical endpoints react sensitively to different modes of action of THSDCs and can be seen as meaningful, population-relevant endpoints. Combined with mechanistic endpoints like TH level measurements, thyroid follicle histopathology or the use of transgenic thyroid fish lines, they could, in many cases, replace the need to run additional amphibian tests.

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O87

PAST EXPOSURE EFFECTS ON FUTURE GENERATIONS' HEALTH: ARE FUTURE GENERATIONS ORGANISMS SAFE AT ALL?

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As the contamination of the environment by endocrine-disrupting chemicals (EDCs) is diminished, endocrine disruption is believed to be minimized. Recent studies clearly demonstrate that EDCs can leave long-lasting and heritable effects if germ cells are exposed to them during their critical stages of increased sensitivity, such as germ cell sex differentiation and spermatogenesis. Such effects can be inherited by somatic cells of the offspring if not corrected during epigenetic reprogramming of the embryo and lead to adverse health outcomes depending on where in the genome the lesions are. Here we show that bisphenol A (BPA), atrazine, ethinylestradiol (EE2), and delta-9 tetrahydrocannabinol (THC) can induce heritable effects in germ cells which leads to reproductive and metabolic disorders in subsequent unexposed generations. BPA exposure of medaka embryos for the first 12 days of life resulted in reproductive impairment in males, dysbiosis of gut microbiota, and non-alcoholic fatty liver disease (NAFLD) in the unexposed grandchildren. Clear germline transmission of altered DNA methylation profile was observed in the paternal sperm and offspring somatic cells predicting dysregulation of transcriptional networks associated with observed phenotypes. Atrazine, EE2, and THC exposure during the same developmental period led to fertility impairment in males in the unexposed third-generation offspring, including transcriptional dysregulation in the brain predictive of increased addiction in future generations by THC exposure. These studies collectively suggest that environmental exposure effects are heritable and can persist in organisms even after the environmental contamination is mitigated. These observations also call for research to determine the health risks caused by past environmental contaminants in humans and wildlife as well susceptibility of current unexposed generation to second or third hit by the chemical contaminants of emerging concern (CECs). It also warrants strategies to mitigate transgenerational diseases in future generations before their onset in adulthood.

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O88

EFFECTS OF ATORVASTATIN AND 17α-ETHINYLESTRADIOL ON BLOOD AND LIVER LIPIDS CONTENTS IN BROWN TROUT JUVENILES

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Hypolipidemic compounds and estrogenic hormones (or their mimics) can cause dyslipidemia in humans and fish due to the similarity in the regulation pathways of lipid metabolism. Therefore, experimental fish models of dyslipidemia, particularly hyperlipidemia, have a biomedical, physiological and toxicological interest. In this context, the present study examined the in vivo effects in juvenile brown trout after intramuscular injections of: a hypolipidemic compound – atorvastatin – ATV (0.3 µg/g), a synthetic estrogenic hormone – 17α-ethinylestradiol – EE2 (2 µg/g) and a mixture of both chemicals – MIX (0.3 µg/g ATV plus 2 µg/g EE2). Control and solvent control corresponded to fish that were injected with a saline solution (0.7% NaCl) and a saline solution fortified with solvent (0.7% NaCl, 0.9% EtOH and 0.1% DMSO), respectively. For each exposure condition, fish (n = 10) were injected with 4 µL/g, two times a week for two weeks (4 injections in total per fish). Endpoints included blood/plasma lipid biochemistry (cholesterol, low-density lipoprotein cholesterol – LDL, high-density lipoprotein cholesterol – HDL and triglycerides – TGL) and hepatic lipid droplets quantification (from histological sections) through software ImageJ, after using osmium tetroxide post-fixation. A total of two sections per fish were photographed (n = 6 photos/section) for image analysis. The liver somatic index increased after exposure to EE2 and with MIX. Cholesterol, LDL and TGL were significantly lower in ATV exposed fish compared with the remaining groups. Moreover, compared with controls, TGL increased after EE2 treatment, and HDL diminished in all experimental groups (ATV, EE2 and MIX). Lipid quantification in osmicated liver tissues was significantly higher in the EE2 and MIX groups. The osmicated liver fragments from fish exposed to ATV, EE2 and MIX showed spatial heterogeneity of lipid droplets distribution. In summary, in vivo exposure of brown trout juveniles through intramuscular injection to ATV, EE2, and a mixture of both chemicals led to lipid metabolism alterations. The changes in blood/plasma lipid profiles and hepatic lipid droplet content and distribution contrast in the ATV and EE2 groups, with a general increase in lipid deposition after EE2 and a decrease in the ATV group. In MIX, ATV and EE2 counteracted each other.

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O89

WHAT DO RIBOSOMES TELL US ABOUT FISH OOCYTE DEVELOPMENT DURING SEX DIFFERENTIATION, OOGENESIS AND XENOESTROGENIC EXPOSURE

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Protein synthesis is allegedly the energetically most demanding process in a cell and it requires of enough ribosomes and tRNAs. While the transcription of 45S ribosomal RNAs (precursor of 5.8S, 18S and 28S rRNA) is mediated by RNA polymerase I (Pol-I), the transcription of 5S rRNA and tRNAs is directed by Pol-III. Pol-III in turn is regulated by activating transcription factors (Gtf3a, b and c) and inhibiting proteins (Maf1). We have demonstrated that 5S rRNA and tRNAs are highly expressed in fish oocytes to the point that they are excellent markers of their presence in a tissue. This includes oocytes within intersex testes of mullets *Chelon labrosus* under exposure to xenoestrogens. 5.8S, 18S and 28S rRNA only begin to accumulate in oocytes during secondary growth, so the dynamics of Pol-III and Pol-I transcribed rRNAs is a valid proxy to identify the ovarian developmental stage in any teleost fish. This is easily done through electrophoretic analysis of gonadal total RNA and calculation of 5S rRNA/18S rRNA and tRNA/5.8S rRNA indices in Bioanalyzer electropherograms as it has been tested in >10 different teleost species. Further, gtf3a is duplicated in teleost genomes with very high ovarian expression rates of one of the paralogs (gtf3ab). This is especially so during previtellogenesis. Also intersex testes show gtf3ab transcription. Gtf3b instead is a multipeptidic transcription factor (Brf1, Brf2, Bdp1 and Tbp). We have identified teleost specific duplications in all the genes coding these factors (brf1a & b, brf2a & b and bp1a & b). Three TATA binding protein (Tbp) coding genes exist in fish as in all vertebrates. Additionally, a duplication in the inhibitor maf1 is present in zebrafish (*D. rerio*) but not in other teleost genomes. Orthologs for all analysed genes were sequenced in *C. labrosus*, our pollution sentinel species, with one single maf1 gene. Transcription of brf1a & b, brf2a & b, bdp1a & b is observed in all mullet and zebrafish tissues, including gonads while tbpl2 is only transcribed in gonads. maf1 is also expressed in all mullet tissues, as it was the case of both zebrafish maf1 paralogs. qPCR analyses of these genes will allow to decipher whether any of the identified paralogs displays higher ovarian than testicular transcription levels, compatible with observed differences in 5S rRNA and tRNA levels. The expression pattern of the 5S rRNA, tRNAs and Pol-III regulators in fish gonads and the indexing approach developed have multiple applications in the study of teleost reproduction. Oocytes need to accumulate molecules that will allow rapid assembly of functional ribosomes in case of fertilisation to sustain early embryo development. Ribosomal intermediates thus, contribute molecular tools to monitor oocyte maturation along oogenesis or intersex testis in fish exposed to xenoestrogens downstream wastewater treatment plants.

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O90

A STRUCTURING APPROACH USING BIOASSAYS TO ASSESS ENDOCRINE DISRUPTION ACTIVITY IN QUEBEC'S EFFLUENTS

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Endocrine disruptors are contaminants capable of altering the normal functioning of hormones such as those involved in the development and reproduction of vertebrates. Several studies have shown that municipal and industrial wastewater can cause endocrine imbalances in fish. Following this work, international initiatives have been launched to identify contaminants with an activity on the endocrine system, but their monitoring and regulation in wastewater is still in its infancy. In partnership with the Ministry of the Environment of Quebec (Canada), this research project aims to develop a structuring approach using bioassays to detect the presence of endocrine disruptors in wastewater effluents in Quebec. The advantage of these bioassays is that it is now possible to quantify the cumulative (global) effect of a complex mixture of contaminants in effluents without having to identify each of the specific contaminants found there. Several aspects of these bioassays will be presented, the preparation of wastewater samples to use these bioassays, the calculations to assess the risk related to the endocrine disrupting activity of a given sample, as well as validation of the proposed approach using Quebec's municipal and industrial effluents. The bioassay approach developed within the framework of this project could enable the Ministry of the Environment of Quebec to monitor the endocrine disruptors present in the effluents, which will make it possible to validate whether the current wastewater treatment methods are adequate and to guide the measures to be taken to improve them if necessary.

POSTER COMMUNICATIONS

P1

GENETIC CHARACTERIZATION OF A ZEBRAFISH INBRED STRAIN GENERATED THROUGH FULL SIB-PAIR MATING

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Genetically homogeneous populations, such as inbred strains, are powerful experimental tools that are ideally suited for studying endocrine systems, endocrine disruption, and genetics of complex traits. In some model organisms, an inbred strain has been established for the use of experiments due to its strong advantage of genetic uniformity. However, loss of heterogeneity reduces the fitness of the population and survival and fertility of offspring of related individuals, which is referred as inbreeding depression. Zebrafish (*Danio rerio*) shows some degree of inbreeding depression, and there have been a few genetically homogeneous strains in this organism. Here, we report the establishment of zebrafish inbred strains from the wildtype India strain and AB strain, defined as the same criterion with the mice, rats and medaka: consecutive full sib-pair mating for more than 20 generations. The reproductive traits in the India-derived inbred strain tended to be lower comparing to the original heterogeneous strains India, although most of them were not statistically significant. On the other hand, the reproductive traits in the AB-derived inbred strain did not show much difference from that of the original AB strain. Sequencing analyses of the India-derived strain and the AB-derived strain revealed that the heterozygosity of the genome (hetero/SNPs) was 0.20% and 0.34%, respectively. These results indicate that our inbred strains, especially the AB-derived inbred strain, can be sufficiently used as a genetically homogeneous population for various experiments, e.g., analysis of the effects of endocrine disrupting substances and various drugs.

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P2

POSSIBLE ROLE OF FAECES IN CHEMICAL COMMUNICATION IN THE MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS)*

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The formation of social hierarchies and the relative position of individuals within the rank determine reproductive readiness and behaviour. In Mozambique tilapia (*Oreochromis mossambicus*), accessibility to mates depends on position of individuals in the hierarchy, which itself is modulated by different sensory inputs such as pheromones. Excretory products such as urine and faeces are potential vehicles for releasing pheromones. Recently, two pregnanetriol-3-glucuronates (P3Gs) have been identified in the urine of male tilapia that act as sex pheromones and prime ovulation in females. Here we hypothesize that, in a dominance hierarchy, female tilapia also signal their ovulation status and attract conspecifics to mate using their faeces as a source of pheromones. To test this hypothesis, we have established family groups with one male and three females. Faeces were taken from pre- and post-ovulatory females. Individual faeces samples were extracted using C18 solid-phase cartridges and eluates were fractionated by HPLC. Olfactory sensitivity of each fraction was assessed by the electro-olfactogram. The fraction(s) giving larger EOG responses were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS). Most olfactory activity was contained in the C18 eluate and in three HPLC fractions from pre-ovulatory females compared to post-ovulatory females. LC-MS identified amino acids in one of the fractions, bile acids cholic acid and taurocholic acid in the other two in much higher concentration in pre-ovulatory than post-ovulatory females. These findings are consistent with a role for faeces in chemical communication, and currently the behavioural response to the identified compounds is being tested.

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P3

THE ENIGMATIC SACCUS VASCULOSSUS. CHARACTERIZATION OF THIS STRUCTURE IN *CICHLASOMA DIMERUS*

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The saccus vasculosus (SV) is a structure present only in fish, located on the floor of the hypothalamus, posterior to the pituitary gland. It is characterized by abundant sinusoidal vessels and a folded neuroepithelium which makes a chamber that connects to the third ventricle. The neuroepithelium consists of coronet cells and supporting cells, with interspersed liquor-contacting neurons. Regarding its function, it was suggested that SV responds to changes in the cerebrospinal fluid and a recent work proposes that it is homologous to the pars tuberalis, participating in the control of seasonal reproduction. In the SV of adults and juveniles *Cichlasoma dimerus*, we detected immunoreactive fibers of gonadotropin inhibitory hormone, and neuropeptide Y. The objective of this study is to characterize the SV of larvae, juvenile, and adults of *C. dimerus*, and to evaluate the presence of the beta-thyrotropin stimulating hormone (β-Tsh) and opsins (CER-982). Adult brains and larvae/juveniles, obtained from 3 independent spawning from hatching to 85 days post-hatching, were sampled and processed for histology, transfer electron microscopy (TEM),
and immunohistochemistry. In this species, the SV starts developing by the time the adenohypophysis is observed. At first, two cell types connecting with the lumen can be distinguished, while blood capillaries associated to the outer layer of the SV at the beginning of juvenile stage. Then, blood capillaries invade the SV, and the cells acquire their typical morphology showing positive β-Tsh immunoreactivity during the gonadal differentiation period. In adults, all cell types are observed showing the typical morphology described for each one, and β-Tsh and rod-opsin immunoreactivity are detected in the crown of the coronet cells in both sexes. The present work proposes that the SV can be involved in the sensing of fish seasonality and comprises the starting point for future experiments about the role of SV in adulthood and during the development of this species.

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P4

A MULTIDISCIPLINARY APPROACH TO INVESTIGATE THE REPRODUCTIVE BIOLOGY OF EUROPEAN HAKE (*MERLUCCIUS MERLUCCIUS*): THE STUDY CASE OF MALES

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An in-depth knowledge of the reproductive biology of the different fish species can provide a sound scientific advice to set up fisheries management, in order to guarantee sustainability and prevent the resources collapse. European hake (*Merluccius merluccius)* is the second most important demersal fish species in the Mediterranean Sea, in terms of abundance and economic value and it is experiencing high fishing pressure, resulting in critical overexploited status. In this scenario, the aim of the study was to characterize, for the first time in the Northern and Central Adriatic Sea (GSA17), the reproductive biology of males of *M. merluccius*, that, unlike females, are currently poorly investigated. Wild European hakes males were randomly sampled throughout the year, to evaluate somatic indices and perform histological analysis. Three additional sampling were carried out on board of bottom trawler fishing vessels in order to take fresh samples (pituitary gland, testis and blood) and perform steroid immunoassay and qPCRs. A new reference scale was set ad hoc for this species. Five stages were distinguished by specific macroscopic and histological features: immature, developing, early spermiogenesis, late spermiogenesis and regenerating, surprisingly, no resting period was detected. The reproductive biology was more thoroughly investigated studying how the brain-pituitary-gonad (BPG) axis acts at different maturity stages. In particular, in pituitary the gonadotropin-releasing hormone receptor 2a (gnrhr2a) showed to be involved at the beginning of sexual maturity. Gonadotropins (fshb and lhb) progressively increased among the stages and peak in late spermiogenesis, suggesting that both hormones play an ongoing role, probably because *M. merluccius* is a multiple spawner with asynchronous testis. In all the stages prior to late spermiogenesis, lhb showed lower values than fshb, probably because lhb acts subsequently. In the testis, fshr and lhr showed earlier expression respect their ligands, presumably to guarantee the presence of the receptor upon arrival of the gonadotropins. Gonadotropins control steroidogenesis thus through androgens, whose mechanism of action is still not clear in the reproduction of fish males. The plasma levels of 11-ketotestosterone showed a gradual increase over the course of the reproductive cycle until reach the maximum in late spermiogenesis. This result can explain the expression pattern of alpha androgen receptor (arα) in testis, that peaked in late spermiogenesis stage, perhaps to better respond to the paracrine hormonal stimuli of 11-KT. According to our previous study carried out on females, in GSA17 this species showed only one peak in spring-summer, as indicated by the highest values of gonadosomatic index (GSI) of males, registered between April and July. The reduced bathymetry of sampling could cause the absence of the winter peak, recorded in other geographic areas, since *M. merluccius* moves to deeper water in winter.

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P5

INHIBITION OF EISENIA FETIDA REPRODUCTION DURING URBAN SEWAGE SLUDGE VERMICOMPOSTING

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Sewage sludge is rich in organic matter and nutrients and therefore current policies further its use as fertilizer, rather to landfill disposal. Composting and vermicomposting of sewage sludge, together with other organic residues, reduce the limitations of the direct sewage sludge application to soil, by improving biological, chemical and physical properties of substrates. However, sewage sludge, and in particular urban sewage sludge, is known to have environmental contaminants of anthropogenic origin, including some that may act as endocrine disruptors. This might be a constraint regarding the vermicomposting of substrates with sewage sludge, as earthworms may be sensitive to these compounds. Therefore, in order to achieve success in the vermicomposting process, it is necessary to reach an equilibrium between what is toxic and what promotes health and growth of earthworms. In the present work, during 31 days, adult *Eisenia fetida* earthworms (29 earthworms/kg of substrate fresh weight) were allowed to vermicompost three mixtures: sewage sludge (M1-45%; M2-35% and M3-25%) + horse manure (M1-45%, M2-55% and M3-65%) + 10% rice husk. A horse manure treatment (M4) was maintained as control and all conditions were performed in triplicate. Earthworm mortality was followed during the vermicomposting period and at the end cocoons were counted. Earthworms were collected and preserved accordingly to posterior analysis. At 31 days of vermicomposting, only M1 showed mortality (100%) and although mortality was not observed in M2 and M3, in these treatments earthworms did not reproduce as no cocoons were found, contrary to the control (M4) that presented an average of 11.2 ± 0.9 per earthworm. Furthermore, annetocin (an oxytocin/vasopressin superfamily peptide) gene expression was lower in the M2 and M3 treatments compared to control. The results suggest that when vermicomposting of sewage sludge is the intent, the substrates should be carefully tested, not only for toxicity, but also for reproductive parameters, as the success to a long-lasting bioprocess requires to maintain a healthy earthworm population. Also, there is evidence that sludge is interfering with the endocrine system of this species, as reproduction was clearly inhibited in its presence

P6

MOLECULAR CHARACTERIZATION AND EXPRESSION OF GDNFA AND GDNFB AND THEIR PUTATIVE RECEPTORS, GFRA1A, GFRA1B AND RET DURING TESTICULAR DEVELOPMENT OF THE EUROPEAN SEABASS *(DICENTRARCHUS LABRAX L.)*

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Spermatogenesis starts when undifferentiated type A spermatogonia (und-SpgA), also known as spermatogonial stem cells, become differentiated SpgA (diff-SpgA). In the mammalian testis, it is well established that Sertoli cells produce GDNF (glial cell line-derived neurotrophic factor) that binds the GFRA1 (GDNF family receptor alpha-1), which is expressed by und-SpgA promoting its self-renewal and maintenance. The Gdnf-Gfra1 complex interacts with the Ret receptor tyrosine-kinase activating the intracellular signaling pathway. A few recent studies have addressed the potential involvement of the Gdnf-Gfra1 system in the regulation of gametogenesis in fish but little is known about Ret receptor. As a first step to determine the Gdnf, Gfra1 and Ret involvement in the self-renewal and maintenance of the und-SpgA in the European seabass, we have characterized and studied their expression at different stages of testicular development. By BLAST analysis against the NCBI whole-genome shotgun contigs (wgs) database for D. labrax (sequences CBXY010000001 to CBXY010037781), and BLAT analysis against the European seabass genome (http://seabass.mpipz.mpg.de) we found the sequences of the paralogs gdnfa and gdnfb, the paralogs gfra1a and gfra1b, and ret. The sequences were confirmed by PCR using testis cDNA and specific primers designed to the respective genomic sequences, showing open reading frames of 762, 807, 1419, 1425 and 3333 bp coding for 254, 269, 473, 475 and 1111 amino acid residues, respectively. Expression analysis by qrtPCR in adult testis during the reproductive cycle showed the highest levels of gdnfa, gdnfb, gfra1a, gfra1b and ret mRNA expression in testes containing only SpgA. The expression of all genes decreased sharply to low levels coinciding with the appearance of type B-spermatogonia, except for gfra1a, whose expression levels barely decrease during later stages of development. These results suggest that Gdnfa/b-Gfra1a/b-Ret regulatory pathway can have key roles during the early stages of testicular development. However, gfra1a could also have important roles throughout spermatogenesis.

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P7

SPEXIN IN THE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX* CHARACTERISATION, BRAIN DISTRIBUTION AND INTERACTION WITH GNRH AND GNIH NEURONS.

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Spexin (Spx) is a recently characterised neuropeptide that has been implicated in multiple physiological processes in vertebrates, including reproduction, food intake, regulation of anxiety and stress. Two orthologs (Spx1 and Spx2) are present in some non-mammalian species, including teleost. However, information about Spx brain distribution and its interactions with other neuroendocrine systems is still scarce in fish. In this work, we cloned and sequenced the sea bass Spx1, which included a signal peptide of 27 aa and a mature peptide of 14 aa that is amidated at the C-termini and contained the GRR motif. *spx1* transcripts were higher in the diencephalon and medulla but were also detected in the olfactory bulbs, telencephalon, optic tectum, cerebellum, and pituitary. The immunohistochemical study revealed Spx1-immunoreactive (ir) cells in different nuclei of the preoptic area, as well as in the dorsal habenular nucleus, ventrolateral thalamic nucleus, lateral nucleus of the valvula, perilemniscular nucleus, and in the proximal pars distalis and the pars intermedia of the pituitary. These Spx1-ir cells profusely innervated the sea bass brain, being ir-fibres particularly evident in the midbrain and the hindbrain. Double immunofluorescence analysis showed Spx1-ir fibres in close contact with isthmic GnIH cells of the secondary gustatory nucleus and in the vicinity of tegmental GnRH2 cells. In addition, GnIH fibres were observed innervating Spx1 cells from the magnocellular and gigantocellular parts of the magnocellular preoptic nucleus and from the lateral nucleus of the valvula, whereas ventral telencephalic Spx1 cells received immunopositive GnRH2 fibres. In the pituitary, GnRH1-ir fibres were observed closely associated with Spx1 cells of the proximal pars distalis. These results suggest that Spx1 could be involved in both reproductive and nonreproductive (e.g., food intake, behaviour) functions in sea bass.

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P8

STUDIES ON THE GREAT POND SNAIL HIGHLIGHT WEAKNESSES IN TWO LINES OF EVIDENCE THAT VERTEBRATE STEROIDS HAVE A HORMONAL ROLE IN THE REPRODUCTION OF MOLLUSKS.

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For the last 70 years, researchers have been attempting to prove that vertebrate sex steroids act as hormones in mollusks in the same way that they do in vertebrates. One common line of evidence is that sex steroids are present in molluscan tissues and that in some studies, their concentrations sometimes show an association with stages of gonad development, sometimes show differences between tissues and sexes, and sometimes appear to be altered in the presence of contaminants. Another line of evidence is that cells in mollusk tissues can be immunohistochemically stained with antibodies raised against mammalian proteins that are associated with sex steroid synthesis or steroid reception. In regard to the first line of evidence, it has been established numerous times that mollusks can readily absorb vertebrate sex steroids from the environment and store them and/or their metabolites for a long time in the form of fatty acid esters. This calls into doubt how much (if any at all) of the vertebrate sex steroids are of endogenous origin in molluscan tissues. Furthermore, it has frequently been pointed out that three crucial steps in the classical vertebrate steroid biosynthetic pathway are either absent or have very weak activity in mollusks. The protein homologues of two enzymes involved in vertebrate sex steroidogenesis, as well as functional sex steroid nuclear receptors, have so far not been found in molluscan genomes. These facts already raise questions about the reliability of the evidence obtained using immunohistochemistry.We investigated the neuronal transcriptome of the widely used model species of invertebrate neuroendocrinology, the great pond snail (*Lymnaea stagnalis*) and confirmed the absence of several of the key protein sequences that would be required to accomplish full sex steroid biosynthesis and sex steroid receptor-mediation as found in vertebrates. Also, we exposed L. stagnalis to radioactive-labelled sex steroids (E2, P, T, EE2) and confirmed that snails can absorb and accumulate them for a long time. Despite the lack of homologous genes, we demonstrated that commercially-available antisera generated against mammalian CYP19A, nPR, and GnRH yielded positive signals in the central nervous system (CNS). Western blotting of CNS extracts showed that the three antibodies stained two or more proteins. Subsequent mass spectrometry analysis demonstrated the lack of homologous sequences to the vertebrate proteins recognized by the antibodies.
In summary, our findings support that the presence of vertebrate-like sex steroids in molluscan tissues is not evidence of endogenous origin and that immunostaining with antibodies generated against vertebrate proteins is a highly unreliable procedure for identifying or localizing specific proteins in invertebrate tissues. These results contribute to the functional and evolutionary understanding of molluscan endocrinology.

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P9

GSDF IS THE ONLY MAJOR PRO-MALE GENE ACTIVATED DURING THE MOLECULAR SEX DIFFERENTIATION PERIOD OF SIBERIAN STURGEON

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Factors controlling the male program of sturgeons during sex differentiation are not well understood. In previous gonadal transcriptomic studies, ten fish at the molecular sex differentiation period of Siberian sturgeons (3 to 6 months of age) were studied. Six fish were at 5 and 6 months of age. Among them, two animals were identified as females by their clear gonad activation of three feminine genes cyp19a1, foxl2, and hsd17b1. The other four fish showed repression of feminine genes and activation of a contig that resulted homologous to gsdf of other fish when it was analyzed by BLAST (NCBI). However, at that time the lack of a sex marker prevented any conclusion when qPCR for gsdf, cyp19a1, foxl2, and hsd17b1 was made in gonads of undifferentiated fish at 3, 4, 5, and 6 months of age (n=30 per age). The recent possibility of sexing the fish at the genomic DNA level allowed to study the molecular basis of testis differentiation in gonads of Siberian sturgeon fish sexed at 3,5, 5, and 6 months of age (n=18, n=19, and n=13 respectively). The genes selected for the evaluation by qPCR were: gsdf and other testicular genes described in fish such as amh, dmrt1, sox9, dax1, dhh, tbx1, tbx2. The gene coding for the enzyme cyp11c involved in the synthesis of the 11-oxygenated androgens, which are the major androgens in fish, was also studied.
Among all the genes analyzed only gsdf showed a sex dimorphic expression with significantly higher levels ($p<0.01$) in gonads of future males at 5 months of age. The phylogenetic studies showed that this gene forms a clade with gsdf of ancient fish such as Latimeria, Protopterus, and other Acipenser genus. The results agree with those obtained using the gonadal transcriptome. In sum, considering the major male genes described to date, gsdf is the only one that resulted activated during male sex differentiation of Siberian sturgeons. More studies are needed to explore the functional significance and the cellular localization of gsdf during Siberian sturgeon testis differentiation.

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P10

REPRODUCTION IS NOT ALTERED BY ENVIRONMENTAL STRESS: NEW INSIGHT IN THE SWORDFISH (*XIPHIAS GLADIUS*) FEMALES

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The Mediterranean swordfish stock (*Xiphias gladius*) is considered in overfishing and declining. In this light, the aim of this study was to evaluate the cross-talk among metabolism, stress response, immune system and reproduction in immature and mature females, combining histological and transcriptomic approaches. The sequenced transcriptome of livers from 3 immature and 3 mature swordfish females was downloaded from website SwordfishOmics (http://www.swordfishomics.com). Read mapping was performed using the A.I.R. (Artificial Intelligence RNA-Seq). For the histological analysis, ovary and liver samples were collected from 50 specimens caught between June and September, in the central and western Mediterranean Sea. A total of 750 genes were differentially expressed between the liver of mature and immature females, 355 up-regulated and 395 down-regulated. The Gene Ontology analysis showed 91 up-regulated and 161 down-regulated biological processes GO terms. Among them, particular attention was done to those related to metabolism, stress response and reproduction. 28 up-regulated biological processes GO terms, such as response to polycyclic arene, long-chain fatty acid transport and response to estradiol, and 29 down-regulated, including xenobiotic metabolic process, complement activation, lipid metabolic process, were selected. Instead, the KEGG Enrichment Analysis revealed a total of 18 enriched pathways, 6 upregulated, including steroid biosynthesis, and 12 downregulated, such as PPAR signalling pathway, metabolism of xenobiotics by cytochrome P450, complement and coagulation cascades. Furthermore, the binding occurring between Estrogen receptors and Aryl Hydrocarbon Receptor Nuclear Translocator, upregulated in mature females, could be liable of the inhibition of cyp450 detoxification pathway, making the organism more susceptible during the reproductive season. Indeed, at histological level, mature females showed a higher density and number of melanomacrophage centres in the liver suggesting a greater exposure to chronic environmental stress. Noteworthy, the reproduction was not affected by environmental stress, since no alterations were evidenced in ovaries. In addition, lipid metabolism shifted from hepatic accumulation before puberty onset to mobilization toward the ovary during the reproductive period to sustain vitellogenesis, as evidenced by lower density of lipids in the mature female livers. The present findings reveal the cross-talk among response to environmental stressors, metabolism and reproduction, underscoring that mature females invest most of energy in reproduction instead of immune response and detoxification.

P11
LEPTIN MODULATES OOCYTE MATURATION VIA CENTRAL AND A DIRECT PATHWAY IN ZEBRAFISH

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Adequate nutritional stores are a prerequisite for appropriately timed maturational events and help ensure normal reproduction. In mammals, the hormone leptin is known to be the adipostat and links body nutritional reserves with central energy homeostasis including the control of reproduction. LEPR loss-of-function (LOF) in mammals leads to diverse phenotypes including morbid obesity and infertility. However, the role of leptin in fish reproduction is unclear and loss of lepr leads to relatively minor phenotypes. Here we investigated the role of leptin in the regulation of reproduction using a Lepr LOF zebrafish line. We found that Loss of Lepr caused dysregulation of gonadotropins namely, luteinizing hormone beta (lhb) and follicle stimulating hormone beta (fshb) in the pituitary of adult females. Despite an impaired HPG axis, the Lepr mutants did not show subfertility. However, they exhibited a delay in ovarian maturation and/or an increased rate of follicular atresia compared to their wild type siblings. Analysis of candidate genes in the ovaries of Lepr mutants showed downregulation of genes involved in the maturational pathway and upregulation of genes involved in follicular atresia. Further, we elucidated the peripheral role of leptin using an in vitro Germinal vesicle breakdown (GVBD) assay. In cultured oocytes, leptin promoted GVBD and attenuated the rate of oocyte degradation. Additionally, Lepr LOF abates the effect of maturation inducing hormone, 17α-20β-dihydroxy-4 pregnen-3-one (DHP), leading to reduced GVBD rates in ovarian cultures of mutants in comparison to wild type. In conclusion, we found that leptin has a central as well as peripheral role in the regulation of the Hypothalamic-Pituitary-Gonadal (HPG) axis in adult zebrafish females. It modulates oocyte maturation and additionally may be involved in the modulation of follicular atresia. On the long run, these findings will provide insights into the role of leptin in energy mobilisation in fish during reproduction.

P12

IS CARBAMAZEPINE TOXIC FOR *A. LACUSTRIS?* ANALYSIS OF THE REPRODUCTIVE PHYSIOLOGY OF FEMALES

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Metabolic and reproductive dysfunction can easily be observed when organisms are subjected to various environmental contaminants. Among those contaminants, carbamazepine, a commonly pharmaceutical drug prescribed as an anticonvulsant and antiepileptic, has been detected in freshwater ecosystems worldwide, and may present its effects through alterations in hormone levels and in the lipid content of aquatic organisms. Although this is known for male teleost species, there is a lack of studies concerning carbamazepine toxic effects on female teleosts. Therefore, this study investigated the effects of environmentally relevant concentrations of this drug on testosterone levels and on the energetic substrates of sexually mature *Astyanax lacustris* females. Female fish (N = 120) were randomly distributed into five treatment groups: control, dimethyl sulfoxide (vehicle), 250 ngL^{-1}, 500 ngL^{-1} and 1250 ngL^{-1} of carbamazepine. After 7 days of exposure, animals were anesthetized for blood withdrawn and euthanized for tissue collection. Testosterone was quantified in plasma samples, the protein content was analyzed in muscle, liver and ovary samples, and lipids in muscle and liver. Data were verified for normality and homogeneity and then, one-way ANOVA was applied. Overall, our results demonstrated that total lipids and protein concentration were almost unaffected by any concentration of carbamazepine tested. Lipid content was not altered due to carbamazepine exposure, either in the muscle or in the liver of the fish. The protein content, similarly, remained unchanged in ovaries and liver samples. Nonetheless, in the muscle, dimethyl sulfoxide might have induced an increase in protein levels of fish in relation to controls. Although significant, this difference may not represent a meaningful biological alteration. Testosterone levels have a tendency to increase in plasma after the lowest and the highest concentrations of carbamazepine exposure. Generally, species of the genus Astyanax are frequently observed in polluted environments. *A. lacustris* and *A. altiparanae*, for example, are widely distributed throughout the hydrographic basins in Brazil and are suggested to be important bioindicators of contamination since they display great phenotypic plasticity and sensitivity toward pollutants. Despite the absence of alteration in the energetic substrate analyzed so far, and the alteration in testosterone levels that are not directly related to carbamazepine concentration, *A. lacustris* females might have elicited different mechanisms to cope with this stressor. Thus, it can be suggested that females adjust their physiology to maintain reproduction.

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BRAIN AROMATASE MUTATION AFFECTS ZEBRAFISH SEXUAL BEHAVIOUR AND REPRODUCTIVE HEALTH

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Sex steroids play critical roles to regulate reproductive physiology and behaviour to optimize breeding success in vertebrates. Aromatase (Cyp19a1) is a steroidogenic enzyme that converts aromatizable androgens into bioactive estrogens, and hence is in a pivotal position to mediate reproduction and sexual behaviour. In teleosts, there are two aromatase paralogs: cyp19a1a that is highly expressed in granulosa and Leydig cells in the gonads, and cyp19a1b that is highly expressed in radial glial cells in the brain. Through the recent creation of cyp19a1 mutant zebrafish lines, Cyp19a1a was shown to critically function in sexual differentiation of the ovary. The importance of Cyp19a1b for sexual differentiation of the zebrafish brain, however, is currently unknown. In this study, cyp19a1 mutant zebrafish lines were used to investigate how independent mutation of cyp19a1a and cyp19a1b paralogs affect zebrafish sexual behaviour and offspring survival and early development. Mutation of cyp19a1b was found to uniquely delay female sexual behaviour and significantly increased the metabolic cost of reproduction through increasing the number of eggs spawned with a concomitant decrease in progeny survival rates. In males, combined mutation of the cyp19a1 paralogs resulted in a significantly lower progeny survival rate. Future experiments will be used to assess the mechanistic pathways of brain estrogen effects on zebrafish sexual behaviour. These data establish the specific importance of estrogens produced in the brain for the regulation of female sexual behaviour and larval survival rates.

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THYROID AXIS IS ACTIVATED IN THE FEMALE-TO-MALE SEX REVERSAL INDUCED BY HIGHER TEMPERATURE IN MEDAKA

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Thyroid hormones (THs) were recently proposed as stress modifiers due to their ability to modulate metabolic and osmotic processes in fish. Although the participation of THs in the response to various types of stress and adaptation has been reported, the role of THs in heat-induced masculinization remains poorly investigated in fish. Therefore, we characterized the expression of genes involved in the synthesis and action of thyroid hormones during sex differentiation and measured T3 levels at critical stage of sex differentiation (stage 36) in embryos under normal (24°C) and thermal stress condition (32°C). Furthermore, to investigate the interaction between THs and cortisol we induced hypothyroidism (methimazole -15mM) or block cortisol pathway (RU486-15ng/L) or both in embryos until 20 dph during heat-stress masculinization. Our analysis showed that high temperature (32°C) modulates the transcript levels of a selected number of genes involved in THs synthesis (dio1, dio2, dio3) and thyroid signaling (tshr, thrα, thrβ) in XX and XY during sex differentiation, as well as, increased T3 levels at 36 stage in both XX and XY when compared to control (24°C). We also showed that both hypothyroidism and inhibition of cortisol down-regulated genes related to male-pathway (amh, amhrII, gsdf, ar) and up-regulated genes related to female-pathway (cyp19a1a, foxl2) in XX but also in XY individuals. In the high temperature (32ºC), 27% of XX individuals developed testes, while methimazole-induced hypothyroidism or inhibition of cortisol decreased female-to-male sex reversal in 16,6% and 8,3%, respectively. Moreover, we demonstrated that concomitant treatment with methimazole and RU486 restored the sex phenotype of XX females under high temperature (32ºC). These results indicated that high temperature (32ºC) activates THs and the increase of THs together with cortisol suppressed the heat-induced masculinization in medaka. Taken together, these results provide clear evidence of the interaction between hypothalamic-pituitary-thyroid (HPT) and cortisol (HPI) axes in the masculinization induced by heat stress.

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GENOMIC AND PHYSIOLOGICAL MECHANISMS OF ANDROGEN SIGNALLING: STEROID-5A-REDUCTASE TYPE 2 KNOCKOUT INVESTIGATION IN FROGS

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With the increased presence of environmental contaminants acting as endocrine disruptors to animals, including humans, it is critical to assess how androgens are regulated and produced to understand the health consequences of altering androgen biosynthesis. Using one of the best aquatic models for endocrine disruptor testing, the Western clawed frog (*Silurana tropicalis*), and the CRISPR gene-editing technology, a unique line of frog mutants to mimic androgen disruption was created. In addition to their metamorphosing ability, frogs represent a unique animal model featuring large externally developing embryos to study all aspects of developmental physiology (compared to any mammalian models). These mutant frogs do not possess the functional enzyme steroid-5α-reductase type 2 (SRD5α2), capable of producing one of the most potent androgens in frogs, the 5α-dihydrotestosterone (5α-DHT). This enzyme is thought to have several roles in frog reproduction. The research objective is to characterize how SRD5α2 regulates gonadal development and differentiation and other reproduction-related aspects like maintaining reproductive capacity and secondary sex characteristics. On a more practical scale, this research may have applications targeting environmental contamination that would act through the activation or deactivation of androgen production in aquatic and semi-terrestrial species. Fundamental discoveries in frogs are relevant for understanding reproductive function in all vertebrates.

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PRELIMINARY DATA ON REPRODUCTIVE GENES OF THE HYPOTHALAMIC-PITUITARY-GONADS AXIS OF THE DUSKY GROUPER *EPINEPHELUS MARGINATUS* (PERCIFORMES: SERRANIDAE)

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The Dusky Grouper, *Epinephelus marginatus*, is an endangered species with great potential for aquaculture. However, when kept in captivity, this hermaphrodite species presents reproductive dysfunctions, limiting the success of its large-scale rearing. We are designing specific primers for this species to perform RT-PCR and analyze the gene expression of the main components of the Hypothalamus-Pituitary-Gonads (H-P-G) axis to better understand the origin of these reproductive dysfunctions in captivity animals. Tissue samples were collected from the brain, pituitary, and gonads of fish from both groups (G1= raised in captivity; G2= sampled from natural environment – as control group), during the reproductive period and outside of the reproductive period. In the brain, we analyzed the gene expression of gnrh (distinct forms) and brain aromatase (cyp19a1b); in the pituitary, gonadotropins fshb and lhb were also analyzed; and in the gonads several genes involved in the steroidogenic pathway and sexual differentiation (sox9, dmrt1, amh, foxl2, 20β-hsd, and cyp19a1a) were analyzed. The genes ef1α and cog5 were used as endogenous references for the analysis of the relative expression of target genes. The samples were processed to conventional PCR for DNA amplification of their respective target genes. The quantification of these genes was performed in Real Time analysis using primers developed and patterned for other species of *Epinephelus* genus. In this study, gonadotropins, fsh and lh, and ef1a have been already performed and apparently were specific for *E. marginatus*. We are designing, by sequencing the DNA fragment that bounds to the primers, and testing new specific primers for gnrh 1, 2, and 3 and cyp19a1b (brain tissue) and sox9, dmrt1, amh, foxl2, 20b-hsd, and cyp19a1a (gonads tissue) for *E. marginatus*. These primers will be specific for this species, and it is the first time that they will be described for *E. marginatus*. It is expected that the results of this study will show whether the endocrine disruption observed in animals kept in captivity is due to changes in the gene expression of neurohormones, neurotransmitters, gonadotropins, and gametogenesis regulators, which coordinate the H-P-G axis. All this information is important to improve the artificial reproduction of E. marginatus through controlled breeding programmers, considering the threatened situation of this species, that requires immediate recommendations to be taken, to avoid the extinction of this important species. Additionally, these results may support future studies on Brazilian aquaculture of marine teleost species.

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P17

A STUDY ON FOLLICULOGENESIS IN LOGGERHEAD SEA TURTLE *(CARETTA CARETTA)*: STRUCTURE AND BIOCHEMICAL CHARACTERIZATION THROUGH HISTOLOGICAL ANALYSES AND FOURIER TRANSFORM INFRARED MICROSPECTROSCOPY IMAGING (FTIRI)

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C. caretta is evaluated as a vulnerable species according to the Red List of IUCN; therefore, it is crucial to prevent the collapse of its populations also to avoid the detrimental consequences of sea turtles' extinction on the marine environment. Despite this species plays a key role in marine ecosystems, some aspects of its reproductive biology need to be clarified. The maturation stages associated with macroscopic gonadic characterization in both sexes have already been described in *C. caretta*. However, the sequence of follicular developmental stages and their histological characterization at each stage has yet to be described in detail. The oocytes' biochemical composition at different maturation phases is missing too for this species. The aim of the present study was to perform a morphological and macromolecular characterization of *C. caretta* folliculogenesis by coupling histological and FTIRI analyses. Ovaries of 23 *C. caretta* found stranded and examined by IZS AM in 2021 along the Abruzzo coasts were sampled and histologically processed to describe the folliculogenesis. Analyses were also performed considering both the period of stranding and the curved carapace length (CCL) which is normally used to identify the maturity stage in sea turtles. FTIR-imaging analysis was performed to determine changes on distribution of the main macromolecules in the follicles at each maturation stage focusing on follicular cells, ooplasm, zona radiata, and yolk vesicles in vitellogenic oocytes. Noteworthy, particular attention was given to atretic follicles and corpora lutea and albicans. The results obtained in the present study represent a clear and comprehensive picture of the *C. caretta* folliculogenesis and suggested that the CCL-based method applied to determine the sexual maturity of sea turtles is not representative of the real gonadal maturity.

P18
OCT4 AND SEXUAL PLASTICITY IN FISH GONAD

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Germ cells hold the genetic information and are the building blocks for future generation. Pluripotent stem cells, expressing various conserved stem cell markers (e.g., Yamanaka factors), are mainly found as an inner cell mass during embryogenesis and can be artificially reprogrammed from various adult cells, differentiated, and turned into all types of cells, including eggs and sperm. In our recent investigation, our group has found that any alteration in the initial germ cell population could ultimately change the phenotypic sex of medaka. Using adult medaka, we found that germ line stem cells (GSC) control the functional sex reversal during adulthood which further points towards pluripotency. Evidently, Oct4 is considered as a stemness regulator across vertebrates. So, in this study, we aimed to determine the Oct4 involvement in sexual physiology.Using medaka (*Oryzias latipes*), Japanese anchovy (*Engraulis japonicus*) and Chub mackerel (*Scomber japonicus*) we found that, irrespective of fish species, Oct4, unlike mammals, expresses in both GSC and germ cells of fish gonad, however, only GSC shows nuclear localization of Oct4. It was observed that, during early stages of sex reversal (from 3-7 days) Oct4 transcriptions were elevated in GSC, which later subsided (from 15-30 days; when GSC differentiation occurred). Surprisingly, we found that mere addition of Oct4 antagonist blocks the functional sex change. This suggests that Oct4 is essential for GSC maintenance. Later, we collected the GSC at various points of sex reversal and performed targeted epigenetic analysis. We found a strong age, sex and steroid dependent Oct4 methylation pattern. Further it was also found that, intronic regions before the nuclear localization signal (NLS) were more epi-methylated from 7-30 days period, which in combination with our western blotting data suggests that Oct4 during GSC differentiation somehow loses its NLS and thus becomes unavailable for GSC maintenance. To prove this, we performed series of targeted knock in and conditional knock out experiment and preliminarily found that deletion of fish specific region (after the NLS) does not affect GSC maintenance but hampers germ cell sex change. Whereas any deletion before NLS inadvertently results in GSC death. Cumulatively, our data suggests that Oct4 is an important regulator of gonadal sexual plasticity. However, further analyses are pertinent to understand the intricate details of Oct4 associated GSC to germ cell differentiation.

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P19
GONADAL DEVELOPMENT IN THR ALPHA OR BETA KNOCK-OUT (*SILURANA TROPICALIS)*

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Thyroid hormones (THs) are known to influence the hormonal axis involved in reproduction. For example, alterations in TH signalling can affect sex ratios, reproduction, and alter sex steroid hormone levels in several vertebrate species. Such complex interplay among endocrine axis makes it even more challenging to predict the effects of endocrine disrupting chemicals (EDCs) in frog species. Many aspects of the endocrine crosstalk are not fully understood. For example, the role, if any, of THs in frog gonadal differentiation remains an open discussion. In this study, we aim to deepen our understanding of TH signalling on gonadal development in amphibians. For that, we will use transgenic *Silurana tropicalis*, lacking functional genes for either or both TH receptors (TRs), tr alpha and tr beta. We will investigate gonad development and differentiation, reproduction success, sexual behaviour, and gene expression profile of key enzymes regulating THs and reproductive hormones in their gonads and brains. Our results can contribute to the field of comparative endocrinology by extending our knowledge of the endocrine crosstalk in amphibians, which are particularly sensitive species to environment insult.

P20

FUNCTIONAL ANNOTATION OF THE TESTICULAR PROTEOME DURING SPERMATOGENESIS IN THE CATSHARK, *SCYLIORHINUS CANICULA*, WITH A FOCUS RELATED TO STEROIDOGENESIS

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Spermatogenesis is a highly specialized process of cell proliferation and differentiation allowing production of spermatozoa from spermatogonial stem cells. Since to its phylogenetic position and anatomy of its testis, catshark is an interesting model to explore stage-based changes in proteins during spermatogenesis and study the evolutionary of regulation of spermatogenesis. Thus, from a cross-section of the testis readily reveals four zones, i.e., the germinative niche and cysts with spermatogonia (zone A), cysts with spermatocytes (zone B), cysts with young spermatids (zone C) and cysts with late spermatids (zone D). In our study, a protein extract of each zone has been analyzed by nLC-TIMS TOF Pro MS (Bruker). Protein identifications was performed with the PeaksXPro software using a sequence database from genome of S. canicula (assembly sScyCan1.1). Then sequences have been blasted against the Mus musculus proteome (ID: UP000000589) in order to perform gene ontology enrichment and pathway analyses using PANTHER and KEGG database, respectively. A total of 3046 proteins were identified and Venn diagram analysis allows zone-specific distribution. Cell process identified agree with the different steps of spermatogenesis such as mitosis and cell differentiation associated with transcriptional molecular process and TGFβ pathway in zone A; meiosis regulation associated with motor proteins and PDGF pathway in zone B; DNA remodelling associated with transcriptional modulators in zone C; cell contractility, response to steroids and apoptosis associated with molecular functions related to cytoskeletal dynamic and catalytic activities in zone D. Interestingly, WNT signalling pathway were retrieved in Zones A, C and D, GnRH signalling pathway in zones A, B and D and thyrotropin-releasing hormone receptor pathway in zone D. Using KEGG system, testicular steroidogenesis has been modelled and main steroidogenesis enzymes (CYP11A, CYP17, 3βHSD, 17βHSD and 20βHSD) were retrieved throughout spermatogenesis except in the early spermatid zone C. In conclusion, such proteomic approach provides a large dataset of testis proteome of the catshark, which might be useful for functional approaches, including for example in vitro response of testicular cells to hormones in order to explore the evolution of the endocrine versus paracrine regulation of spermatogenesis in Vertebrates.

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P21

EFFECTS OF THE NONYLPHENOL ON *IN VITRO* SPERMATOGENESIS OF THE CRITICALLY ENDANGERED CYPRINID *GNATHOPOGON CAERULESCENS*

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Nonylphenol (NP) is widely used in a variety of industrial and agricultural processes and is known to be one of the major endocrine disrupting chemicals (EDCs). The influx of NP into the aquatic environment adversely affects the reproduction of aquatic organisms including fishes and the ecological system. NP has been reported to induce hormonal imbalance, feminization of males, and reduction of germ cell production by *in vivo* studies but the mechanism and how NP affects spermatogenesis remains unknown. In this study, we investigated the effect of NP on spermatogenesis using *in vitro* differentiation system of *Gnathopogon caerulescens*, which is an endangered endemic fish of Lake Biwa in Japan. We collected spermatogonia from non-spawning honmoroko and subjected to *in vitro* culture for 3 weeks. Spermatogonia differentiated into flagellated sperm within 3 weeks regardless of the presence of NP. NP treatment decreased the number of germ cells dose-dependently, whereas the number of somatic cells decreased only with a high dose of NP (1 μM). Flow cytometric analysis, which enables to separate germ cells by developmental stage, revealed the decrease in germ cell number is attributed to the haploids: spermatids and sperm, and the number of spermatogonia and spermatocytes were not changed by NP treatment. This result is consistent with the idea that NP might induce apoptosis of haploids after the second meiosis. This study demonstrated that the combination of *in vitro* germ cell differentiation and flow cytometric analysis is useful to evaluate the direct effect of EDCs on germ cell differentiation in endangered endemic fish.

P22

NEUROPEPTIDE CASCADES DURING ECDYSIS IN THE SHORE CRAB, CARCINUS MAENAS. BURS AND CCAP NEURONS SIMULTANEOUSLY RELEASE ALLATOSTATIN-C AND -CC, DURING ECDYSIS

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Growth and development in arthropods is dependent on periodic shedding of the exoskeleton-ecdysis, which is controlled by a precisely timed release of a cocktail of neuropeptides. Whilst much is now known about this process in insects, much less is known for crustaceans, and it is of interest to determine which neuroendocrine pathways and functions have been conserved or abandoned during arthropod evolution. In crabs, we have shown that ecdysis is initiated by a surge in crustacean hyperglycemic hormone (CHH) from gut endocrine cells, which promote dipsogenesis and cuticular splitting. This release is closely followed by simultaneous release of crustacean cardioactive peptide (CCAP) from the pericardial organs (PO) which results in stereotyped eclosion behaviour and cardioacceleration. At the same time bursicon (BURS) is released from the PO which leads to post-ecdysis cuticle hardening. Since, in flies the so-called CAMB neurons express CCAP, Allatostatins, Myoinhibitory peptides and BURS as part of the neuroendocrine cocktail involved in ecdysis, we were firstly interested in determining the occurrence of allatostatins in the CNS. We found that every single neuron expressing CCAP and BURS also express both Allatostatin-C and -CC (AST-C, -CC), and that release kinetics measured by RIA, and levels of both peptides in these neurons exactly mirror those of BURS and CCAP clearly implicating them in ecdysis. We are now deorphaning AST-C receptor(s) to note tissue distribution in a prelude to potential knockdown of peptide and receptor mRNA in an attempt to determine the roles of AST-C peptides in ecdysis.

P23

GROWTH HORMONE (GH) INDUCES NEUROPROTECTIVE EFFECTS IN THE EMBRYONIC CHICKEN CEREBELLUM EXPOSED TO HYPOXIC INJURY, IN VITRO AND IN VIVO

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It is known that growth hormone (GH) has neuroprotective effects in response to neural damage. This work aimed to study the mechanisms involved in GH actions against hypoxia-ischemia (HI) injury in the chicken cerebellum, both in vitro and in vivo. It was found that HI insult provoked a significant increase of local GH and GH receptor (GHR) mRNA expression in cerebellar cell cultures. Also, treatments with GH or IGF-1 clearly augmented cell viability and decreased apoptosis and necrosis in cultures exposed to hypoxia and re-oxygenation. In vivo, it is still controversial if GH is able to cross the blood-brain barrier (BBB) and perform its protective effects in neural tissues. Here, we observed that in ovo microinjection of Cy3-labeled GH resulted in a wide distribution of the fluorescent signal within several brain areas (choroid plexus, pallium, hypothalamus, periventricular regions, hippocampus and cerebellum, among others), both under normoxic and hypoxic incubation conditions, although in the latter the signal was higher. In the cerebellum, Cy3-GH and GHR immunoreactivities co-localized in the granular and Purkinje cell layers, and also in the deep cerebellar nuclei. These data indicate that GH crosses the BBB and has direct effects through binding to its specific receptor in the target tissues. Administration of GH to embryos exposed to hypoxia-reoxygenation injury resulted in a significant reduction in apoptosis and lipoperoxidation (an indicator of oxidative stress), as well as a decrease in the mRNA expression of various inflammatory mediators (TNFα, IL-6, IL-1β, iNOS). In contrast, GH upregulated the expression of several neurotrophic factors (IGF-1, VEGF, BDNF) and, remarkably, of locally expressed GH and GHR mRNAs, suggesting the existence of an endogenous protective mechanism against HI damage. Overall, our results show that GH exerts antiapoptotic, antioxidative, anti-inflammatory, neuroprotective and regenerative actions in the embryonic chicken cerebellum exposed to hypoxia.

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GONADAL SEX DIFFERENTIATION IN JAPANESE EEL: EXPRESSION PROFILES OF CYP19A1, ERS AND GTHS IN THE BRAIN/PITUITARY

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Gonochoristic feature with environmental sex determination and gonadal differentiation that occurs during the yellow stage in eels provides a unique model to investigate the mechanisms of sex differentiation and development. We proposed a diagram of sex-related gene profiles in the gonads during sex differentiation of Japanese eels (*Anguilla japonica*). We suggest that cyp19a1 and gsdf play a critical role in early female and male gonadal differentiation, respectively. However, the expressions of sex related genes in the brain/pituitary during gonadal sex differentiation in eels are still unclear. The aims of this study were to investigate the expressions of cyp19a1, foxl2s, ers, gnrh1, gnrhrs and gths in the brain/pituitary during gonadal sex differentiation of Japanese eel. Based on our histological study, the control eels developed as males and E2 was used for feminization. The gonadal status could be divided into undifferentiated, differentiating and differentiated stages. Our results showed that aromatase proteins were detectable in virous parts of brain in undifferentiated eels. The foxl2s, cyp19a1 transcripts and aromatase proteins in brain were significantly increased during testicular development. The transcripts of ers, except gperb in midbrain (with pituitary) also were increased significantly during testicular development. Forebrain gnrh1 transcripts increased during gonadal development of both sexes, the transcripts of gnrhrs in pituitary (with midbrain) were stable during gonadal development except the gnrhr1a transcripts decreased during testicular development. The expression of glycoprotein-a, fshb, lhb and gh in pituitary (with midbrain) were higher in males as compared with E2-feminized females, and the transcripts levels of glycoprotein-a, fshb, lhb and gh were significantly increased during testicular development. These results suggest that endogenous estrogens may play an important role in the brain/pituitary during testicular differentiation and development, foxl2s and ers may have a role on cyp19a1 regulation in the midbrain of Japanese eel. Regarding the GnRH-GTH axis, gths, especially fshb, may be regulated by ers and involved in regulating testicular differentiation by stimulating the synthesis of sex steroids in Japanese eel.

P25
PUBERTAL DEVELOPMENT IN EUROPEAN SEA BASS: EVALUATION OF GERM CELL MOLECULAR MARKERS

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The European sea bass, *Dicentrarchus labrax*, is a highly valued species in Mediterranean aquaculture. The expansion of its culture requires basic studies to improve the understanding of pathways and factors involved in the first sexual maturation and, therefore, the onset of puberty. These studies will make it possible to delay the onset of puberty, generally linked to growth retardation, and thus obtain larger fish with a higher economic value by marketing time. This work is aimed to contribute to the knowledge of the molecular mechanisms that regulate the onset of pubertal development, in particular on sea bass males, and identifying the presence and expression of possible molecular markers involved in this process. In addition, the expression of these markers in pre-pubertal females was also assessed. The study focused on the initial stages of testicular maturation, mainly stage I and stage II-III, identifying highly expressed *dazl*, *rec8* and *vgll3* as possible molecular markers. Two isoforms of *dazl* were expressed in testes, both the complete and newly alternative splicing generated isoform (exon 7; *dazl_Δ7*), whereas, in ovaries, only *dazl_Δ7* could be found. Likewise, *vgll3* in testes expressed two isoforms, one complete and another probably formed by an alternative splicing event derived from the retention of an intron around 100-200 bp. The high expression levels of *dazl* and *rec8* allowed for the re-assembling and curation of the sequences initially annotated in Ensembl. Moreover, an *in silico* analysis allowed us to describe the structure and phylogeny of these genes in vertebrates. In particular, the high transcription levels of *rec8* -a *stra8* homologue with a prominent role as a meiosis gatekeeper in other vertebrates- during the onset of meiosis suggest that this gene may be regulating meiosis entry in this and other fish species as well. Additional studies are underway to address this hypothesis fully.

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T3 EFFECTS UPON MYELINATION DEPEND ON THE DEVELOPMENTAL STAGE OF ZEBRAFISH

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During vertebrate central nervous system (CNS) development, the myelination of neuronal axons is controlled by several extrinsic and intrinsic factors, including thyroid hormones (THs). The bioactive TH, triiodothyronine (T3), promotes the differentiation of the oligodendrocyte (OL) precursor cells (OPCs) and regulates the expression of myelin-related genes in mature OLs. Moreover, it has been suggested that T3 exert its actions in these events mainly through its binding to the different isoforms of TH receptors (TRs), which are thought to be expressed in a stage-specific manner along the differentiation and maturation of OLs, although this mechanism has not been fully described. Taking this into account, we are interested in addressing the role of THs and TRs during oligodendrogenesis and myelination of the developing CNS in the zebrafish model. Zebrafish OPCs and myelinating OLs have been identified as early as 30 and 72 hours post-fertilization (hpf), respectively. Because the onset of TH responsiveness and its temporality have not yet been determined, we initially studied the effects of T3 (0.025 nM) administered by immersion to F0 mbp:egfp transgenic zebrafish at different time points within 3 days post-fertilization (dpf) and evaluated the effect of T3 upon CNS myelination. Three different experimental groups were analyzed: embryos treated with T3 at 0 hpf, at 24 hpf and at 48 hpf. In all cases, T3 exposure lasted 24 h and animals were euthanized at 72 hpf. Controls included untreated wild type uninjected or mbp:egfp injected zebrafish embryos. Gene expression results showed that myelin protein zero (mpz), a gene associated to myelination, was positively regulated when T3 was administered at 24 or 48 hpf, when OPCs are proliferating and their differentiation to OLs is ongoing. These results correlate with an increase in flourescence signal (myelin content) in the brain of mbp:egfp zebrafish larvae. Our most interesting results were observed in zebrafish larvae exposed to T3 at 0 hpf during 24 h. These larvae showed a marked decrease of fluorescence signal at 72 hpf. Since myelin synthesis starts after 60-72 hpf, it is possible that an excess of T3 could modify the fate of OPCs in the CNS. Through immuno-staining techniques that identify neurons, radial glial cells and OPCs, we showed that only the OPCs population was significantly decreased after the T3 treatment and only in the larvae treated at 0 hpf, suggesting that the effects of T3 upon myelination depend on the developmental stage of the larvae, being detrimental when administered at the onset of zebrafish development, time in which the fate of OPCs development and maturation could be disarrayed.

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IDENTIFICATION AND PROFILING OF STABLE MICRORNAS IN HEMOLYMPH OF YOUNG AND OLD LOCUSTA MIGRATORIA FIFTH INSTARS

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Since the discovery of the first microRNA (miRNA) in the nematode *C. elegans*, numerous novel miRNAs have been identified which can regulate presumably every biological process in a wide range of metazoan species. In accordance, several insect miRNAs have been identified and functionally characterized. While regulatory RNA pathways are traditionally described at an intracellular level, studies reporting on the presence and potential role of extracellular (small) sRNAs have been emerging in the last decade, mainly in mammalian systems. Interestingly, evidence in several species indicates the functional transfer of extracellular RNAs between donor and recipient cells, illustrating RNA-based intercellular communication. In insects, however, reports on extracellular small RNAs are emerging but the number of detailed studies is still very limited. Here, we demonstrate the presence of stable sRNAs in the hemolymph of the migratory locust, *Locusta migratoria*. Moreover, the levels of several extracellular miRNAs (ex-miRNAs) present in locust hemolymph differed significantly between young and old fifth nymphal instars. In addition, we performed a 'proof of principle' experiment which suggested that extracellularly delivered miRNA molecules are capable of affecting the locusts' development.

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PRELIMINARY RESULTS REGARDING THE ROLE OF THYROID HORMONE ON GROWTH PERFORMANCE, AND MORPHOLOGY OF STELLATE STURGEON (*A. STELLATUS*) LARVAE DURING METAMORPHOSIS

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The stellate sturgeon is native to the Black, Azov, Caspian, and Aegean Sea basins, but wild populations of stellate have decreased catastrophically as a result of overfishing, water contamination, and loss of spawning grounds. Stellate is one of the three most important species for caviar, and its flesh is considered an expensive delicacy in the world. There are surprisingly few studies of larval development in sturgeons and if there is a thyroid hormone (TH) driven metamorphosis like in teleost fishes is largely unstudied. Heavy mortality is recorded at early developmental stages of the sturgeon and the present study is focused on the growth and early development of the stellate sturgeon. Due to the positive effects of TH on larval growth and survival, the present study was performed to analyze the role of the thyroid axis on growth and development of stellate larvae. For this purpose, 12 day post hatch (dph) larvae were transferred into six tanks (total 1800: two treatments with three replicates) (15 L). Then, methimazole (MMI) dissolved in dimethyl sulfoxide was added to three tanks to give a final concentration of 0.03 mM, and the other three tanks were the untreated control. Fish were sampled at 12, 16, 20, 24, 28, 32, and 44 dph. Based on the results, higher growth performance and a lower mortality rate was recorded for the control groups compared to the MMI-treated larvae. The effect on the larval development of manipulating the thyroid axis with MMI was studied by assessing the external and internal morphology during development. At 28 and 38 dph, the developmental status and pigmentation ratio was higher in the control group compared to the MMI-group. These preliminary data indicate that THs are most likely involved in regulating the growth, development, and external morphology of stellate larvae during development and at metamorphosis.

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EFFECTS OF TEMPERATURE MANIPULATION ON GROWTH AND SKELETAL DEVELOPMENT OF STERLET STURGEON (*A. RUTHENUS*) DURING THE FIRST TWO MONTHS OF LIFE

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While in many parts of the world sturgeons have become endangered because of overexploitation and pollution, interest in aquaculture production has increased. The sterlet (*Acipenser ruthenus*) is one of the commercially important sturgeon species because of its availability, relatively small size and fast sexual maturation it is used as a model in many aspects to develop sturgeon culture. Larval production is one of the most important phases in sturgeon culture, as they are susceptible to environmental changes that can lead to high mortality. Furthermore, temperature can modify sex ratios and induce skeletal malformations. Here we analysed the effect of temperature on early development. We divided fertilized sterlet eggs of the same batch into 4 groups grown in well water - control (ambient temperature, range 17-19 ºC), 21ºC, 24ºC and 27 ºC. From 32 dpf and until 64 dpf the temperature in the 4 groups was kept at ambient. The larvae were fed with Artemia nauplii, followed by gradual feeding of chironomids and weaning to a commercial feed. Cumulative mortality was high in all groups and ranged from 68% in the control group to 87% in the 24ºC group. Final length, weights, and specific growth rate increased at higher temperature. The formation of calcified structures was observed earlier at higher temperatures compared to the control group. Also, the number of soft and hard rays of fins showed significant difference in different groups. We conclude that higher temperatures during the first month after fertilization accelerate growth and development of calcified structures in sterlet sturgeon. We are also analysing the effect of temperature on germ cell development and gene expression.

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REGULATION OF GROWTH PERFORMANCE, GH/IGFS AXIS AND MUSCLE DEVELOPMENT MARKERS BY DIET AND EXERCISE IN JUVENILES OF GILTHEAD SEA BREAM.

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Gilthead sea bream is a widely farmed species in the Mediterranean Sea and understanding how its growth is regulated by the diet and the culture conditions is crucial to improve its production. In a previous experiment, we observed that this species subjected to sustained exercise enhanced the use of dietary lipids, resulting in a better growth performance. This work aims to examine the combined effects of swimming activity (voluntary swimming vs. sustained exercise) and diet composition (high-protein: 56% proteins, 8.6% carbohydrates; vs. high-carbohydrate: 48% proteins, 24.9% carbohydrates) on growth performance, growth hormone (GH)/insulin-like growth factors (IGFs) axis and muscle development markers. After 6 weeks, exercise decreased the hepatosomatic and mesenteric fat indexes in both dietary groups and increased the final body weight in fish fed with the high-protein diet. The hepatic gene expression of the anabolic GH receptor (ghr1) was significantly higher in fish subjected to exercise and fed with the high-protein diet than in the other groups, in agreement with the greater growth of this group. The high-protein diet increased the gene expression of different molecules of the GH/IGFs axis in liver (igf-1, igf-2, igf-1rb, igfbp-2, igfbp-4 and igfbp-5) and in muscle (igf-1 and igf-1rb), suggesting a better anabolic endocrine status. In muscle, the high-protein diet also upregulated the gene expression of myogenic and proliferative markers like pcna, myf5 and myod2, which would indicate an increased muscle growth by hyperplasia regardless of swimming condition. The transcriptional profile of the proteolytic systems molecules was barely affected by diet and swimming activity. Overall, these results suggested that sustained exercise improved growth performance, and in fish fed with the high-protein diet it induced an endocrine status that resulted in higher body weight.

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DETECTION OF MYELIN RICH REGIONS IN THE BRAIN OF AXOLOTL (*AMBYSTOMA MEXICANUM*)

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The Mexican axolotl (*Ambystoma mexicanum*) has gained attention because it has the ability to regenerate several tissues including the brain. However, some aspects of the brain such as its basic anatomy, cell composition and connectivity remain unclear. Based on previous results from our research group, we recently detected myelin in the axolotl brain. In this sense, we explored in more detail the myelin-rich regions in pre-metamorphic axolotl. The brain of juvenile axolotls was fixed and cryopreserved for coronal sections. The slices were stained with Black Gold II and observed under microscope. The brain of axolotl contains heterogeneity in terms of myelin-rich regions. In rhombencephalon, we detected staining in the white matter of medulla oblongata but not in the grey matter, confirming the specificity of the staining method. Also, we were able to detect myelin in the cerebellum. In mesencephalon, we observed staining in tegmentum and in axons connecting left and right optic tectum. In the diencephalon we found myelin in specific regions of the hypothalamus dorsalis, pars dorsalis hypothalamic, the floor of the third ventricle, dorsal thalamus and ventral thalamus. In telencephalon, we found low myelin rich regions; we detected myelin accumulations only at the level of the lateral/medial forebrain bundle. No myelin signal was detected in the pallium, subpallium and olfactory bulb. All together, these results suggest that the brain of the juvenile axolotl is not full myelinated. Comparative investigations of brain myelin content in metamorphic animals are necessary to better understand if this is a feature associated to the neotenic nature of this species.

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MATERNAL LOSS OF SOMATOSTATIN 2 IMPAIRS EARLY EMBRYONIC DEVELOPMENT IN ZEBRAFISH

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Somatostatin (*ss*) is a neuropeptide and a key regulator of growth and development. Six *ss* encoding genes (*ss1* to *ss6)* have been identified in teleost fish genomes but little is known about their specific function. Ss2 (also known as Cortistatin in mammals) has been implicated in in the immune and central nervous systems. To investigate the role of Ss2 in fish, we established a zebrafish CRISP/Cas9 *ss2* deficient line and found that maternal, but not paternal, loss of *ss2* mRNA impaired embryo cleavage and gastrulation resulting in 80% embryo mortality by 48 hours after fertilization. This indicates a maternal origin and an essential role for Ss2 in cleavage and gastrulation of embryos. Immunostaining showed cellular morphological defects and reduction of cell proliferation in *ss2* mutants. We also found a significant expression reduction of polycomb group ring finger protein 6 (*pcgf6*) and downstream pluripotency genes *oct4*, *nanog* and *sox2* in *ss2* mutants during early embryonic development. We suggest that maternal loss of *ss2* may impair the pluripotency maintenance complex and lead to the failure of cleavage and gastrulation. We are now investigating the interaction network of Ss2 signaling and the pluripotency maintenance complex during early embryonic development.

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AN HYDROXYTYROSOL-RICH EXTRACT FROM OLIVE JUICE AMELIORATES THE OBESOGENIC EFFECTS OF A HIGH-FAT DIET IN GILTHEAD SEA BREAM

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In the context of improving aquaculture sustainability, and in addition to the shift toward the use of vegetable ingredients in fish feeds formulation, the inclusion of phytocompounds in functional diets is nowadays uptrend. Hydroxytyrosol (HT) is a polyphenolic compound that can be found in olive oil and other derivatives with proved antioxidant, anti-inflammatory and anti-obesogenic properties in mammals. However, these beneficial characteristics of HT have not been fully investigated in fish. The aim of this study was to evaluate the effects of the inclusion of an HT-rich extract obtained from olive juice as an additive (0.5 g HT/Kg feed) in a high-fat (24% lipids) diet in gilthead sea bream (*Sparus aurata*) juveniles. The two experimental diets were administered at a standard (3% of total biomass in the tank) or at a restricted ration (40% daily reduction) for 8 weeks. Hepatosomatic and mesenteric fat indices, plasma metabolites, histological analysis of adipose tissue and mRNA levels of lipid metabolism-related genes in liver and adipose tissue were analyzed. At the end of the trial, visceral fat weight and the body fat percentage measured with a fatmeter were significantly lower in fish fed with the restricted ration, regardless of the diet. Nevertheless, plasma levels of glucose and triglycerides remained unaltered, concerning either diet composition or ration regime. In adipose tissue, feeding the diet containing the HT-rich extract downregulated the mRNA levels of the fat transporter cd36, suggesting reduced fatty acid uptake. However, area and number of adipocytes, and lipogenesis and lipolysis-related gene expression in this tissue did not change in response to any of the two factors. At hepatic level, according to transcriptional data, a reduced lipid turnover was induced by HT-containing diets, and specifically, fatty acid uptake markers (lpl, cd36, fatp1 and fabp11a) mRNA levels were lower in the fish fed the HT-rich extract, supporting the anti-obesogenic potential of this phytocompound. Overall, this study provides new insights into the beneficial use of HT as a dietary additive in aquafeeds to modulate adiposity in farmed fish.

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EVIDENCE FOR THE PRESENCE OF FATTY ACID SENSING MECHANISMS BASED ON CHANGES IN INTRACELLULAR METABOLIC PATHWAYS IN THE RAINBOW TROUT GASTROINTESTINAL TRACT

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In mammals, it is well known that intestinal cells have receptors able to sense the presence of nutrients (carbohydrates, lipids/fatty acids and proteins/amino acids) in the lumen and respond to such a presence with the release of signaling molecules that ultimately modulate food intake. Recent studies from our research group have demonstrated the presence of equivalent nutrient sensors in the gastrointestinal tract (GIT) of the rainbow trout. In addition to this sensing pathway, sensing mechanisms independent of receptors but based on changes in intracellular metabolic pathways are know to operate in other tissues, e.g. the brain, in both mammals and fish. However, whether these mechanisms are also present in the fish GIT is yet to be investigated. This research aims to study the putative intestinal presence of fatty acid (FA) sensing mechanisms based on changes in pathways involved in lipid metabolism in rainbow trout. For this, rainbow trout were intragastrically administered with fatty acids of different length and degree of unsaturation [i.e., octanoate (8-carbon saturated FA), oleate (18-carbon monounsaturated FA), α-linolenate (ALA, 18-carbon PUFA) and butyrate (4-carbon saturated FA)], and the activity and mRNA abundance of key enzymes related to lipid metabolism [i.e., ATP citrate synthase (Acly) and fatty acid synthase (Fas), both involved in fatty acid biosynthesis, and carnitine palmitoyltransferase 1c (Cpt1c), which converts long-chain acyl-CoA species to their corresponding long-chain acyl-carnitines for transport into the mitochondria to be used for energy through β-oxidation)] were assessed in different regions of the GIT. Results from thisr study demonstrated that the luminal presence of fatty acids leads to important changes in the mRNA abundance and activity of the three enzymes analyzed. These observations provide the first evidence demonstrating that the luminal presence of fatty acids induce changes in parameters related to lipid metabolic pathways in the rainbow trout GIT, which could relate to putative fatty acid sensing systems based on the intracellular lipid metabolism within intestinal cells.

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MUSCARINIC SYSTEM REGULATES THE EXPRESSION OF INSECT INSULIN-LIKE PEPTIDE GENES

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The insulin/insulin-like peptide signalling (ILP signalling) pathway is an old and evolutionarily conserved pathway that widely regulates metabolism throughout the whole metazoa kingdom. It is involved in the control of metabolism *sensu stricte*, as well as in many other aspects of life, such as growth, reproduction, lifespan, resistance to stress conditions and immune activity. Thus, its activity is crucial for reproduction success of population and determines if the population size will increase or decrease. Of course, ILP signalling is a part of bigger and more complicated network in which muscarinic receptors are also involved. Our preliminary studies showed that activation or inhibition of muscarinic receptors change the expression of genes encoding insulin-like peptides in differentiated way. The observed changes are tissue-specific and specific for individual insulin-like peptides.

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THE INFLUENCE OF MYOTROPIC INSECT NEUROPEPTIDES ON MAMMALIAN CARDIOMYOCYTES – PRELIMINARY RESULTS

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Neuropeptides play a crucial role in regulation of physiological processes. They act as neurotransmitters, neuromodulators and neurohormones. Thus, they modulate the activity of neural and non-neural tissues in complex manner, in two major ways 1) by changes of cell membrane bioelectrical properties, and 2) by changes of concentration of secondary messengers. Each year, numerous insect neuropeptides are identified. Big number of them was proved to be myotropic. Some properties of insect neuropeptides can be specified based on homology to their mammalian counterparts. It is very interesting, if insect and mammalian homologs can show cross-reactivity, if insect neuropeptides can activate mammalian receptors.
To check the influence of neuropeptides on cell electrophysiology, we use a multielectrode arrays system (MEA system) which allows to measure the extracellular action potential (EAP). Videomicroscopy technique was used to measure the frequency of contractions of cardiomyocytes in vitro derived from induced pluripotent stem cells. The cells were cultured on 6-well MEA plates coated with gelatin and showed stable activity. Application of proctolin to culture medium increased the frequency of cells contraction. Moreover, we noticed changes in the amplitude of extracellular action potential (EAP). CCAP also increased frequency of cardiomyocytes contractions, but the application of the peptide significantly deregulates the contraction rhythm. Other tested neuropeptides slightly changed the contraction frequency of the tested cells.

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IDENTIFICATION OF SULFAKININ RECEPTORS (SKR) IN TENEBRIO MOLITOR BEETLE AND THE INFLUENCE OF SULFAKININS ON CARBOHYDRATES METABOLISM

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Insects sulfakinins (SKs) are neuropeptides structurally and functionally homologous to the mammalian gastrin/cholecystokinin (CCK) family. SKs together with sulfakinin receptors (SKRs) cooperate in sulfakinin signaling in variety of biological functions, including food intake, carbohydrate and lipid metabolism or muscle contraction. In the present study, we determined the distribution of SKRs in *Tenebrio molitor* larvae and revealed the impact of nonsulfated and sulfated SKs on carbohydrates and insulin-like peptides (ILPs) level in beetle hemolymph. Our results indicate the presence of both sulfakinin receptors, SKR1 and SKR2, in the nervous system of *T. molitor*, but distribution of SKR2 transcripts in peripheral tissues such as fat body, gut or hemolymph was more widespread than SKR1. Moreover, we evidenced that SKs regulate carbohydrates and ILPs level in insect hemolymph, and that presence of sulfation is not crucial for peptides activity. Our study confirms the role of SKs in maintaining of energy homeostasis in insects.

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IMPACT OF DIETARY VEGETABLE PROTEIN CONTENT ON AMINO ACID SENSING AND FEED INTAKE REGULATION IN RAINBOW TROUT JUVENILES

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In fish nutrients trigger different endocrine and metabolic responses within the gastrointestinal tract (GIT), which have an important role in controlling feed intake. In previous studies, we demonstrated that the individual administration of specific amino acids impacts some of such mechanisms in a way similar to that described in mammals. However, the impact of a whole diet (where interactions between different nutrients occur) on nutrient-triggered endocrine and metabolic responses remains unexplored. In this study, we evaluated the response of amino acid sensing systems along the GIT of rainbow trout (Oncorhynchus mykiss) fed with two diets differing in their amount of fishmeal and vegetable protein: one containing 20% fishmeal and 10% soy protein concentrate (NF/LV), and another containing 10% fishmeal and 20% soy protein concentrate (LF/HV). Feed intake was registered daily during 4 weeks with no significant differences observed. At the end of the feed intake trial, we collected samples of different areas of the GIT (stomach, anterior intestine and posterior intestine) and hypothalamus at different times: after 48h fastening (time 0); and at 1h (time 1), 4h (time 2) and 24h (time 3) after refeeding. We evaluated the enzyme activity of pepsin in the stomach (at times 0 and 1); and trypsin and chymotrypsin in the proximal intestine (both at times 0 and 2). Results showed an increase in pepsin activity 1h after refeeding in fish offered the LF/HV diet, and an increase in both trypsin and chymotrypsin activity 4h after refeeding in NF/LV-fed fish. Moreover, gastrointestinal levels of mRNAs encoding amino acids receptors and transporters, key gut hormones (GHRL, CCK, PYY, GLP-1) and hypothalamic neuropeptides (NPY, AgRP, CART and POMC) were measured by RT-qPCR. Results showed some changes mainly in receptors and transporters mRNA levels among different sampling times. Finally, these results together show how the different vegetable protein content of these 2 diets affects amino acid sensing systems.

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SEROTONIN RHYTHMS IN RAINBOW TROUT INTESTINE. CIRCADIAN INFLUENCE AND ROLE OF PHOTOPERIOD AND FEEDING

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The gastrointestinal tract (GIT) is a key element in the absorption of nutrients from food and its regulation involves multiple neuroendocrine processes. The GIT is rich in monoaminergic neurotransmitters, in particular serotonin (5-hydroxytryptamine, 5HT), which centrally modulates feeding behavior. In addition, 5HT from the TGI locally regulates intestinal motility and secretory processes. The synthesis of 5HT takes place from L-tryptophan and the enzyme tryptophan-5-hydroxylase (TPH) is a key step in this pathway. In fish, there are two TPH isoforms, TPH1 and TPH2, with different functional relevance. Degradation of 5HT produces mainly 5-hydroxy-indolacetic acid (5HIAA), through the action of monoamine oxidase and aldehyde dehydrogenase.Digestive activity occurs on a daily rhythmic basis under the control of local circadian oscillators. In relation to serotonin, a daily rhythmic profile of serotonergic activity has been described in the mammalian digestive tract, but there are no data in fish. Its relationship with local circadian oscillators, as well as the influence of environmental variables, is also unknown. In this study, rainbow trout (Oncorhynchus mykiss) were used to study the existence of daily rhythms in serotonergic activity of the GIT, its dependence on the circadian system and the influence of photoperiod and feeding. HPLC techniques were used to measure 5HT and 5HIAA levels, as well as RT-qPCR for the expression of Tph1 and Tph2 genes. Trout were distributed into four groups: 1) Control, 12L:12D photoperiod and feeding at ZT3 (ZT = zeitgeber time; ZT0 = lights on); 2) constant darkness and fasting for 48 h (DD+F); 3) DD and refeeding at CT3 (CT0 = subjective day start) for the last 72h; 4) 12L:12D and feeding at ZT15. Fish were sacrificed throughout the 24h daily and samples of foregut and midgut were collected. The results obtained reveal the existence of Tph1 and Tph2 expression rhythms in the intestine of trout maintained under standard (control) conditions with peaks at the beginning of the night. The absence of light and/or food strongly decreased these rhythms and their amplitude. Levels of 5HT and 5HIAA also increased during the night in the control group, although light and/or food significantly influenced the rhythmic profile. These results corroborate the existence of daily rhythms of serotonergic activity in the trout TGI and point to regulation by a local circadian oscillator, although the role of light and food in these rhythms is unclear.

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DIURNAL NEUROPEPTIDERGIC OSCILLATIONS IN THE BRAIN OF AN ANURAN, *EUPHLYCTIS CYANOPHLYCTIS*

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Circadian rhythms (CR) synchronize daily biological clock with the external environment clues. Most of the physiological activities are regulated by circadian rhythms via hormones and neuropeptides. Regulation of physiological activities by circadian clock is well elucidated in mammals. In spite of photoperiod dependent behavioral activities of anurans, the oscillations in the neuropeptides coordinating circadian clock in anurans is underexplored. Neuropeptide NPY and CART are known to regulate feeding and reproductive behaviour in anurans. Therefore, an attempt has been made to study the natural diurnal oscillation in these peptides in frog *Euphlyctis cyanophlyctis*. In the present work, frogs were fixed on field at noon, midnight (in natural conditions, photoperiod- ~13 hr) and immunohistochemical localization of CART and NPY was performed. We observed a differential expression of CART and NPY in various brain regions of *E. cyanophlyctis*. CART expression was higher in the POA, Hypothalamus, AV, EW and Pars distalis of Pituitary in the night compared to day. On the contrary, expression of NPY was reduced in these regions. During night, CART expression increased in the Habenula, Raphe Nucleus and Nucleus isthmus. While, during day, CART and NPY expression increased in pineal only. In *Torus semicircularis*, both the CART and NPY expression increased during night as compared to the day. Oscillations in the levels of CART and NPY indicate that neuropeptides exhibit diurnal oscillation. Neuropeptidergic oscillations in the limbic regions suggests their probable role in diurnal behaviors.

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ASSOCIATION BETWEEN MICROPLASTIC OCCURRENCE AND CYTOKINE SIGNALING IN THE GASTROINTESTINAL TRACT OF COMMERCIAL FISH SPECIES

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There is a growing scientific evidence that microplastic (MP) particles can accumulate mainly in the gastrointestinal tract of aquatic organisms. However, there is still limited knowledge on the potential effects of MPs' accumulation in the gut to cause aquatic toxicity. The aim of the present study is to evaluate MPs accumulation and their immunotoxic effects in the digestive tract of three species showing different ecological traits: the red mullet (Mullus barbatus), the European hake (Merluccius merluccius) and the Atlantic mackerel (Scomber scombrus). Infrared spectroscopy (FTIR-ATR), micro-Raman and electron scanning microscope (SEM) were used to correctly identify the main plastic polymers detected in gut contents. In addition, we investigated the association between MP uptake and intestinal inflammation by evaluating expression and secretion of proinflammatory cytokines. MP abundance ranged from 1–20 items/individual in red mullet, from 2–15 items/individual in European hake and from 3-25 item/individual in the Atlantic mackerel. The majority of ingested MPs were fibers, while the dominant colors were black and blue in all species. We found that the most common polymer types were polyethylene and polypropylene. Moreover, it was observed that MP abundance was highly positive correlated to pro-inflammatory cytokines and antioxidant enzyme transcript levels suggesting ROS generation and an infiltration of immune cells in the gut. Our findings provide evidence that the induction of cytokine signaling is one aspect of the complex mechanism by which MPs affect the gut system in fish.

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ANXIOGENIC EFFECTS OF DIFFERENT FEEDING CONDITIONS IN GOLDFISH

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Stress and food intake seem to be interrelated by their regulation at the hypothalamic level. In fish, stressors commonly cause alterations in feeding behavior, although the sense of these alterations (orexigenic/anorexigenic) is variable, and the underlying pathways are unclear. On the other hand, the effect of feeding status in anxiety is still being explored, and a relationship between orexigenic peptides as Neuropeptide Y and Agouti-related protein and anxiety behavior has been proposed. The aim of this work was to determine the effect of fasting and different feeding regimes on anxiety-like behavior and exploration in goldfish (Carassius auratus). First, anxiety-like behavior studied by both the black/white and open field behavioral tests was compared in 3-h postprandial fish and 48-h fasted fish. Regarding the black/white test, scototaxis (time spent in the black side of the tank) tends to decrease in the fed group. In accordance, the open field test revealed that fasted fish present increased thigmotaxis, venturing less to the center of the tank and swimming faster in this area, indicating a higher level of anxiety. In a second experiment, two groups of fish were fed once a day either following a fixed schedule or at non-expected random times. The food anticipatory activity was lost on randomly fed fish. After 3-4 days under random feeding, fish also showed a higher thigmotaxis response and an increase in the latency in entering the center zone of the open field test. In summary, present data suggest that fasting is an anxiety-like state in goldfish, that has been associated with the "wanting" hedonic component of food in mammals. Moreover, scheduled feeding reduces anxiety-like responses in goldfish, supporting the relevance of the relationship among temporal homeostasis and welfare.

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AUTOPHAGY: AN IMPORTANT PLAYER IN GERM CELL MAINTENANCE AND GONADAL SEXUALITY

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Autophagy, or cellular self-digestion, is an essential cellular process imperative for energy homeostasis, development, differentiation, and survival. It was recently observed that autophagy genes are relatively abundant in steroidogenic tissues and its associated processes are sex-biased and estrogen-responsive in fish. Previously, we also reported that, autophagy is instrumental for gamete maintenance in adult fish. So, in this investigation, we explored the possibility of autophagic involvement in embryonic germ cell development and sexual assignment in medaka. In depth analysis show that, all candidate autophagy gene are prevalent in the germ cells, and homozygous knockout (KO) of ULK1b, ATG13, LC3a, SIRT1 and DOR results in complete germ cell depletion and thus renders sterility. Interestingly, Beclin1-/-, HK4-/- and HK2(+/-)/HK4(+/-) mutants show severe defects in sperm characteristics (motility, etc.) and oocyte development (micropyle formation, etc.), thus affecting the fertilization process. This could only be rescued by overexpression of respective gene in concern. However, mutation of HK1, HK2, ULK1a and LC3g does not have any effect on fertility. To understand the autophagy-steroid connection in gonad, we used estrogen receptor KO fish and found that, apart from significant alterations in autophagic genes, the micropyle formation, sperm motility, fertility, and sex ratio (at adulthood) were highly affected in both fish groups, and had a strong correlation with ATG13, LC3a, especially in the ERb2-KO fish gonads. RNA-seq analysis and subsequent validation suggested that autophagy-estrogen-early germ cell development are interlinked. Earlier using ERb2-KO medaka, we found that calcium ion signaling associated alternate (independent of hexokinase/AMPK pathway) autophagy pathway also affects the germ cell health and female development. Cumulatively, our data suggests that germ cell autophagy is critical for early gamete development and further analysis are required to unveil the steroid responsive autophagy regulatory switches to confirm the gender-skewed autophagy. Expectedly, this study may furnish newer appreciation for fertility management, and gender-specific medicine research and therapeutics.

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STRESS RESPONSE INDUCED BY HYPERSALINE CHALLENGE IS MODULATED BY CORTICOSTEROIDS, CORTISOL AND DEXAMETHASONE, IN THE GILTHEAD SEABREAM (*SPARUS AURATA*)

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In fishes, the stress system involves different mechanisms at several levels, being the cortisol, as the final product of hypothalamus-pituitary-interrenal axis, the primary corticosteroid involved in stress response. The influence of dietary treatment for 30 days with control fish feed (CT), supplemented with cortisol (F; 400 mg/kg fish feed) or with the synthetic glucocorticoid dexamethasone (DEX; 300 mg/kg fish feed) on molecular biomarkers of stress system before and after hyperosmotic challenge (direct transfer from seawater -SW, 38 ppt- to high salinity water -HSW, 60 ppt- during 3 days), was evaluated.DEX significantly reduced plasma cortisol at 38 ppt, while F group presented intermediate levels between CT and DEX groups. After HSW challenge, cortisol was significantly higher in CT. In the brain, in SW, all evaluated genes (trh, crh, and crhbp) were unaltered. Transfer to HSW significantly up-regulated trh expression, but only in CT. Overall, expression of the three genes was increased in all the groups due to HSW challenge but without significant differences as observed for trh in CT. In the hypophysis, pomcb gene expression enhanced significantly in DEX acclimated to SW compared to CT, while F treatment presented intermediate values. However, this expression was significantly down-regulated in DEX group after HSW challenge. No significant differences were observed for pomca1 and pomca2 expressions due to hormonal treatments for 30 days or after HSW challenge. In DEX, gh gene expression was significantly up-regulated (linked to a lower growth performance, -11 % at day 30). Interestingly, its expression increased in all the groups after HSW transfer for 3 days, hence supporting an osmoregulatory role for this gene. Lastly, no significant differences were observed for star expression in the head kidney at SW or after HSW transfer. Our results provided new insights into the fish physiological disruption due to exogenous synthetic corticosteroids on different molecular biomarkers of the stress system but also after an additional hyperosmoregulatory stress. In addition, the roles of trh, pomcb, as well as of gh, in stress response is supported in the CT group.

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ADIPOKINETIC HORMONE REGULATES DEFENCE REACTIONS AGAINST HONEYBEE VENOM IN THE AMERICAN COCKROACH *PERIPLANETA AMERICANA*

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Originally, honeybees developed their sting apparatus and venom as a very sophisticated mechanical and chemical weapon against other honeybees and predatory insects. The use of these weapons against birds and mammals appeared on the evolutionary scene much later; nevertheless, it is well known that they are comparably potent due to their non-specific mechanism. Interestingly, relatively little is known about the biological and biochemical defence responses to bee venom in the insect body. Therefore, in this study, we monitored physiological reactions in the insect model, the American cockroach *Periplaneta americana*, after the application of bee venom. Injection of crude venom into the body of the cockroach caused a strong stress response (LD50 = 1,063 µl of venom) associated with significant increases in the levels of the adipokinetic hormone (AKH, insect stress neurohormone) in both the central nervous system (CNS) and the haemolymph. The venom application also elicited extensive destruction of the ultrastructure of muscle cells, especially myofibrils and sarcomeres. In addition to that, the simultaneous co-application of venom and cockroach AKH (= Peram-CAH-II) significantly reduced the destructive effect; in other words, the muscle tissue ultrastructure was much less damaged. Furthermore, bee venom modulated the levels of carbohydrates, lipids, and proteins in the haemolymph, as well as the activity of digestive amylases, lipases, and proteases in the midgut. The level of vitellogenin in the female haemolymph was significantly decreased after venom treatment. The energy was most likely not wasted on egg development, but rather on removing stressor. Surprisingly, dopamine and glutathione levels (GSH and GSSG) increased just slightly in the CNS after the injection of venom, but dopamine levels increased significantly after both the co-application of AKH + venom, and application AKH itself, while GSH and GSSG levels increased significantly, only when both agents were co-administrated. In summary, the results demonstrate the overall response of the cockroach body to bee venom treatment and at least a partial role of AKH in these reactions.

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IONOTROPIC GLUTAMATE RECEPTORS ARE POSSIBLE MEDIATORS OF PHEROMONE SIGNALLING IN ECHINODERMS

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Ionotropic glutamate receptors (iGluRs) are a group of ligand-gated ion channels activated by glutamate that regulate synaptic communication in the nervous systems and have been shown to be mediators of pheromone signaling in insects. These receptors are composed of three to five protein subunits that assemble in homo and heterodimers to form an ion-conducting pore in the center, and are expressed in different organs, including endocrine cells. In metazoans, six main subfamilies have been characterized: α-amino-3-hydroxy-5-methyl-4-isoxa-zolepropionic acid (AMPA) receptors (Gria/GluA), kainate receptors (Grik/GluK), N-methyl-d-aspartate (NMDA) receptors (Grin/GluN), delta receptors (Grid/GluD) and two are specific to invertebrates the phi receptors (Grif/GluF) and epsilon receptors (Grie/GluE). Recently, we described a new subfamily of iGluRs named GluH that are specific to echinoderms and largely expanded in holothurians. Three main echinoderm receptor clades, GluHA, GluHB and GluHC, emerged during evolution via lineage and species-specific tandem gene duplications. The GluH S1 and S2 domains within the ligand-biding domain share only 39%–46% amino acid sequence similarity with the vertebrate iGluRs, suggesting they may be activated by distinct molecules. Transcriptome analysis revealed that GluH genes are the most expressed type iGluRs subunit genes in the sea cucumber (*Holothuria arguinesis*), some are exclusive to tentacles, an organ with a chemosensory role. The existence of multiple GluH subunits may provide alternative receptor combinations in tentacles thus expanding the functional possibilities and widening the range of pheromonal compounds detected during echinoderm aggregation and spawning.

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IMMUNOENDOCRINE RESPONSE OF THE HEAD-KIDNEY TO A PATHOGEN CHALLENGES IN *N. ROSSII*

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Immune and endocrine system interactions are well established in mammals but has been less studied in teleost fish. The current state-of-the-art in fish hints at bidirectional communication between hormones and cytokines. In this study, the Antarctic Notothenioid *Notothenia rossii*, which radiated from a single benthic ancestor around 10 million years ago was used to study the immunoendocrine response to a pathogen challenge. Experiments were performed in the Great Wall Station in King George Island by immersing *N. rossii* (30 ± 4.2 cm length and 312 ± 124.7 g weight) in sea water containing a bacterial endotoxin (E. coli LPS 0111:B4) or viral agonist (Poly I:C). The head-kidney, that contains cytokine producing lymphoid tissue and endocrine cells producing corticoids, was collected 8h after an immersion challenge. Enzymatic markers of the immune response and cortisol were measured in plasma and transcriptome sequencing of the RNA from the head-kidney was performed. Plasma cortisol was not significantly different between the control or treatment groups indicating the immune challenge did not provoke a stress response. Blood antiprotease activity, and the haematocrit were significantly ($p < 0.05$) decreased after the Poly I:C challenge suggesting a systemic immune response was stimulated. Differentially expressed genes (DEGs) between the head-kidney of control and immune challenged fish were numerous and greater than ten thousand DEGs (FDR 0.05) were identified (FDR < 0.05) and included both immune and endocrine related factors. An effective immune challenge was elicited and interferon and other cytokines like lysozyme g, complement C4, interleukin-1β, tumor necrosis factor α, toll-like receptors 3 and 5 modified are already described in literature but we cannot distinguish between the different treatments as we observed in other immune tissues. Significant down-regulation of transcripts encoding the growth hormone receptor (ghr), insulin-like growth factor II (igf2) receptor, insulin-like growth factor-binding protein 5 (igfbp5), estrogen-related receptor gamma (esrrg), Ras-related and estrogen-regulated growth inhibitor (rerg), thyroid receptor-interacting protein 11 (trip11) and prolactin regulatory element-binding protein (preb) occurred in fish exposed to LPS and Poly I:C by immersion. Our results indicate that endocrine factors may play a role in immune system defence in head-kidney under both immune challenges with slightly differences namely in hormone-sensitive lipase (hsl) and thyroid hormone receptor-associated protein 3 (thrap3) exclusively observed after LPS challenge. Further experiments need to be performed to establish their role in immune system of *N. rossii*.

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PUTATIVE ROLE OF DNA METHYLATION IN THE GH RESPONSE OF GILTHEAD SEABREAM (*SPARUS AURATA*) TO OSMOTIC CHALLENGE

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Contradictory results have been obtained on the synthesis and release of growth hormone (GH) during *S. aurata* adaptation to hypo-osmotic environments. Evidence in mammals and fish on the possible epigenetic regulation of gh gene encourages us to evaluate the putative role of DNA methylation in the GH response to changes in osmotic conditions. Moreover, it was hypothesized that if changes in GH-related DNA methylation occur, it would be influenced by the transcriptional response of the pituitary to the environment, probably through chromatin accessibility. Hypo-osmotic transfer of sea bream juveniles from seawater to brackish water (37 to 15 psu) significantly decreased osmolality from 380 to 340 mOsm after 8 h. Thereafter, osmolality increased again but at 24 h was still lower than before transfer. Sodium and GH concentrations in plasma mirrored osmolality changes, suggesting a decrease or no change in GH secretion. Thereafter, pituitary in vitro assays were performed using media simulating physiological variations in plasma osmolality (340 and 380 mOsm), and DNA methylation remodeling agents (genistein, SAM, AZA) for 24 h under these two conditions. GH secreted by the pituitaries after 24 h of exposure to the agents and changing osmolality was influenced by both the presence of methylation remodeling agents and media osmolality, but not by their interaction. Osmolality had by far the major single effect after 24 h of exposure: a reduction in media osmolality tend to decrease, as observed in vivo, GH secretion irrespective of the presence of agents, though a statistical difference was only fund for AZA. The same pattern was observed 3 days later even when all glands were kept under control (380 mOsm) osmolality. Yet, the effects seem to fade even for AZA, though gh gene expression at this time was still significantly lower in AZA treated glands. Interestingly, this occurred only if glands experienced a change in medium osmolality. Results sustain the hypothesis that the epigenetic effects of these substances depend on the environmental context. While some of used agents may exert effects other than on DNA methylation, AZA is a well-known methylation inhibitor. Decreased expression of gh after exposure to AZA under certain conditions suggests that intragenic methylation would play a role in GH regulation, though effects may occur also at other genes/regions upstream GH. Noteworthy, under our experimental conditions, the effects of AZA on pituitaries seem to be target-specific, as it did not produce an overall decrease in DNA methylation.

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CAN CRUSTACEAN ECDYSIS BE 'TRIGGERED'?: EXPLORING THE ECLOSION HORMONE (EH) AND ECDYSIS TRIGGERING HORMONE (ETH) SYSTEM IN *CARCINUS MAENAS*

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Ecdysis, the shedding of the exoskeleton, is a defining characteristic of growth and development in arthropods, which is controlled by a highly coordinated release of neuropeptides. In insects, the process of ecdysis is initiated by the release of ecdysis triggering hormone (ETH) from specialised Inka cells, which leads to secretion of eclosion hormone (EH) and a positive feedback loop with ETH results in a predictable behaviour pattern to release the exoskeleton from the underlying cuticle. Despite the evidence of similar behaviour patterns in crustaceans, the precise roles, if any, of these key insect neuropeptides has yet to be established. Transcriptome analysis of the green shore crab *Carcinus maenas* throughout the molt cycle has identified two transcripts encoding EH-like peptides, and one encoding an ETH-like molecule named carcikinin (CK), leading us to investigate whether crustaceans do, in fact, possess an EH/ETH system analogous to insects. We present here the neural architecture of both EH and ETH using immunohistochemistry (IHC) and in situ hybridisation (ISH), as well as the gene expression in key nervous tissues. The presence of one EH transcript (CamEH1), expressed solely in the eyestalk and adjacent to a neurohemal release centre, as well as the presence of CK in pericardial organ (PO), would suggest that both molecules are secreted neuropeptides. The second EH (CamEH2) is widely distributed throughout non-neuronal tissue and bears less similarity with that of insects but was intriguingly upregulated in the Y-organ (molting gland) of late pre-molt crabs. CK expression was significantly upregulated in late pre-molt for ES, CG and VG, however protein synthesis, measured by time-resolved fluorescence immunoassay, was sustained into early pre-molt. These results strongly suggest a role in ecdysis for both EH-like peptides and CK. We are now investigating release of these neuropeptides in the hemolymph to establish their position in the sequence of the hormone release cascade, such as those previously determined for CHH, bursicon, CCAP and Ast-C, with a further aim to carry out knockdown studies and ascertain a definitive functional role.

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EVOLUTION OF OXYTOCIN AND VASOTOCIN RECEPTOR GENES IN VERTEBRATES

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Oxytocin's name literally means "quick birth" and refers to the peptide's ability to induce contractions of the uterus. Later oxytocin was found to stimulate lactation. However, the ancestral peptide arose hundreds of millions of years before the uterus and the mammary glands. The ancestral peptide gene was duplicated in early vertebrate evolution and gave rise to oxytocin (OT) and vasotocin (VT, in mammals called vasopressin) (1). The receptor side of business is more complicated. We showed several years ago that the ancestral jawed vertebrate had no less than six related receptors that convey the broad range of actions of these peptides (2). This receptor repertoire arose by a local gene duplication resulting in the ancestor of VTR1/OTR and the ancestor of VTR2. Subsequently this gene pair was quadrupled in the early vertebrate genome doublings. Then one copy of each ancestral gene was lost, resulting in one subfamily consisting of the three members VTR1A, VTR1B and OTR, and the other consisting of three VTR2 genes named VTR2A, VTR2B and VTR2C. Our conclusion was based on both sequence analyses and conserved chromosomal synteny with many other gene families. This parsimonious scheme has been questioned by other researchers who have instead proposed serial duplications (3). Therefore, we have investigated a large number of additional vertebrate species from new high-coverage genome assemblies, including species that occupy key phylogenetic positions: the polypteriform fish reedfish (*Erpetoichthys calabaricus*), the cartilaginous fish thorny skate (*Amblyraja radiata*) and a more recent high-quality assembly of the Western clawed frog (*Xenopus tropicalis*) genome. Our analyses forcefully corroborate our previous conclusion: the evolution of the OTR/VTR gene family can be most parsimoniously explained by a local gene duplication followed by two genome doubling events resulting in six ancestral genes (4). Later, differential gene losses of VTR2 genes have occurred in different lineages. For instance, the VTR2 family in mammals has retained only VTR2A whereas birds have retained only VTR2C. Some evolutionary lineages have expanded the receptor repertoire further by genome doubling resulting in 9 genes in some teleosts (8 in zebrafish) and 10 genes in a sturgeon and the American paddlefish. Thus, there is much more complexity on the receptor side than the peptide side of this system.

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IN VITRO RESPONSIVENESS OF MALE AND FEMALE SKIN FIBROBLAST CELLS UNDER THE INFLUENCE OF ESTRADIOL OR TESTOSTERONE

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Steroid hormones, like estrogen and testosterone have multiple effects on skin including the promotion of wound repair. The effects of these hormones are mediated when they bind to their specific receptors, which are present in skin fibroblasts. Fibroblasts are the principal cells of the dermis, and together with other skin cells they are responsible for maintaining its normal structure and function. Skin fibroblasts also play a central role in damage repair and studies of these cells can provide insight into the potential response of skin to steroid hormones or other novel compounds. Despite the differing hormonal background (estrogen or testosterone) of cells harvested from males and females most in vitro studies do not consider cell origin in their design. In the present study the effect of two steroid hormones, 17 β-Estradiol (17βE) and testosterone (T), on two fibroblast cell lines of distinct origin, male (BJ5ta) and female (HDF) was characterized. Our results showed that, while the estrogen receptors, ESR1 and GEPR, and the testosterone receptor, AR, were present in both male and female cell lines the estrogen receptor, ESR2, was only present in the male fibroblast cell line (BJ5ta). Gene expression analysis also showed that the expression of these receptors differed between cell lines. Exposure of HDF cells to 17βE slightly increased receptor expression while no change in expression occurred in the presence of testosterone. Exposure of BJ5ta cells to 17βE or T slightly decreased expression of all cognate receptors except for ESR2, that was unaffected. Preliminary results showed that 17βE and T did not influence BJ5ta cell proliferation but promoted HDF cell proliferation. The effect of 17βE and T on cell migration was assessed using a scratch assay and the ECIS (Electric Cell-substrate Impedance Sensing) system. Exposure of both cell lines to 17βE or T increased cell migration and reduced the time for wound recovery. Immunofluorescence assays developed at different stages of cell migration revealed both cell lines presented cytological characteristics typical of migrating cells.

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THE 'CRUSTACEAN CARDIOACTIVE PEPTIDE' SIGNALING SYSTEM IS CRUCIAL FOR ECDYSIS AND INHIBITS ECDYSTEROIDOGENESIS IN THE DESERT LOCUST

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In insects, the process of molting is tightly controlled by hormones. Towards the end of the molting cycle, a set of neuropeptides coordinates the innate behavioral sequence designated as 'ecdysis', *i.e.* the shedding of the old cuticle. In this context, the crustacean cardioactive peptide (CCAP), a structurally conserved neuropeptide, plays a crucial role. In the desert locust, *Schistocerca gregaria*, we identified the genes encoding the CCAP precursor and three G protein-coupled receptors that are activated by CCAP with EC_{50} values in the (sub)nanomolar range. Analysis of spatiotemporal expression profiles revealed that these receptors are expressed in a variety of tissues, including the prothoracic glands, which are the endocrine organs where ecdysteroidogenesis occurs. RNAi-mediated knockdown of CCAP precursor or receptors showed that the CCAP signaling system is not only essential for ecdysis, but also influences the ecdysteroid production in these endocrine glands. Interestingly, when compared to control treatment, the depletion of CCAP precursor or receptors resulted in significantly elevated transcript levels of several *Halloween* genes, which code for ecdysteroid biosynthesis enzymes, and in elevated ecdysteroid levels one day prior to ecdysis. Moreover, prothoracic gland explants exhibited decreased secretion of ecdysteroids in the presence of CCAP, indicating that this peptide can elicit a hitherto unknown prothoracicostatic activity. These results suggest the existence of an intricate crosstalk between developmental hormones, such as ecdysteroids, and neuropeptides controlling innate behaviors, such as CCAP.

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TESTING THE TROUT LIVER RTL-W1 CELL LINE POTENTIAL TO STUDY THE INFLUENCES OF TEMPERATURE ON THE EFFECTS OF ENDOCRINE DISRUPTORS

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Global warming is an undeniable reality, and one consequence is a continuous increase in the temperature of different water bodies. This phenomenon affects numerous processes in the aquatic ecosystems, including the impacts of the widely present endocrine-disrupting chemicals (EDCs). Within EDCs, ethinylestradiol (EE2) and progestins, such as levonorgestrel (LEV), are commonly detected in environmental waters. It is thus imperative to study the effects of temperature changes in the toxicology of EDCs to aquatic animals, including fish. Endocrine disruption research often relies on *in vitro* models, complying with the 3Rs principles. Primary hepatocytes are commonly used but also are specific and well-characterized cell lines, such as the rainbow trout liver-derived RTL-W1. This cell line presents several functional features of differentiated hepatocytes. To test the cell line potential to study the way temperature models the effects of EDCs, we exposed RTL-W1 cells (72h) to EE2, LEV or a mixture of both synthetic hormones (at 10 µM). Exposures were carried out at 18 ºC and 21 ºC, in 24-well plates, 80.000 cells per well. The cells grew at either temperature. At the end of exposures, cells were trypsinized, and the viability measured using the trypan blue exclusion assay. Cell suspensions were then centrifuged at 200 rcf, for 5 min, and pellets were frozen at -80 ºC for gene expression analysis. No differences were found in cell viability among experimental groups. The expression of two CYP P450 enzymes enrolled in xenobiotic metabolism was analyzed by qRT-PCR. The Two-way ANOVA results revealed that CYP1A was affected by temperature as its expression was lower at 18ºC than at 21ºC. As to CYP3A27 expression, it was independent of temperature but was significantly higher in the EE2 + LEV mixture compared with control and solvent control conditions. Given these results, the RTL-W1 cell line seems like a promising model to study the interactions of temperature and exposure effects of EDCs. Studies are undergoing to include genes from other relevant signalling pathways such as estrogenic, progestogenic and lipid metabolism.

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THE EFFECTS OF TWO PROGESTINS AND 17A-ETHINYLESTRADIOL ON CULTURED HEPATOCYTE SPHEROIDS OF BROWN TROUT (*SALMO TRUTTA*)

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Progestins are classified as emerging micropollutants in freshwater ecosystems. The concentrations of this class of contaminants in water have been increasing, since they are of anthropogenic origin and their use in human and veterinary medicine is in expansion. Apart from well-studied effects of progestins on fish, such as alterations of behavioral processes, impaired reproduction and involvement in intersex, the effects on structure and metabolism of liver are understudied; namely when progestins and estrogens are concurrent. Thus, this study is exploring sub-chronic effects of in vitro exposure hepatocyte spheroids to progestins and 17α-ethinylestradiol. Primary hepatocytes were isolated from brown trout (n = 3) and cultured for 12 days in non-adhesive plates which were constantly agitated with frequency of 60 times per minute at 18 °C. Shortly after plating, hepatocytes started to spontaneously form spheroids (3D cellular aggregates), which increased their size over time. At day 12, spheroids were exposed to either 17α-ethinylestradiol (0.3 μM), levonorgestrel and megestrol acetate in two concentrations (0.3 and 0.6 μM) or binary mixtures of these chemicals in 0.1% ethanol as a solvent. After a 6 days exposure, spheroids were measured and sampled for biochemical (lactate dehydrogenase and resazurin assays), biometrical, immunohistochemical and gene expression analyses. Both biochemical assays did not show differences between exposed groups or between exposed and control and solvent control groups, which supported that viability of exposed spheroids was not compromised. For biometry, no significant differences in sphericity, area and diameter of spheroids were found between groups. However, when anti-vitellogenin antibody was applied on histological sections, spheroids from all exposed groups had increased expression comparing with controls, proving all three chemicals could modify the hepatocyte function. Besides estrogenic targets, genes related with lipid-metabolism pathways are under study. As a perspective, analysis of the modeling effect of temperature in the same targets is being considered.

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P55
BROWN TROUT PRIMARY HEPATOCYTE SPHEROIDS – A MODEL TO ASSESS ESTROGENIC EFFECTS

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Hepatocyte monolayer cultures (2D) are a useful tool to assess mechanistic effects and liver toxicity caused by drugs. In contrast, tridimensional cultures (3D) have a greater similarity to in vivo models, which has contributed to an increasing use of these systems in mammals. Despite this, 3D hepatocyte cultures are not well established in fish. To improve the knowledge of 3D fish models, we aimed to culture for up to 25 days spheroids of primary hepatocytes obtained from 1 year-old brown trout (*Salmo trutta fario*) juveniles. Furthermore, we aimed to obtain a functional characterization of those spheroids along the time in culture, specifically focusing on estrogenic targets. For that purpose, spheroids collected at distinct days (8, 12, 16, 20 and 25 post-isolation) were pooled and the mRNA levels of estrogen receptor α (ERα), vitellogenin A (VtgA) and zona pellucida glycoprotein 2.5 (ZP2.5) were assessed by RT-qPCR. Cell viability was checked at those days using the lactate dehydrogenase (LDH) assay. Cell density was 5x105 cells/mL in 6-well microplates using 3 mL of DMEM/F-12 with 15 mM HEPES, 10 mL/L of antibiotic/antimycotic solution and 10% charcoal stripped FBS. Spheroids were maintained at 18 ºC, without additional supply of O2/CO2 and at constant agitation (~100 rpm). The LDH was measured in the culture medium, and a decrease in absorbance values was noticed on later culture days, suggesting that mature spheroids have greater membrane integrity. ERα, VtgA and ZP2.5 mRNA expressions were constant along the 25 days in culture, mostly between the 12th and the 20th day. Data suggest that spheroids were viable for up to 25 days and that levels of expression of three estrogenic targets were stable. In conclusion, primary hepatocyte spheroids from brown trout have potential to be used as a first line model to verify the impacts – and likely other aspects such as metabolism and mode of action – of estrogenic compounds in fish livers.

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P56

EFFECTS OF ANTIINFLAMMATORY DRUGS DICLOFENAC AND IBUPROFEN ON THE RESPONSES OF REPRODUCTIVE, METABOLIC AND STRESS HORMONES OF *ASTYANAX LACUSTRIS* (TELEOSTEI:CHARACIADAE).

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The occurrence of non-steroidal anti-inflammatory drugs (NSAIDs), such as Ibuprofen (IBU) and diclofenac (DCF), in aquatic compartments has been a major issue to non-targeted species. Although several studies reported NSAIDs harmful effects to aquatic biota, the information related to their toxicity and endocrine disruption remains unclear. This study evaluated the effects of IBU and DCF in the reproductive, metabolic and stress hormones profile in *Astyanax lacustris* females. Animals were exposed to 3.08 mgL-1 DCF and 13.7 mgL-1 IBU, concentrations based on previous toxicity tests. A total of 96 females were used, 4/aquarium, 4 groups: control (CTL), DCF, IBU and MIX (DCF+IBU) during 24 and 96 hours, when blood samples were collected. Estradiol (E2), testosterone (T) and cortisol (F) were measured in plasma using ELISA CAYMAN Kits and thyroxine (T4) and triiodothyronine (T3) using ELISA ARBOR Kits. The kits were validated for the species and data was analyzed using ANOVA one way. The concentration of T4 enhanced in females exposed to IBU compared to CTL after 24h, while T3 levels enhanced in MIX group compared to DCF after 96h. The plasma levels of F, E2 and T did not change after 24 or 96 hours. T4/T3 ratio increased in females exposed to IBU compared to CTL in 24h and was reduced in MIX compared to CTL in 96h. Despite the absence of changes in F, E2, T, in *A. lacustris* females, T4 and T3 decreased after XXX exposure to nanoproxen, another NSAID, and *A. lacustris* males also presented reduction in T and E2 plasma levels after DCF treatment. These results elucidated that NSAIDs can disrupt the endocrine axis in different ways, and the responses also depend on species and gender. Thereby, investigations are still needed at different levels to better understand the endocrine disruption mechanism of NSAIDs.

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ASSESSMENT OF ENDOCRINE DISRUPTING CHEMICALS BY USING PITUITARY CELL CULTURE OF EUROPEAN SEA BASS.

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Coastal and estuary environments are subjected to various pollutions resulting from human activities. Among the different chemicals spilt in the water not all have been tested for their endocrine disrupting potential on aquatic organisms. The pituitary is a key endocrine gland in vertebrates involved in the regulation of the main physiological functions controlling animal fitness. It plays a central role in the regulation of growth, metabolism, reproduction, stress, osmoregulation, by the expression and release of hormones in the bloodstream. We set up a 6 days in vitro primary pituitary cell culture of sea bass (*Dicentrarchus labrax*) in order to identify and evaluate the endocrine disrupting activities of emergent chemicals. We investigated the direct in vitro effect of two different chemicals, the homosalate (HMS) and the triclocarban (TCC), alone or in combination with oestradiol (E2). We measured their effects at high and environmental doses on the pituitary hormone gene (gh, fshb, lhb, tshba, pomc, sl, prl) and aromatase (cyp19b) expression by qPCR. Our primary results indicated that whatever the doses, HMS did not induce any significant effect on the target gene expression. In contrast, significant downregulation of tshba, lhb and cyp19b expression was observed after TCC exposure at high dose. Pituitary cell culture can be considered as a valuable tool for screening emergent chemical disrupting effects on pituitary endocrine signalling.

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DISRUPTION OF THE SEA BASS SKIN-SCALE BARRIER BY ANTIDEPRESSANT FLUOXETINE AND ESTRADIOL: IN VIVO AND IN VITRO EVIDENCE

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Fluoxetine (FLX) is a highly prescribed selective inhibitor of serotonin-reuptake and an emerging pollutant affecting fish behaviour, stress and reproduction, but little is known about possible actions and mechanisms in barrier tissues. We combined in vivo and in vitro approaches to demonstrate multi-level impacts of FLX on the sea bass (*Dicentrarchus labrax*) skin-scale barrier and on the estrogenic system. Juvenile sea bass intra-peritoneally injected with FLX had significantly increased levels of FLX and its metabolite nor-FLX. In contrast to the natural estrogen E2, FLX did not increase plasma calcium, phosphorus (P) or vitellogenin, although a slight decrease in scale P content was detected. Quantitative SWATH-MS proteomics of the scales identified 134 proteins that were affected by FLX. Modified proteins were mainly related to extracellular matrix and protein turnover and energy production, 31 of which were also affected by E2. Multiple estrogen receptors and genes related to serotonin activity, transport and degradation were expressed in sea bass scales and transcript abundance of some of them was modulated by E2 and/or FLX. Using a minimally invasive in vitro bioassay with cultured sea bass scales and adhering epithelia we showed direct effects of FLX exposure on enzymatic activity associated with mineral mobilization, while the expression of estrogen receptors was not significantly affected. In in vitro receptor-reporter assays, FLX alone did not activate any of the three sea bass nuclear estrogen receptors but had antiestrogenic effects on Esr1/2b when in co-treatment with E2, and directly activated both plasma membrane G-protein-coupled estrogen receptors. The combination of in vitro and in vivo assays substantiated the notion that FLX disrupted scale physiology through several different processes, with probable consequences for fish health, and revealed that some of the mechanisms of disruption can result from direct interaction with multiple estrogen receptors.

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SINGLE AND COMBINED EFFECTS OF TRICLOSAN AND ULTRAVIOLET RADIATION IN METAMORPHOSING SOLE

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The ultraviolet radiation (UV) and the bactericide triclosan (TCS) are known to affect the early development of the flatfish Solea senegalensis. However, the mechanisms responsible for such effects are still not understood, particularly during the thyroid-regulated metamorphosis, a critical life stage of flatfish. In this context, this work aims to study the single and combined effects of exposure to UV and TCS on metamorphosing sole. Exposure of S. senegalensis to sub-lethal UV dose (5.89 kJ m-2) and/or sub-lethal TCS concentrations (0.546 and 1.090 mg L-1) was performed during 48h at the beginning of metamorphosis (13 days after hatching, dah) with a subsequent period in clean medium until complete metamorphosis (24 dah). Malformations and metamorphosis progression were evaluated along the test. Total length and behavior were evaluated at 24 dah and expression of thyroid-axis related genes were quantified at 15 dah and 24 dah. Triclosan exposure induced malformations, decreased swimming activity, and altered metamorphosis progression, inducing an acceleration at 15 dah, followed by a delay at 24 dah. In general, the combination of the stressors TCS and UV, induced a faster metamorphosis in the transient stages in relation to isolated stressors, suggesting an exacerbation of the effect of TCS by the UV exposure. However, this was not observed at the end of metamorphosis. At the transcriptomic level, a down-regulation of thyroid axis-related genes was observed immediately after the exposure to TCS (15 dah). After 9 days in clean medium (24 dah) only 2 genes were still down-regulated (NIS and THRβ), suggesting a partial recovery of the hypothalamus-pituitary-thyroid-axis. In addition, UV potentiates the effect of TCS by further down-regulating NIS immediately after 48h of exposure. In general, even after a period of maintenance in clean medium exposure to these stressors, single or in combination, resulted in adverse effects on metamorphosing sole, which might have further implications on the ecological performance of the species

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GLYPHOSATE EXPOSURE INDUCES SEX-SPECIFIC OUTCOMES IN ZEBRAFISH ADULT: INSIGHTS ON HEPATIC AND GONADAL TOXICITY

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The herbicide glyphosate and its formulation Roundup® are commonly used for weeing control in crops, gardens and municipal parks. Despite its wide environmental application, few studies have been carried out so far showing its toxicity on wildlife and humans. In this study, male and female adult zebrafish were exposed to a 700 µg/L Glyphosate or to Roundup® containing an equivalent concentration of glyphosate (700 µg/L) and the results were compared to those of a control (C) group.At hepatic level, the impact of GLY was found to be sex-specific: in treated female, the exposure affected purine metabolism by decreasing the levels of AMP, GMP, UMP and inosinic acid, consequently increasing uric acid levels. In male, GLY exposure decreased aminoadipic acid levels. These changes were associated with an increased stress response in both sexes, as suggested by higher nr3c1 mRNA expression. Moreover, in male a reduction of hsp70.2, sod1, sod2, cat and gpx1a mRNA, as signs of the impaired oxidative stress response, was observed. In female, mRNA levels of the pro-inflammatory interleukins litaf and cxcl8b.1, increased. Taken together, the results provide evidence of disrupted nucleotide hepatic metabolism, increased stress inflammatory response in female, and disruption of oxidative stress response in male. Thus, considering these alterations, an impairment also at the reproductive level was hypothesized. Hepatic vtg isoform mRNAs levels were analyzed in both male and female livers and a significant decrease was seen in both sexes. Furthermore, alteration of gametogenesis was observed. In particular in male, testis of exposed fish presented an increase of Spermatogonia A, B and spermatids and a decrease in spermatozoa abundance. On the contrary, no differences were found regarding the abundance of previtellogenic-, vitellogenic and mature follicles between C and treated ovaries. A deeper investigation was made to gain evidence regarding glyphosate hormonal behavior, thus the expression of genes involved in gonadal steroidogenesis, star, cyp11a1 and cyp19a, gonadotropin receptors and gamete maturation, pgrmc1 and pgrmc2 was analyzed, evidencing the herbicide hormone-like activity, and confirming the sex-specific effect of this widely used pollutant.

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GENOMIC AND PHYSIOLOGICAL MECHANISMS OF ANDROGEN SIGNALLING: STEROID-5A-REDUCTASE TYPE 2 KNOCKOUT INVESTIGATION IN FROGS

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With the increased presence of environmental contaminants acting as endocrine disruptors to animals, including humans, it is critical to assess how androgens are regulated and produced to understand the health consequences of altering androgen biosynthesis. Using one of the best aquatic models for endocrine disruptor testing, the Western clawed frog (*Silurana tropicalis*), and the CRISPR gene-editing technology, a unique line of frog mutants to mimic androgen disruption was created. In addition to their metamorphosing ability, frogs represent a unique animal model featuring large externally developing embryos to study all aspects of developmental physiology (compared to any mammalian models). These mutant frogs do not possess the functional enzyme steroid-5α-reductase type 2 (SRD5α2), capable of producing one of the most potent androgens in frogs, the 5α-dihydrotestosterone (5α-DHT). This enzyme is thought to have several roles in frog reproduction. The research objective is to characterize how SRD5α2 regulates gonadal development and differentiation and other reproduction-related aspects like maintaining reproductive capacity and secondary sex characteristics. On a more practical scale, this research may have applications targeting environmental contamination that would act through the activation or deactivation of androgen production in aquatic and semi-terrestrial species. Fundamental discoveries in frogs are relevant for understanding reproductive function in all vertebrates.

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EFFECTS OF SWIMMING EXERCISE ON PERFOMANCE OF ATLANTIC SALMON

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Producing Atlantic salmon (*Salmo salar*) smolts that allow to shorten the time at sea has been a major objective to reduce environmental and welfare challenges in the Norwegian aquaculture industry. However, even when salmon appear optimally smoltified, health and welfare problems in the early sea phase are common, resulting in major financial losses and indicating a lack of robustness of the produced smolts. One strategy that has shown to have positive effects on health and wellbeing of farmed salmon at an industrial scale is the use of active swimming training. Swimming training in salmon has shown to increase growth and facilitate recruitment of white musculature, improve heart health, decrease feed waste, and reduce mortality compared with reference fish in net (i.e., no swimming training).To study the mechanisms behind the beneficial effects observed in previous studies, we conducted an experimental trial analyzing effects of different and constant water velocities on the Atlantic salmon performance. Atlantic salmon (98.1 ± 1.3 g; 20.4 ± 1.3 cm) were provided by Lerøy Seafood Group AS (Kjærelva, Norway). At the swimming lab at the Department of Biological Sciences (UiB, Bergen, Norway), fish were randomly assigned into six tanks (80 fish per tank), with freshwater at 12.5 °C, LD24:0, and oxygen above 80%. Fish were fed in excess from 9:00 to 15:00 with a commercial feed. After 4 weeks of acclimation period, three different water velocities (0.5, 1.0 and 1.5 body length per sec) were assigned in duplicated tanks. The fish were then subjected to swimming training for 5 weeks LD12:12 followed by 6 weeks LD24:0. At the end of the freshwater phase, they were transferred into brackish water (26 ‰) and monitored for 4 weeks. The effects of exercise were assessed based on morphometric parameters, oxygen and feed consumption, as well as biochemical and molecular parameters related to energy metabolism, smoltification and growth. Preliminary results suggests that the cardio somatic index increased with increased swimming speed, while the hepatosomatic index and specific growth rate decreased. With the ongoing analyses, we will assess and document to what extent swimming training can be used as a tool to ensure the production of healthier and more robust farmed salmon.

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A COMPARATIVE APPROACH TO MEASURE SEX AND AGE-RELATED DIFFERENCES IN SHOULDER MORPHOLOGY AND BODY SIZE

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For the human scapula, we have previously described a significant sexual dimorphism (Mathews et al., BMC Musculoskelet Disord. 2017;18:9), in line with the scarce data set. However, when it comes to correlation with body size, database is still weak, particularly for women and smaller individuals. For instance, previous studies from our group (e.g. Häusler and co-workers, University of Zurich 2001; J Hum Evol 2004;46:433-65; 2007;53:383-405) in 100 skeletons with emphasis on small-bodied individuals (65 men, 35 women) originating from Africa, Asia and Europe have revealed contradictory results for the ratio of glenoid size to body size depending on the calculation method. Furthermore, in the elderly, potential osseous shrinking processes have to be considered. Thus, there is a demand to compare the individual scapula size with body size in a larger data set, with special emphasis on aged and female individuals. In this multimodality study, we systematically explored the gleno-humeral joint using morphological and CT-based measurements, and compared the data with the donor body size using different methodological approaches: This study combines dissection with anatomical measurements and radiological CT data in order to correlate the scapula and glenoid size. Computed tomography (CT) scans included the shoulder girdle and arms and were performed prior to dissection. Glenoid morphological parameters and size were determined on subsequently isolated scapulae and 3D-CT reconstructions of the gleno-humeral joint according to the method of Friedman and co-workers (J Bone Joint Surg Am 1992;74A:1032-7) as described (Mathews et al. 2017; Serrano et al. BMC Musculoskelet Disord 2021;22:849). Body length was correlated with the glenoid size to create a data set to extrapolate glenoid and body size in the elderly. We aim at establishing a systematic basis of glenoid parameters and body sizes for future studies in physiology, comparative and translational research and in diverse pathologies.

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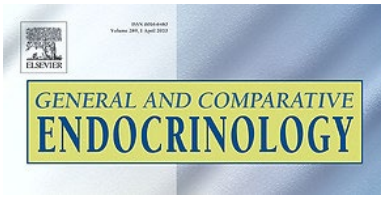
NOTES



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