18th International Congress of Comparative Endocrinology (ICCE18)

held jointly with

4th Biennial Conference of the North American Society for Comparative Endocrinology (NASCE)

and

9th International Symposium on Amphibian and Reptilian Endocrinology and Neurobiology (ISAREN)

Chateau Lake Louise
Banff National Park, Alberta, Canada
June 4-9, 2017

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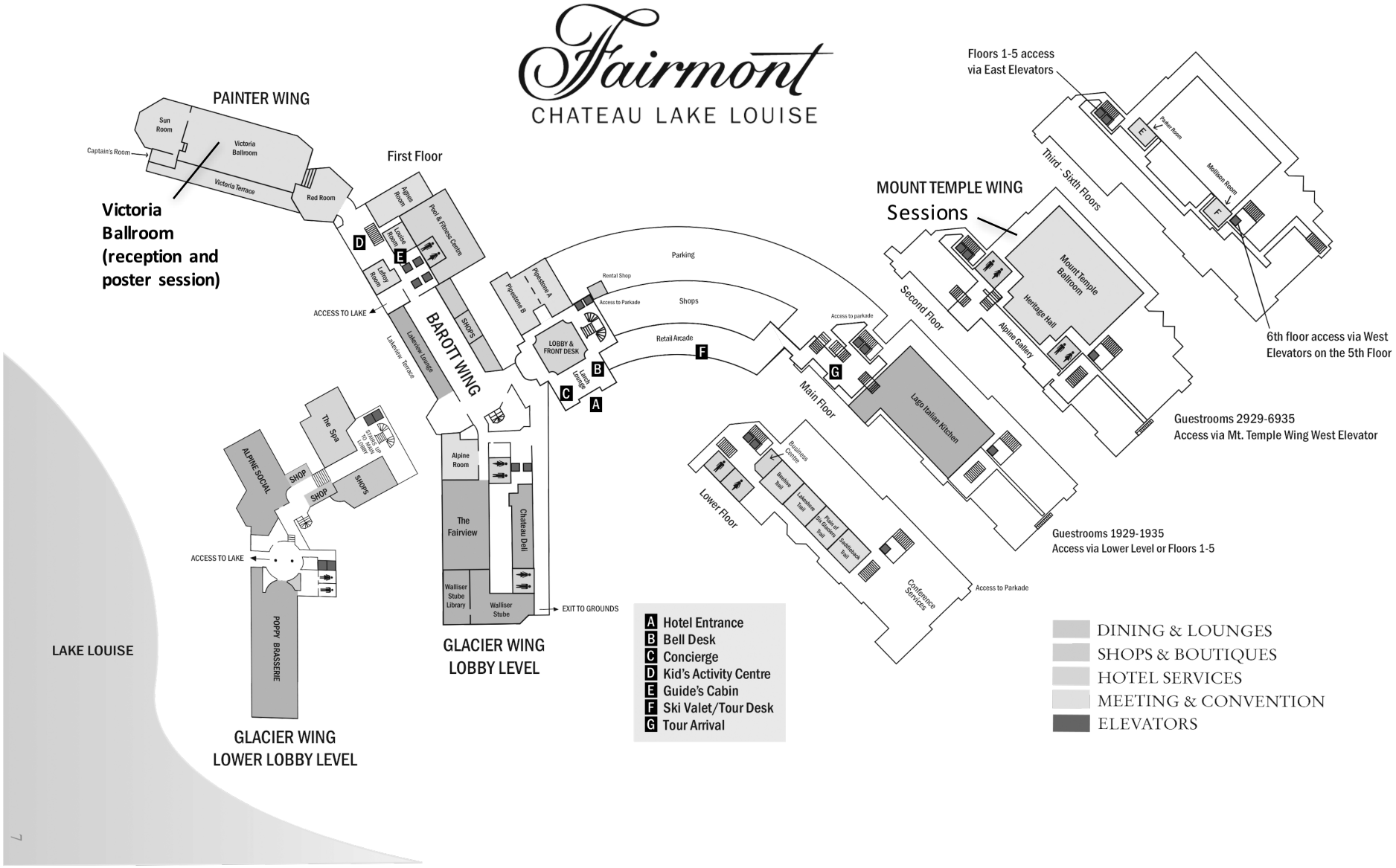
Student presentation awards

Four awards sponsored by The International Federation of Comparative Endocrinological Societies (IFCES), North American Society for Comparative Endocrinology (NASCE) and ICCE18 will be presented to students for best oral and poster presentations. Student presentations will be evaluated by a student presentation evaluation subcommittee, chaired by Professor Matt (Mathilakath) Vijayan.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CHATEAU LAKE LOUISE
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Red Room
Victoria Ballroom (reception and poster session)
First Floor
Alpine Room
Pool & Fitness Centre
Louise Room
Lobby
D
E
ACCESS TO LAKE
Lakeview Lounge
Lakeview Terrace
BAROTT WING
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Lewis Lounge
A
B
C
F
G
Access to parkade
Main Floor
Lago Italian Kitchen
Floors 1-5 access via East Elevators
Third - Sixth Floors
Parker Room
Mollison Room
MOUNT TEMPLE WING
Sessions
Second Floor
Mount Temple Ballroom
Heritage Hall
Alpine Gallery
6th floor access via West Elevators on the 5th Floor
Guestrooms 2929-6935
Access via Mt. Temple Wing West Elevator
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Access via Lower Level or Floors 1-5
Lower Floor
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Lakeshore Trail
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EXIT TO GROUNDS
GLACIER WING
LOBBY LEVEL
LAKE LOUISE
A Hotel Entrance
B Bell Desk
C Concierge
D Kid's Activity Centre
E Guide's Cabin
F Ski Valet/Tour Desk
G Tour Arrival
DINING & LOUNGES
SHOPS & BOUTIQUES
HOTEL SERVICES
MEETING & CONVENTION
ELEVATORS

Program

ICCE18

	Monday June 5			Tuesday June 6			Wednesday June 7			Thursday June 8			Friday June 9		
Sunday June 4	8:00 – 8:30 Opening Ceremony Temple A &B			8:00 – 8:45 PL2 Grace Pickford Temple A &B			8:00 – 8:45 PL4 Gorbman-Bern Lecture Temple A &B			8:00 – 8:45 PL5 Gorbman-Bern New Invest. Temple A &B			8:00 – 8:45 PL7 ISAREN Plenary Lec Temple A &B		
	8:30 – 9:15 PL1 Bargmann-Scharrer Temple A &B			8:50-9:20 SOA7 Temple A &B	8:50-9:20 SOA8 Lakeshore/ Beehive	8:50-9:20 SOA9 Plains6/ Saddleback	8:50-9:20 SOA13 Temple A &B	8:50-9:20 SOA14 Lakeshore/ Beehive	8:50-9:20 SOA15 Plains6/ Saddleback	8:50-9:20 SOA22 Temple A &B	8:50-9:20 SOA23 Lakeshore/ Beehive	8:50-9:20 SOA24 Plains6/ Saddleback	8:50-9:20 SOA28 Temple A &B	8:50-9:20 SOA29 Lakeshore/ Beehive	8:50-9:20 SOA30 Plains6/ Saddleback
	9:20:10:20 S1 Temple A &B	9:20:10:20 S2 Lakeshore/ Beehive	9:20:10:20 S3 Plains6/ Saddleback	9:20:10:20 S7 Temple A &B	9:20:10:20 S8 Lakeshore/ Beehive	9:20:10:20 S9 Plains6/ Saddleback	9:20:10:20 S10 Temple A &B	9:20:10:20 S11 Lakeshore/ Beehive	9:20:10:20 S12 Plains6/ Saddleback	9:20:10:20 S16 Temple A &B	9:20:10:20 S17 Lakeshore/ Beehive	9:20:10:20 S18 Plains6/ Saddleback	9:20:10:20 S19 Temple A &B	9:20:10:20 S20 Lakeshore/ Beehive	9:20:10:20 S21 Plains6/ Saddleback
	10:20-10:50 Coffee Break (Heritage Hall)			10:20-10:50 Coffee Break (Heritage Hall)			10:20-10:50 Coffee Break (Heritage Hall)			10:20-10:50 Coffee Break (Heritage Hall)			10:20-10:50 Coffee Break (Heritage Hall)		
	10:50-11:50 S1 Temple A &B	10:50-11:50 S2 Lakeshore/ Beehive	10:50-11:50 S3 Plains6/ Saddleback	10:50-11:50 S7 Temple A &B	10:50-11:50 S8 Lakeshore/ Beehive	10:50-11:50 S9 Plains6/ Saddleback	10:50-11:50 S10 Temple A &B	10:50-11:50 S11 Lakeshore/ Beehive	10:50-11:50 S12 Plains6/ Saddleback	10:50-11:50 S16 Temple A &B	10:50-11:50 S17 Lakeshore/ Beehive	10:50-11:50 S18 Plains6/ Saddleback	10:50-11:50 S19 Temple A &B	10:50-11:50 S20 Lakeshore/ Beehive	10:50-11:50 S21 Plains6/ Saddleback
	11:50-12:20 SOA1 Temple A &B	11:50-12:20 SOA2 Lakeshore/ Beehive	11:50-12:20 SOA3 Plains6/ Saddleback	11:50-12:20 SOA10 Temple A &B	11:50-12:20 SOA11 Lakeshore/ Beehive	11:50-12:20 SOA12 Plains6/ Saddleback	11:50-12:20 SOA16 Temple A &B	11:50-12:20 SOA17 Lakeshore/ Beehive	11:50-12:20 SOA18 Plains6/ Saddleback	11:50-12:20 SOA25 Temple A &B	11:50-12:20 SOA26 Lakeshore/ Beehive	11:50-12:20 SOA27 Plains6/ Saddleback	12:00-12:30 Presentation of Awards Closing Ceremony Temple A &B		
	12:20-14:0 Lunch Lago / Fairview			12:20-14:40 Lunch and Poster Session 1 Victoria Ballroom/Terrace/ Red Room			12:20-14:0 Lunch Lago / Fairview			12:20-14:40 Lunch and Poster Session 2 Victoria Ballroom/Terrace /Red Room					
13:30-14:00	14:00-14:30 SOA4 Temple A &B	14:00-14:30 SOA5 Lakeshore/ Beehive	14:00-14:30 SOA6 Plains6/ Saddleback				14:00-14:30 SOA19 Temple A &B	14:00-14:30 SOA20 Lakeshore/ Beehive	14:00-14:30 SOA21 Plains6/ Saddleback						
Registration	14:30-15:30 S4 Temple A &B	14:30-15:30 S5 Lakeshore/ Beehive	14:30-15:30 S6 Plains6/ Saddleback				14:30-15:30 S13 Temple A &B	14:30-15:30 S14 Lakeshore/ Beehive	14:30-15:30 S15 Plains6/ Saddleback						
	15:30-15:50 Coffee Break			14:40-15:25 PL3 ICCE Plenary Lec Temple A &B			15:30-15:50 Coffee Break			14:40-15:25 PL6 ICCE Plenary Lec Temple A &B					
Victoria Ballroom Red Room	15:50-16:50 S4 Temple A &B	15:50-16:50 S5 Lakeshore/ Beehive	15:50-16:50 S6 Plains6/ Saddleback	15:40 GCE Editorial Board meeting MT Temple C			15:50-16:50 S13 Temple C	15:50-16:50 S14 Lakeshore/ Beehive	15:50-16:50 S15 Plains6/ Saddleback	15:40 IFCES and NASCE Business meeting MT Temple C		15:40-17:30 Workshop High resolution Expression Quantification Plains6/ Saddleback			
	17:00 PM IFCES Business meeting MT Temple C														
19:30 - 21:00 Opening Reception Victoria Ballroom/Terrace /Red Room										19:30 – 23:30 Banquet Victoria Ballroom/Red Room / Terrace					

Monday June 5, 2017 - Morning Session			
8:00-8:30	**Opening Ceremony** (MT Temple A &B)		
Plenary Lecture 8:30-9:15	**Intoduction: Kazuyoshi Tsutsui (IFCES Chair of the awards Committee)** **PL1- Stacia Sower (USA) - ICCE18 Bargmann-Scharrer Lecture (**MT Temple A &B) **THE ORIGINS OF THE VERTEBRATE HYPOTHALAMIC-PITUITARY AXIS: INSIGHTS FROM LAMPREYS** **Chair: Richardson, Samantha**		
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session **Chair**	**S1 - Signaling and Neuroendocrine Control** **John Chang and Anderson Wong**	**S2 - Insulin and insulin-like peptides, vertebrate and invertebrate** **Angela Lange and Cunming Duan**	**S3 - Neurochemical Regulation of Instinctive Behavior** **Kazuyoshi Tsutsui and Takayoshi Ubuka**
9:20-9:40	**S1-1) Wong, Anderson (Hong Kong)** INSULIN AS A FUNCTIONAL LINK BETWEEN FOOD INTAKE AND SPEXIN EXPRESSION: RECENT PROGRESS ON SPEXIN AS A SATIETY FACTOR IN FISH MODEL Ma A, He M, Bai J, Wong MKH, Ko WKW, Wong AOL	**S2-1) Brown, Mark (USA)** OF THE INSULIN-LIKE PEPTIDES IN MOSQUITOES: ILP3 PREVAILS Brown MR, Strand MS	**S3-1) Ubuka, Takayoshi (Malaysia)** GNIH GENE KNOCKOUT REDUCES PAIN, ANXIETY, AND ENABLES INTENSIVE EXERCISE IN MICE Ubuka T, Okada S, Yamazaki D, Narihiro M, Taguchi R, Kiyohara M, Parhar I, Tsutsui K
9:40-10:00	**S1-2) Son, You Lee (Japan)** MOLECULAR MECHANISM OF GONADOTROPIN-INHIBITORY HORMONE ACTION ON REPRODICTION Son YL, Tsutsui K	**S2-2) Defferrari, Marina (Canada)** INSULIN-LIKE PEPTIDES IN RHODNIUS PROLIXUS, THE VECTOR OF CHAGAS DISEASE Defferrari MS, Orchard I, Lange AB	**S3-2) Ando, Hironori (Japan)** PERIODIC CONTROL OF KISSPEPTIN AND ITS RECEPTOR GENE EXPRESSION BY PHOTIC AND NON-PHOTIC ENVIRONMENTAL CUES IN THE GRASS PUFFER, A SEMILUNAR SPAWNER Ando H, Shahjahan MD, Kitahashi T
10:00-10-20	**S1-3) Orchard, Ian (Canada)** NEUROENDOCRINE SIGNALLING: DISCOVERIES FROM THE BLOOD-GORGING KISSING BUG, *RHODNIUS PROLIXUS* Orchard I, Lange AB	**S2-3) Kamei, Hiroyasu (Japan)** ROLES OF INSULIN RECEPTOR SUBSTRATES IN CONTROLLING EMBRYONIC GROWTH IN RESPONSE TO CHANGING ENVIRONMENTAL OXYGEN TENSION Kamei H	**S3-3) Soga, Tomoko (Malaysia)** SEROTONERGIC REGULATION OF GONADOTROPIN-INHIBITORY HORMONE (GNIH) NEURONS IN SOCIAL STRESS Soga T, Parhar IS
10:20-10:50	**Coffee Break**		
10:50-11:10	**S1-4) Michalec, Ola (Canada)** TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP), AN ANCESTRAL PEPTIDE AND ITS EFFECT ON CALCIUM ION FLUX, A MECHANISM OF CELL SIGNALING IN ASTROCYTES Michalec OM, Lovejoy DA	**S2-4) Bogerd, Jan (Netherlands)** Igf3 AND Amh, TWO Fsh-RESPONSIVE GROWTH FACTORS, REGULATE SPERMATOGONIAL DIFFERENTIATION IN A CONCERTED MANNER Bogerd J, Morais R, Nóbrega R, Crespo D, Van de Kant H, Male R, Schulz R	**S3-4) Takeuchi, Hideaki (Japan)** MOLECULAR BASIS UNDERLYING SOCIAL COMPETENCE IN MEDAKA FISH Takeuchi H
11:10-11:30	**S1-5) Rocco, David (Canada)** NOVEL INSIGHTS ON THE FUNCTIONAL ROLE OF THE GLYCOPROTEIN HORMONE GPA2/GPB5 AND ITS RECEPTOR IN THE ADULT MOSQUITO, *AEDES AEGYPTI* David A. Rocco, Jean-Paul V. Paluzzi	**S2-5) Jia, Yudong (PRC)** DEVELOPMENTAL EXPRESSION PATTERNS OF INSULIN-LIKE GROWTH FACTORS DURING TURBOT METAMORPHOSIS Jia YD, Meng Z, Hu P, Huang B	**S3-5) Jarvis, Erich (USA)** DISSECTING THE MOLECULAR MECHANISMS OF VOCAL LEARNING AND SPOKEN LANGUAGE Jarvis E
11:30-11:50	**S1-6) Hollander Cohen, Lian (Israel)** FUNCTIONAL INVESTIGATION OF GNRH, FSH AND LH IN ZEBRAFISH Hollander Cohen L, Revah O, Golan M, Mollard P, Gutnick M, Levavi Sivan B	**S2-6) Vahkal, Brett (Canada)** POTENTIAL ROLE OF INSL5 AND RXFP4 IN THE IMMUNE SYSTEM Vahkal B, Good SV	**S3-6) Knapp, Rosemary (USA)** BRAIN TRANSCRIPTIONAL PROFILES OF MALE ALTERNATIVE REPRODUCTIVE TACTICS IN BLUEGILL SUNFISH WITH A FOCUS ON ENDOCRINE CANDIDATE GENES Knapp R, Partridge CG, MacManes MD, Neff BD
SOA Lecture 11:50-12:20	**SOA1 - Chang, John (Canada)** BIASED SIGNALING IN GONADOTROPIN-RELEASING HORMONE STIMULATION OF GOLDFISH PITUITARY GONADOTROPES AND SOMATOTROPES Chang JP, Pemberton JG	**SOA2 - Duan, Cunming (USA)** A NOVEL MECHANISM UNDERLYING CALCIUM DEFICIENCY-INDUCED IIS-PI3K-AKT-TOR SIGNALING AND EPITHELIAL CELL PROLIFERATION Duan C, Liu C, Dai W, Xin Y and Bai Y	**SOA3 - Tsutsui, Kazuyoshi (Japan)** GNIH CONTROL OF REPRODUCTIVE PHYSIOLOGY AND BEHAVIOR Tsutsui K
12:20-14:00	**Lunch**		

Monday June 5, 2017 - - Afternoon Session			
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session **Chair**	**S4 - Tackling Endocrine Mysteries in Domestic Animals** **Daniel J. MacPhee and Prasanth Chelikani,**	**S5 - The Hormonal Control of Osmoregulation in Vertebrates** **Jason P. Breves and Steve Perry**	**S6 - Invertebrate sex steroids and peptides** **Pei-San Tsai and David Norris**
SOA Lecture 14:00-14:30	**SOA4 - Adams, Gregg (Canada)** ENDOCRINE ROLE OF OVULATION-INDUCING FACTOR/NERVE GROWTH FACTOR (OIF/NGF) Adams GP, Carrasco RA	**SOA5 - Takei, Yoshio (Japan)** HORMONAL CONTROL OF WATER AND ION ABSORPTION BY THE DIGESTIVE TRACT OF SEAWATER-ACCLIMATED EELS Takei Y, Wong MKS, Shimada T, Ando M	**SOA6 - Markov, Gabriel (France)** ORIGINS AND EVOLUTION OF THE NUCLEAR RECEPTOR/ HORMONE COUPLE ILLUSTRATED BY THE CASE OF STEROIDS Markov GV
13:30-14:50	**S4-1) Singh, Jaswant (Canada)** EFFECT OF KISSPEPTIN ON BOVINE FOLLICULAR FUNCTION Singh J, Leonardi CEP, Dias CFC, Adams GP	**S5-1) McCormick, Steve (USA)** THE ROLE OF CORTICOSTEROIDS IN OSMOREGULATION OF BASAL VERTEBRATES McCormick SD, Shaugnessy C, Regish A	**S6-1) Tsai, Pei-San (USA)** GONADOTROPIN-RELEASING HORMONE-RELATED PEPTIDES AND THEIR RECEPTORS IN A GASTROPOD MOLLUSK Tsai PS, Sun B, Dai X, Kavanaugh SK, Johnson JI
14:50-15:10	**S4-2) McMillan, Chantal (Canada)** INCRETIN HORMONES IN CATS McMillan C, Zapata RC, Tong J, Snead EC, Chelikani PK	**S5-2) Breves, Jason (USA)** MULTIFACTORIAL CONTROL OF BRANCHIAL CLC-2C GENE EXPRESSION IN TILAPIA: EFFECTS OF SALINITY, PROLACTIN AND EXTRACELLULAR OSMOLALITY. Breves JP, Keith PLK, Hunt BL, Pavlosky KK, Inokuchi M, Yamaguchi Y, Lerner DT, Seale AP, Grau EG	**S6-2) Alexander Scott (UK)** VERTEBRATE STEROIDS PRESENT IN MOLLUSK TISSUES ARE MORE LIKELY TO BE OF EXOGENOUS THAN ENDOGENOUS ORIGIN Scott AP, Schwarz TI, Maskrey BH, Katsiadaki I
15:10-15:30	**S4-3) Snead, Elisabeth (Canada)** SAFETY AND EFFICACY ASSESSMENT OF A GLP-1 MIMETIC: INSULIN GLARGINE COMBINATION FOR TREATMENT OF FELINE DIABETES MELLITUS Snead EC, Scuderi M, Ribeiro Petito M, Unniappan S, Waldner C, Mehain S	**S5-3) Gilmour, Kathleen (Canada)** REGULATION OF CARBONIC ANHYDRASE IN IONOCYTES OF THE FISH GILL. Gilmour KM	**S6-3) Thitiphuree, Tongchai (Japan)** GAMETOGENESIS AND mRNA EXPRESSION OF STEROIDOGENIC ENZYME GENES DURING REPRODUCTIVE CYCLE IN JAPANESE SCALLOP Thitiphuree T, Nagasawa K, Osada M
15:30-15:50	**Coffee Break**		
15:50-16:10	**S4-4) Thundathil, Jacob (Canada)** NUTRITIONAL MODULATION OF HORMONE PRODUCTION AND REPRODUCTIVE DEVELOPMENT IN BULLS Thundathil J, Dance A, Kastelic J	**S5-4) Seale, Andre (USA)** OSMOTIC REGULATION OF PROLACTIN 188 AND PROLACTIN 177 AND THEIR RECEPTORS IN TWO TILAPIA CONGENERS WITH DISTINCT SALINITY TOLERANCES. Seale AP, Yamaguchi Y, Breves JP, Haws MC, Lerner DT, Grau EG	**S6-4) Mita, Masatoshi (Japan)** HORMONAL ACTION OF RELAXIN-LIKE GONAD-STIMULATING PEPTIDE: REGULATORY MECHANISM OF STARFISH OOCYTE MATURATION AND OVULATION. Mita M
16:10-16:30	**S4-5) Danks, Janine (Australia)** PARATHYROID HORMONE RECEPTOR 1 AS A PROGNOSTIC INDICATOR IN CANINE OSTEOSARCOMA Al-Khan AA, Day MJ, Nimmo J, Tayebi M, Ryan S, Saad ES, Richardson SJ, Danks JA	**S5-5) Shaughnessy, Ciaran (USA)** 11-DEOXYCORTISOL PROMOTES SEAWATER TOLERANCE IN METAMORPHOSING SEA LAMPREY (*PETROMYZON MARINUS*) Shaughnessy CA, Barany-Ruiz A, McCormick SD	**S6-5) Paluzzi, Jean-Paul (Canada)** CAPA NEUROPEPTIDES IN THE DISEASE VECTOR, AEDES AEGYPTI: HORMONAL ACTIONS AND FUNCTIONAL ANALYSIS OF MULTIPLE RECEPTOR VARIANTS. Curcuruto C, Sajadi F, Uyuklu A, Paputsis C, Paluzzi JP
16:30-16:50	**S4-6) Mo, Chunheng (PRC)** CHARACTERIZATION OF CHICKEN GRP, NMB AND THEIR RECEPTORS: DISCOVERY OF THE ENDOGENOUS LIGAND FOR ORPHAN RECEPTOR BRS3 AND EVIDENCE FOR GRP BEING A NOVEL POTENTIAL PITUITARY HORMONE IN CHICKENS Mo C, Huang L, Cui L, Lv C, Lin D, Song L, Ge M, Li J, Wang Y	**S5-6) Hwang, Pung-Pung (Taiwan)** MOLECULAR PHYSIOLOGY OF HORMONAL ACTIONS ON BODY FLUID IONIC AND ACID-BASE HOMEOSTASIS: USING ZEBRAFISH AS A MODEL Hwang PP	**S6-6) Liutkeviciute, Zita (Austria)** OXYTOCIN/VASOPRESSIN SIGNALLING IN ANTS Liutkeviciute Z, Gil-Mansilla E, Di Giglio MG, Cremer S, Gruber CW
17:00-18:30	**IFCES Business meeting (MT Temple C)**		

Tuesday June 6, 2017 - Morning Session			
Plenary Lecture 8:00-8:45	**PL2 - Kataaki Okubo (Japan) - Grace Pickford Medal Lecture (**MT Temple A &B) **SEXUAL DIFFERENTIATION AND PLASTICITY OF THE BRAIN OF TELEOST FISH** **Chair: Robert Denver**		
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session **Chair**	**S7 - Fish sexual development** **Bon-chu Chung, Chun Peng**	**S8 - Early Life Adversity and the Stress Response/Stress coping mechanisms** **Nick Bernier, Patrick Prunet, Gilmour K**	**Recent Progress in Cartilaginous Fish Endocrinology (NASCE President's Symposium)** **Robert M. Dores, Akiyoshi Takahashi**
SOA Lecture 8:50-9:20	**SOA7 - Ge, Wei (Macau)** GENETIC ANALYSIS OF EARLY FOLLICULOGENESIS IN THE ZEBRAFISH Ge W, Zhang Z	**SOA8 - Bernier, Nicholas (Canada)** EARLY LIFE ENVIRONMENTAL CHALLENGES AND THE STRESS RESPONSE IN ZEBRAFISH Bernier NJ, Ivy CM, Mikloska KV, Williams TA	**SOA9 - Dores, Robert M. (USA)** CARTILAINGOUS FISH COMPARATIVE ENDOCRINOLOGY OVER THE PAST 50 YEARS: RECENT STUDIES ON MELANOCORTIN RECEPTORS Dores RM
9:20-9:40	**S7-1) Tanaka, Minoru (Japan)** CHARACTERIZATION OF A FEMINIZING POWER BY GERM CELLS DURING SEX IFFERENTIATION IN MEDAKA Tanaka M	**S8-1) MacDougall-Shackleton, Scott (Canada)** SEX-SPECIFIC EFFECTS OF DEVELOP-MENTAL STRESS ON SONGBIRDS MacDougall-Shackleton SA, Farrell TM, Kriengwatana B, Schmidt KL	**S9-1) Takahashi, Akiyoshi (Japan)** MELANOCORTIN SYSTEMS OF STINGRAY, A CARTILAGINOUS FISH Takahashi A
9:40-10:00	**S7-2) Canário, Adelino (Portugal)** THE TIMING OF PUBERTY IN FISH: NOVEL INSIGHTS FROM TRANSCRIPTOMICS. Martins RST, Pinto P, Carrillo M, Zanuy S, Louro B, Gomez A, Tine M, Machado R, Reinhard R, Canário AVM	**S8-2) Crespi, Erica (USA)** EFFECTS OF EARLY-LIFE ADVERSITY ON POST-METAMORPHIC PHYSIOLOGY AND BEHAVIOR IN AMPHIBIANS: PATTERNS AND MECHANISMS Crespi EJ	**S9-2) Hyodo, Susumu (Japan)** MOLECULAR AND FUNCTIONAL EVOLUTION OF NEUROHYPOPHYSIAL HORMONE SYSTEM: WITH SPECIAL REFERENCE TO A POSSIBLE FUNCTION OF NEWLY DISCOVERED V2B RECEPTOR IN CATSHARK. Hyodo S, Inoue N, Kaiya H, Kuraku S, Yamaguchi Y
10:00-10-20	**S7-3) Wang, Deshou (PRC)** ADVANCES IN ENDOCRINE CONTROL OF TILAPIA SEX DIFFERENTIATION. Wang DS	**S8-3) Brunton, Paula (UK)** PROGRAMMING OF THE STRESS AXIS AND BEHAVIOUR BY PRENATAL STRESS: MALADAPTIVE AND ADAPTIVE STRESS COPING RESPONSES Brunton PJ	**S9-3) Kobayashi, Yasuhisa (Japan)** TRANSITION FROM OOCYTES TO THE ESTROGEN-PRODUCING CELLS: ANALYSIS OF THE OVARY IN THE RED STINGRAY, *DASYATIS AKAJEI.* Kobayashi Y, Tsutsui N, Nakao M, Sakamoto T
10:20-10:50	**Coffee Break**		
10:50-11:10	**S7-4) Chun, Bon-chu (Taiwan)** SEXUALLY DIMORPHIC GENE EXPRESSION DURING EARLY ZEBRAFISH GONADAL DEVELOPMENT Chung BC	**S8-4) Prunet, Patrick (France)** EARLY LIFE ENVIRONMENTAL STRESS MODIFI ES PHYSIOLOGY AND ABILITY TO COPE WITH ACUTE STRESS IN JUVENILE RAINBOW TROUT Leguen I, Peron S, Le Calvez JM, Goardon L, Labbé L Prunet P	**S9-4) Wheaton, Catharine (USA)** CHALLENGES, PITFALLS AND SURPRISES: MEASURING STRESS, REPRODUCTIVE AND THYROID HORMONES IN ELASMOBRANCHS Wheaton CJ, Mylniczenko ND
11:10-11:30	**S7-5) Piferrer, Francesc (Spain)** THERMAL INFLUENCES ON FISH SEXUAL DEVELOPMENT Piferrer F, Ribas L, Anastasiadi D, Valdivieso A, Pla S, Sánchez N	**S8-5) Whitehouse, Lindy (Canada)** THE ONTOGENY OF THE HPI-AXIS AND ITS RESPONSE TO STRESS IN EMBRYONIC AND POST HATCH LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS)* Whitehouse LM, Faught E, Vijayan M, Manzon RM	**S9-5) Nozu, Ryo (Japan)** SEASONAL CHANGES IN SEX STEROID HORMONES AND FOLLICLE SIZE IN THE ZEBRA SHARK, *STEGOSTOMA FASCIATUM* Nozu R, Murakumo K, Matsumoto R, Yano N, Yanagisawa M, Sato K
11:30-11:50	**S7-6) Rafael, Nobrega (Brazil)** IDENTIFICATION OF PLURIPOTENCY GENES IN ZEBRAFISH TESTIS AND EFFECTS OF FSH AND GDNF ON SPERMATOGONIAL FATE FROM A SERTOLI CELL Nóbrega RH, Butzge A, Doretto LB, Martinez ERM, Digmayer M, Ricci JMB, Branco GS, Tovo-Neto A, Oliveira MA, Costa DF, Sene VF	**S8-6) Herron C (USA)** SPLENIC OXIDATIVE BURST ACTIVITY IN JUVENILE CHINOOK SALMON IS INCREASED AFTER FISH ARE EXPOSED TO A STRESSOR Herron C, Dolan BP, Schreck CB	**S9-6) Davis, Perry (USA)** HPI AXIS OF THE ELEPHANT SHARK: DETECTION OF AN MRAP1 ORTHOLOG AND THE PHARMACOLOGICAL INTERACTIONS OF THIS ACCESSORY PROTEIN WITH ELEPHANT SHARK, MC2R AND MC5R Davis P, Deyarmond M, Dores MR, Iki A, Hyodo S, Dores RM
SOA Lecture 11:50-12:20	**SOA10 - Denslow, Nancy (USA)** THE IMPACT OF OMICS TECHNOLOGIES ON ENVIRONMENTAL ASSESSMENTS OF CONTAMINATION Denslow N	**SOA11 - Schreck, Carl (USA)** FISH STRESS RESPONSE: CONSISTENCIES AND INCONSISTENCIES IN ENDOCRINE DATA Schreck C	**SOA12 - Lovejoy, David (Canada)** ROLE OF ELASMOBRANCHS AND HOLOCEPHALANS IN UNDERSTANDING PEPTIDE EVOLUTION IN THE VERTEBRATES: LESSONS LEARNED FROM GnRH AND CORTICOTROPIN-RELEASING HORMONE (CRH) PHYLOGENIES Lovejoy, D

Tuesday June 6, 2017 - Afternoon Session	
12:20 -14:40	**Lunch and** **Poster Session 1** **Odd poster numbers** **Victoria Ballroom/Terrace/ Red Room**
Plenary Lecture 14:40 -15:25	**PL3 - Marilyn Renfree (Australia) - ICCE18 Plenary Lecture (**MT Temple A &B) **NEW PARADIGMS FROM THE ENDOCRINOLOGY OF MARSUPIAL SEXUAL DIFFERENTIATION** **Chair: Jean Joss**
15:40 – 17:30	**GCE Editorial Board meeting (MT Temple C)**

Wednesday June 7, 2017 - Morning Session			
Plenary Lecture 8:00-8:45	**PL4 - NASCE Gorbman-Bern Lecturer: Robert Denver (USA) (**MT Temple A &B) **KRÜPPEL-LIKE FACTORS ARE EFFECTORS OF NUCLEAR RECEPTOR SIGNALING** **Cair: Robert Dores**		
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session **Chair**	**S10 - ISAREN: Environmental & genetic influences on amphibian and reptilian endocrine systems** **Caren Helbing and John Clulow**	**S11 - Old questions, new technological approaches in thyroid hormone signaling and function** **Aurea Orozco and Deborah Power**	**S12 - Integrating factors of appetite, energy balance and growth** **Oliana Carnevali, Suraj Unnappian, Encarnacion Capilla**
SOA Lecture 8:50-9:20	**SOA13 - Ito, Michihiko (Japan)** MAINTENANCE & DECONSTRUCTION OF MASS-IN-LINE STRUCTURES BY SEX STEROIDS OR TGFβ DURING ERALY GONADAL DIFFERENTIATION IN THE AFRICAN CLAWED FROG –IS DEFAULT SEX FEMALE OR MALE? Ito M, Wada M, Mawaribuchi S, Tamura K, Takamatsu N	**SOA14 - Demeneix, Barbara (France)** SCREENING ENVIRONMENTAL CHEMICALS FOR EFFECTS ON THYROID SIGNALLING AND EARLY BRAIN DEVELOPMENT IN XENOPUS Demeneix B	**SOA15 - Unniappian, Suraj (Canada)** BIOLOGY OF NESFATIN-1: A DECADE AND BEYOND Unniappan S
9:20-9:40	**S10-1) Clulow, John (Australia)** BOYS ARE EASY, GIRLS ARE HARD – OBTAINING GAMETES FROM AUSTRALIAN TEMPERATE FROGS BY HORMONAL INDUCTION. Clulow J, Pomering M, Upton R, Calatayud N, Clulow S, Mahony MJ, Trudeau VL	**S11-1)** Power, Deborah (Portugal) FLATFISH EVOLUTION: TILTING THE BALANCE OF THYROID AND RETINOIC ACID SYSTEM SIGNALLING Power DM, Shao C, Bao B, Chen S	**S12-1) Oliana Carnevali (Italy)** EFFECTS OF GUT MICROBIOTA VARIAT–ION ON HOST'S ENERGY BALANCE AND DEVELOPMENT IN FISH MODEL Falcinelli S, Rodiles A, Hatef A, Picchietti S, Cossignani L, Merrifield DL, Unniappan S, Carnevali O
9:40-10:00	**S10-2) Woodley, Sarah (USA)** TESTING HYPOTHESES ABOUT INDIVIDUAL VARIATION IN PLASMA CORTICOSTERONE IN FREE-LIVING SALAMANDERS Woodley SK, Thomas JR, Magyan AJ, Freeman PE	**S11-2) Buchholz, Daniel (USA)** INSIGHTS INTO THYROID HORMONE RECEPTOR FUNCTION FROM GENE KNOCKOUT STUDIES Buchholz DR	**S12-2) Sheridan, Mark (USA)** INTEGRATION OF FEEDING, GROWTH, AND METABOLISM: INSIGHTS FROM STUDIES IN FISH Sheridan MA
10:00-10-20	**S10-3) Gabor, Caitlin (USA)** THE ROLE ANTHROPOGENIC STRESSORS PLAY IN MEDIATING STRESS AND DISEASE IN AMPHIBIANS Gabor C, Roznik B, Knutie S, Rohr J	**S11-3) Orozco, Aurea (Mexico)** IN THYROID HORMONE-MEDIATED GENE TRANSCRIPTION, 3,5-T2 HAS A SAYING Orozco A	**S12-3) Lange, Angela (Canada)** ADIPOKINETC HORMONE AND INSULIN SIGNALLING PATHWAYS IN THE BLOOD-GORGING DISEASE VECTOR, *RHODNIUS PROLIXUS* Lange AB, Orchard I
10:20-10:50	**Coffee Break**		
10:50-11:10	**S10-4) Miura, Ikuo (Japan)** EVOLUTIONARY CHANGE OF GONADAL SEX-REVERSAL SENSITIVITY TO SEX STEROIDS AND ITS RELATION TO TURNOVER OF SEX CHROMOSOMES IN A FROG Miura I, Ohtani H, Ogata M, Ezaz T	**S11-4) Olvera Vidal, Aurora Maria (Mexico)** DIFFERENTIAL TRANSCRIPTOME REGULATION IN TILAPIA *ORECHROMIS NILOTICUS* BRAIN & LIVER BY 3,5-T2 & 3',3,5-T3 Olvera A, Buisine N , Martyniuk CJ, Villalobos P, Jiménez-Jacinto V, Sanchez-Flores A, Sachs L, Oroz A	**S12-4) Michel, Maximilian (USA)** MODELING OF ENERGY HOMEOSTASIS IN ZEBRAFISH Michel M, Cone RD
11:10-11:30	**S10-5) Orton, Frances (UK)** REPRODUCTIVE BIOLOGY IN *XENOPUS TROPICALIS* Orton F, Säfholm M, Janson E, Carlsson Y, Eriksson A, Fick J, Verbruggen B, Economou T, Uren-Webster T, Berg C, Tyler CR	**S11-5) Tamura, Kei (Japan)** THYROID HORMONE NEGATIVELY REGULATES MYOGENIC DIFFEREN–TIATION IN TADPOLE TAIL-DERIVED MYOBLAST CELLS IN *XENOPUS LAEVIS* Tamura K, Kitagishi C, Takamatsu N, Ito M	**S12-5) Ukena, Kazuyoshi (Japan)** IDENTIFICATION AND BIOLOGICAL ACTION OF A NOVEL SMALL SECRETORY PROTEIN, NEUROSECRETORY PROTEIN GL, IN THE CHICKEN HYPOTHALAMUS Ukena K, Shikano K, Kato M, Taniuchi S, Bessho Y, Furumitsu M, Iwakoshi-Ukena E
11:30-11:50	**S10-6) Koide, Emily (Canada)** VISUALIZING THE EFFECTS OF THYROID HORMONE: IDENTIFICATION OF AFFEC–TED METABOLITES USING MATRIX-ASSISTED LASER DESORPTION/ IONIZA–TION-MASS SPECTROMETRY IMAGING (MALDI-MSI) IN BULLFROG TADPOLES Koide EM, Baker TC, Wang X, Han J, Borchers CH, Helbing C	**S11-6) Subash, Peter (India)** UNDERSTANDING THE THYROID HORMONE-DRIVEN INTEGRATIVE AND DIFFERENTIAL NA+ SIGNALING IN FISH IONOCYTES Subhash P	**S12-6) Habroun, Stacy (USA)** EFFECTS OF FOOD CONSUMPTION ON CELL PROLIFERATION IN THE BRAIN OF *PYTHON REGIUS* Habroun SS, Strand CR
SOA Lecture 11:50-12:20	**SOA16 - Jean-Francois Baroiller (France)** SEX REVERSALS IN GONOCHORISTIC FISH: INSIGHTS AND CONSEQUENCES FOR WILD POPULATIONS. Baroiller JF, D'Cotta H	**SOA17 - Darras, Veerle (Belgium)** TRANSIENT AND PERMANENT SILENCING OF THYROID HORMONE TRANSPORTERS AND DEIODINASES IN CHICKEN AND ZEBRAFISH Darras VM, Vancamp P, Houbrechts AM	**SOA18 - Carr, James (USA)** THE VISUAL SYSTEM IS A TARGET FOR PEPTIDES REGULATING FOOD INTAKE Carr JA, Prater C, Islam R, Harris BN
12:20-14:00	**Lunch**		

Wednesday June 7, 2017 - Afternoon Session			
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session **Chair**	**S13 - Environmental Regulation of Reproductive Processes'** **Vance Trudeau and Glen Van Der Kraak**	**S14 - Eco-Evo-Devo: The Physiology of Phenotypic Variation** **Christen Mirth and Nadia Aubin-Horth**	**S15 - Neuroendocrinology of invertebrate deuterostomes - a crucial link between protostomes and vertebrates** **Dan Larhammar and Maurice Elphick**
SOA Lecture 14:00-14:30	**SOA19 - Van Der Kraak, Glen (Canada)** DEFINING THE ENDOCRINE TARGETS THAT MEDIATE THE ENVIRONMENTAL REGULATION OF REPRODUCTION Van Der Kraak G	**SOA20 - Abouheif, Ehab (Canada)** WHAT ANTS TEACH US ABOUT MECHANISMS OF SIZE, GROWTH, AND ALLOMETRY DURING DEVELOPMENT AND EVOLUTION Rajakumar R, Couture M, Fave MJ, Chen T, Lillico-Ouachour A, Koch S, Abouheif E	**SOA21 - Satake, Honoo (Japan)** PEPTIDERGIC REGULATION OF FOLLICLE MATURATION AND OVULATION IN A PROTOCHORDATE, CIONA INTESTINALIS Satake H
13:30-14:50	**S13-1) Meuti, Megan (USA)** THE CIRCADIAN CLOCK'S CONTROL OF OVERWINTERING DORMANCY AND SEASONAL DIFFERENCES IN MOSQUITO REPRODUCTIVE PHYSIOLOGY Megan E. Meuti	**S14-1) Aubin-Horth, Nadia (Canada)** AN INTEGRATIVE APPROACH TO STUDY A BEHAVIOR-MODIFYING PARASITE AND ITS HOST Grécias L, Hébert FO, Berger CS, Grambauer S, Barber I, Landry CR, Aubin-Horth N	**S15-1) Lee, Leo (Macau)** EVOLUTION OF AMPHIOXUS PTH AND PACAP/GLUCAGON RECEPTOR FAMILY AND THEIR POTENTIAL ROLE IN REGULATING GH-LIKE GENE EXPRESSION On JSW, Chow BKC, Lee LTO
14:50-15:10	**S13-2) Yamamoto, Yoji (Japan)** GENOTYPIC AND TEMPERATURE-DEPENDENT SEX DETERMINATION IN PEJEREY Yamamoto Y, Zhang Y, Sarida M, Hattori RS, Strüssmann CA	**S14-2) Mirth, Christen (Australia)** FROM PLASTICITY TO ROBUSTNESS: COORDINATING ORGAN SIZE AND PATTERN Oliveira MM, Nogueira Alves A, Koyama T, Shingleton AW, Mirth CK	**S15-2) Elphick, Maurice (UK)** THE EVOLUTION AND COMPARATIVE PHYSIOLOGY OF NEUROPEPTIDE SIGNALLING: INSIGHTS FROM ECHINODERMS Elphick MR
15:10-15:30	**S13-3) Greives, Timothy (USA)** SONGBIRDS AS A MODEL FOR UNCOVERING MECHANISMS REGULATING VARIATION IN SEASONAL REPRODUCTIVE TIMING IN THE WILD Greives TJ, Ketterson ED	**S14-3) Lavine, Laura (USA)** ENDOCRINE CONTROL OF CONDITION-DEPENDENT TRAITS. Lavine LC, Gotoh H, Warren IA, Zinna RA, Emlen DJ	**S15-3) Larhammar, Dan (Sweden)** NEUROPEPTIDES AND RECEPTORS IN THE DEUTEROSTOME PREDECESSOR OF VERTEBRATES Larhammar D, Bergqvist CA, Xu B, Lagman D, Ocampo Daza D
15:30-15:50	**Coffee Break**		
15:50-16:10	**S13-4) Martyniuk, Chris (USA)** METABOLIC PROFILING IN RADIAL GLIAL CELLS: A NOVEL APPROACH TO STUDY REGULATION BY ENDOCRINE DISRUPTORS AND SEX STEROIDS Souders II C, Schmidt J, Da Fonte D, Xing L, Trudeau VL, Martyniuk CJ	**S14-4) Shingleton, Alexander (USA)** BREATH CONTROL: THE SYSTEMIC REGULATION OF GROWTH IN RESPONSE TO HYPOXIA IN *DROSOPHILA* Shingleton AW, Saleh Ziabari O, Zhu Y, Broeker H, Tank P, Petranek C, Harrison, JF	**S15-4) D'Aquila, AL (Canada)** **THE ROLE OF THE TENEURIN C-**TERMINAL ASSOCIATED PEPTIDE (TCAP) FAMILY IN ENERGY PRODUCTION IN PROTOCHORDATES AND CHORDATES. D'Aquila, AL(1), Reid, RML(3), Biga, PR(3), Locke, M(2), Lovejoy, DA(1)
16:10-16:30	**S13-5) Norris, David (USA)** ARE INTERSEX FISHES IN NORTH AMERICAN RIVERS A RECENT PHENOMENON? Norris DO, Bolden AL, Vajda AM	**S14-5) Renn, Suzy (USA)** MECHANISMS OF BEHAVIORAL PLASTICITY ON MULTIPLE TIMESCALES. Renn S	**S15-5) Osugi, Tomohiro (Japan)** IMAGING MASS SPECTROMETRY ANALYSIS OF MULTIPLE NEUROPEPTIDES IN THE BRAIN OF CIONA INTESTINALIS Osugi T, Shiraishi A, Sugiura Y, Sasakura Y, Sakamoto N, Kageyama A, Satake H
16:30-16:50	**S13-6) Maclatchy, Deborah (Canada)** RESPONSES TO ESTROGENIC ENDOCRINE DISRUPTORS ARE VARIABLE IN COMMON MODEL TELEOSTS MacLatchy D, Lister A, Kanagasabesan T	**S14-6) Laslo, Mara (USA)** EXPRESSION OF THYROID HORMONE RECEPTORS AND DEIODINASES IN THE DIRECT-DEVELOPING FROG *ELEUTHERODACTYLUS COQUI* Laslo M, Hanken J	**S15-6) Taylor, Elias (Canada)** THE EFFECT OF THYROID HORMONES ON LARVAL SKELETOGENESIS IN THE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS* Taylor E, Heyland A

Thursday June 8, 2017 - Morning Session			
Plenary Lecture 8:00-8:45	**PL5 - NASCE Gorbman-Bern New Independent Investigator: Valerie Langlois (Canada)** (MT Temple A &B) **THE INSIDE STORY OF STEROID 5-REDUCTASES IN FROGS** **Chair: Vance Trudeau**		
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session Chair	**S16 - Growth Hormone and prolactin: Neuroprotective and developmental actions** **Stephen Harvey and Carlos Arámburo de la Hoz**	**S17 - Development of the neuroendocrine system** **Deborah Kurrasch and Per-Erik Olsson**	**S18 - Steroid Receptor Actions and Their Signaling: Nongenomic vs. Genomic** **Yong Zhu and Peter Thomas**
SOA Lecture 8:50-9:20	**SOA22 - Capilla, E (Spain)** GROWTH POTENTIAL IMPROVEMENT IN GILTHEAD SEA BREAM BY RECOMBINANT BOVINE GROWTH HORMONE (rBGH) Capilla E, Vélez EJ, Perelló M, Azizi Sh, Lutfi E, Navarro I, Blasco J, Fernández-Borràs J, Gutiérrez J	**SOA23 - Shah, Nirao (USA)** MODULAR GENETIC AND NEURAL CONTROL OF SEXUALLY DIMORPHIC SOCIAL BEHAVIORS Shah, NM	**SOA24 - Thomas, Peter (USA)** ROLE OF THE NOVEL MEMBRANE PROGESTIN, ESTROGEN, AND ANDROGEN RECEPTORS (GPER, PAQR7, AND ZIP9) IN THE REGULATION OF FISH OVARIAN FUNCTIONS Thomas P
9:20-9:40	**S16-1) Aramburo, Carlos (Mexico)** NEUROPROTECTIVE ACTIONS OF GH IN THE EMBRYONIC AND POSTNATAL CHICKEN RETINA Martínez-Moreno CG, Carranza M, Ávila-Mendoza J, Luna M, Harvey S, Arámburo C	**S17-1) Kurrasch, Deborah (Canada)** THE INFLUENCE OF HORMONES ON HYPOTHALAMIC NEURAL PROGENITORS DURING EMBRYONIC DEVELOPMENT Thornton H, Nesan D, Kurrasch DM	**S18-1) Shi, Yunbo (USA)** NUCLEAR ACTION OF TRA CONTROLS METAMORPHIC TIMING AND RATE DURING *XENOPUS* DEVELOPMENT Wen L, Shibata Y, Su D, Fu L, Luu N, Shi Y
9:40-10:00	**S16-2) Stephen Harvey (Canada)** GROWTH HORMONE PROTECTS AGAINST KAINATE EXCITOTOXICITY (IN VITRO AND IN VIVO) AND INDUCES BDNF AND NT3 EXPRESSION IN NEURORETINAL CELLS Martinez-Moreno CG, Fleming T, Carranza M, Avila-Mendoza J, Luna M, Harvey S, Arámburo C	**S17-2) Heyland, Andreas (Canada)** SEA URCHIN HISTAMINE RECEPTOR 1 REGULATES PROGRAMMED CELL DEATH IN LARVAL STRONGYLOCENTROTUS PURPURATUS Luteka K, Heylanda A	**S18-2) Vijayan, Matt (Canada)** NONGENOMIC CORTISOL SIGNALING IN RAINBOW TROUT HEPATOCYTES Das C, Wildering W, Vijayan M
10:00-10-20	**S16-3) Morales, Teresa (México)** NEUROPROTECTIVE ACTIONS OF PROLACTIN HORMONE AGAINST NEUROTOXIN LESIONS IN THE CNS OF RODENTS Morales T, Ramos E, Anagnostou I, Reyes-Mendoza J	**S17-3) Dewey, Deborah (Canada)** BRAINS AND BEHAVIOUR IN YOUNG CHILDREN EXPOSED PERINATALLY AND IN EARLY CHILDHOOD TO ENDOCRINE DISRUPTING CHEMICALS Dewey D, Ejaredar M, Liu J, Grohs M, Ten Eckye K, Giesbrecht GF, Letourneau N, Lebel C, Martin JW	**S18-3) Zhao, Xiao Fan (PRC)** G-PROTEIN-COUPLED RECEPTOR TRANSMITS STEROID HORMONE SIGNAL ON CELL MEMBRANE Wang D, Zhao W, Cai M, Ren J, Liu W, Jing Y, Wang J, Zhao, Xiao Fan
10:20-10:50	**Coffee Break**		
10:50-11:10	**S16-4) Luna, Maricela (México)** NEUROPROTECTIVE ACTIONS OF GH AND IGF-I AGAINST HYPOXIA-ISCHE–MIA INDUCED BRAIN DAMAGE Luna M, Baltazar-Lara MR, Armenta ME, Carranza M, Martínez-Moreno CG, Arámburo C	**S17-4) Nishimura, Hiroko (Japan)** STRESS AND REDUCED NUTRITION DURING DEVELOPMENT PROGRAM ABNORMAL GROWTH OF THE EMBRYO Nishimura H, Gomez RA	**S18-4) Pang, Yefei (USA)** COORDINATE CONTROL OF MEIOTIC ARREST OF ZEBRAFISH OOCYTES BY GPER- AND NATRIURETIC PEPTIDE RECEPTOR 2-MEDIATED SIGNALING Pang Y, Thomas P
11:10-11:30	**S16-5) Causey, Dwight (UK)** DIFFERENT ROUTES TO RAPID GROWTH: PROTEOME-WIDE OUT–COMES OF SELECTIVE BREEDING VS. GROWTH HORMONE TRANSGENESIS Causey DR, Kim JH, Alzaid A, Stead DA, Martin SAM, Devlin RH, Macqueen DJ	**S17-5) Nesan, Dinushan (Canada)** INVESTIGATING THE ACTIONS OF STEROID HORMONES AND XENOESTROGENS ON HYPOTHALAMIC NEUROGENESIS DURING DEVELOPMENT Nesan D, Thornton HF, Kurrasch DM	**S18-5) Zhu, Yong (USA)** GENERATION AND CHARACTERIZATION OF ZEBRAFISH KNOCKOUT MODELS FOR STUDYING FUNCATIONS OF GENOMIC AND NONGENOMIC PROGESTIN RECEPTORS Zhu Y, Liu DT, Wu XJ
11:30-11:50	**S16-6) Biga, Peggy (USA)** VARIABLE ORGANISMAL GROWTH POTENTIAL CORRESPONDS TO DIFFERENTIAL GROWTH HORMONE SIGNALING MECHANISMS Biga P, Reid R, Latimer M	**S17-6) Miller, Annie (USA)** SEX AS AN ENRICHMENT TO RESCUE REPRODUCTIVE DEFICITS IN MALE TRANSGENIC MICE Miller AV, Brooks LR, Tsai PS	**S18-6) Mohapatra, Sipra (USA)** ESTROGEN RECEPTORS: MAJOR PLAYERS IN SEX-BIASED REGULATION OF AUTOPHAGY IN FISH Mohapatra S, Chakraborty T, Shimizu S, Ohta K
SOA Lecture 11:50-12:20	**SOA25 - Björnsson, Björn Thrandur (Sweden)** ENVIRONMENTAL ENDOCRINOLOGY OF GROWTH HORMONE IN SALMONIDS Björnsson BTh	**SOA26 - Belosevic, Miodrag (Canada)** DEVELOPMENT OF MYELOID CELLS IN BONY FISH Belosevic M	**SOA27 - Nelson, Erik (USA)** A CHOLESTEROL METABOLITE PROMOTES BREAST CANCER PROGRESSION; NEW PERSPECTIVES ON SELECTIVE ESTROGEN RECEPTOR PHARMACOLOGY Nelson, E

Thursday June 8, 2017 - Afternoon Session		
12:20 -14:40	**Lunch and** **Poster Session 2** **Even poster numbers** **Victoria Ballroom/Terrace/ Red Room**	
Plenary Lecture 14:40 -15:25	**PL6 - ICCE18 Plenary Lecturer: Xavier Bellés (Spain)** (MT Temple A &B) **REGULATION OF INSECT METAMORPHOSIS BY HORMONAL SIGNALING AND MicroRNAs** **Chair: Angela Lange**	
15:40 – 17:30	**IFCES and NASCE Business meeting** **(MT Temple C)**	**Workshop (sponsored by Bio-Rad) -** (Plains6/ Saddleback) **High resolution Expression Quantification**

19:30 – 23:30

Banquet (separate ticket required) / Live music - Harp music (7:30-9:30)
Victoria Ballroom/Red Room / Terrace

Live music – Dance (9:30-11:30)

Friday June 9, 2017 - Morning Session			
Plenary Lecture 8:00-8:45	**PL7 - ISAREN Plenary Lecturer: Taisen Iguchi (Japan)** (MT Temple A &B) **TRPV4 ASSOCIATES ENVIRONMENTAL TEMPERATURE AND SEX DETERMINATION IN THE AMERICAN ALLIGATOR** **Chair: Caren Helbing**		
	Temple A &B	**Lakeshore/ Beehive**	**Plains6/ Saddleback**
Session Chair	**S19 - Endocrine Disruption in Aquatic Vertebrates - Lessons Learned and Future Prospects -A Tribute to Professor Louis J. Guillette, Jr.** **Charles R. Tyler and Thea Edwards**	**S20 - Biological Rhythms, circadian clock** **Akiyoshi Takahashi, Takashi Yoshimura, Horst-Werner Korf**	**S21 - Kisspeptins: mandatory or optional for reproduction** **Berta Sivan Sivan and James Nagler**
SOA Lecture 8:50-9:20	**SOA28 - SA28 Tyler, Charles (UK)** THE FEMINIZATION OF NATURE – CHEMICALS, MECHANISMS AND CONSEQUENCES Tyler CR	**SOA29 - Korf, Horst-Werner (Germany)** IMPACT OF ENDOGENOUS MELATONIN ON CIRCADIAN ORGANIZATION Korf HW, Wicht H, Pfeffer M	**SOA30 - Kriegsfeld, Lance (USA)** THE ROLES OF KISSPEPTIN AND GONADOTROPIN-INHIBITORY HORMONE THE SEASONAL CONTROL OF REPRODUCTION Kriegsfeld, LJ
9:20-9:40	**S19-1) Edwards, Thea (USA)** GRANULATED MAST CELLS, AN UNEXPECTED OCCUPANT IN TILAPIA HEPATO-PANCREAS Edwards TM	**S20-1) Yoshimura, Takashi (Japan)** UNDERSTANDING THE MOLECULAR BASIS OF VERTEBRATE SEASONAL ADAPTATION. Yoshimura T	**S21-1) Parhar, Ishwar (Malaysia)** CELLULAR IDENTITY AND REPRODUCTIVE FUNCTION OF DEEP BRAIN PHOTORECEPTORS, SEROTONIN AND KISSPEPTIN SYSTEM Parhar I
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Workshop
Thursday June 8
15:40 – 17:00
High resolution Expression Quantification
Plains6/ Saddleback

Meysam Abbasi, PhD
Application Scientist
Bio-Rad Laboratories

High Resolution Detection of Rare Transcripts by Droplet Digital PCR (ddPCR)

ddPCR is the next generation of PCR which allows for absolute quantitation of nucleic acids without the requirement for standard curves. This technology applies sample partitioning to generate 20,000 individual PCR reactions, allowing for unrivalled precision, accuracy, and sensitivity. Emerging applications include Gene Expression, Mutation Detection, Pathogen Detection, Copy Number Variation, and Genome Editing. Publications using this technology have increased well over 500 during the past 4 years and it has proven to be a truly quantitative technique for detection of rare transcripts. In this seminar we will provide a brief overview of this technology, discuss the complimentary nature of ddPCR to qPCR, and review published data using ddPCR in the field of Endocrinology.

A Highly Quantitative Western Blot method using Total Protein Normalization

The traditional method for normalization of target protein expression in Western Blot is to normalize to housekeeping proteins such as Actin, Tubulin, and GAPDH. However, these proteins typically saturate on the membrane and are not reliable sources for protein normalization. Furthermore expression of these proteins is rarely stable and often varies based on treatment. Many publications have emphasized on this and introduced normalization to total protein as a solution to this problem. Furthermore, JBC has now stated Total Protein Normalization as the preferred normalization method for semi quantitative western blots. In this seminar we will provide details of Bio-Rad's quantitative western blot workflow that allows for fast and reliable quantitation and normalization of target protein expression.

Abstracts
ICCE18

Plenary Lectures

PL1 - Monday June 5, 8:30 am

ICCE18 Bargmann-Scharrer Lecturer: Stacia Sower (USA)

THE ORIGINS OF THE VERTEBRATE HYPOTHALAMIC-PITUITARY AXIS: INSIGHTS FROM LAMPREYS

Sower S
A Department of Molecular, Cellular and Biomedical Sciences and Center for Molecular and Comparative Endocrinology, University of New Hampshire, Durham, NH, USA.

The hypothalamic-pituitary (HP) system, which is specific to vertebrates, is considered to be an evolutionary innovation that emerged prior to or during the differentiation of the ancestral jawless vertebrates (agnathans) leading to the neuroendocrine control of many complex functions. Along with hagfish, lampreys represent the oldest lineage of vertebrates, agnathans (jawless fish). Generally, gnathostomes (jawed vertebrates) have one or two hypothalamic gonadotropin-releasing hormones (GnRH) while lampreys have three hypothalamic GnRHs. These GnRHs regulate reproduction in all vertebrates via the pituitary. In gnathostomes, there are three classical pituitary glycoprotein hormones (luteinizing hormone, LH; follicle stimulating hormone, FSH; and thyrotropin, TSH) interacting specifically with three receptors, LH-R, FSH-R, and TSH-R, respectively. In general, FSH and LH regulate gonadal activity and TSH regulates thyroidal activity. In contrast to gnathostomes, lampreys have only two heterodimeric pituitary glycoprotein hormones, lamprey GpH and thyrostimulin, and two lamprey glycoprotein hormone receptors (lGpH-R I and –R II). The existing data suggest the existence of a primitive, overlapping yet functional hypothalamic-pituitary-gonadal (HPG) and HP-thyroidal (HPT) endocrine systems in lampreys. The study of basal vertebrates provides promising models for understanding the evolution of the hypothalamic-pituitary-thyroidal and gonadal axes in vertebrates. We hypothesize that the glycoprotein hormone/glycoprotein hormone receptor systems emerged as a link between the neuroendocrine and peripheral control levels during the early stages of gnathostome divergence. Discovery of a functional HPG axis in lamprey has provided important clues for understanding the forces that ensured a common organization of the hypothalamus and pituitary as essential regulatory systems in all vertebrates. This talk will present the discoveries and latest information including phylogenomic analyses on the origins, co-evolution and divergence of ligand and receptor protein families of gonadotropin-releasing hormones (GnRH) and pituitary glycoprotein hormones (GpH).
Supported by: NSF IOS-1257476 and AES NH00624 to SAS

PL2 - Tuesday June 6, 8:00 am

ICCE18 Grace Pickford Medal Lecturer: Kataaki Okubo (Japan)

SEXUAL DIFFERENTIATION AND PLASTICITY OF THE BRAIN OF TELEOST FISH

Okubo K
Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Male and female animals exhibit differences in a wide range of physiological and behavioral traits, including endocrine secretion patterns, stress response, aggression, and reproductive behavior. Many of these differences certainly reflect underlying differences in brain structure and function between males and females. Numerous studies in mammals and birds have shown that the brain is sexually differentiated early in development, under the influence of gonadal hormones and sex chromosome genes. This is an irreversible process, and consequently the sexual phenotype of the brain is permanently fixed. However, this appears not to hold for the brain of teleost fish. The phenotypic sex of teleost fish, including sex-specific behavioral patterns, can be altered by treatment with exogenous hormones, even in adulthood. In addition, many teleost species spontaneously change their phenotypic sex in response to various social and physiological factors. These phenomena indicate that the brain of teleost fish have distinctive mechanisms of sexual differentiation, which enable it to maintain a considerable degree of sexual plasticity throughout life. However, the molecular and cellular basis for sexual differentiation and plasticity of the teleost brain remains largely unknown. We recently identified female-specific, sex steroid hormone-responsive peptidergic neurons in regions of the teleost brain that have been implicated in reproductive behavior. A subsequent series of analyses suggested that these neurons contribute substantially to sexual differentiation and enduring sexual plasticity of the teleost brain. Acknowledgements: I am truly grateful to all my mentors, lab members, and collaborators who have generously helped me over the years. This work has been supported in part by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan, the Japan Society for the Promotion of Science (JSPS), the Suntory Foundation for Life Sciences, and the Towa Foundation for Food Science and Research.

PL3 - Tuesday June 6, 2:40 pm

ICCE18 Plenary Lecturer: Marilyn Renfree (Australia)

NEW PARADIGMS FROM THE ENDOCRINOLOGY OF MARSUPIAL SEXUAL DIFFERENTIATION

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School of BioSciences, The University of Melbourne, Melbourne, Victoria, Australia

Marsupials are only 6% of living mammals but have many characteristics that can inform us about the endocrine control of reproduction and development. A striking characteristic is that marsupials give birth to highly altricial young after a relatively short gestation period supported by the chorio-vitelline placenta. They complete much of their development within the pouch, dependent on a long and highly sophisticated lactation during which the composition of the milk changes dynamically to coordinate the specific growth requirement of the developing young. We have focussed on the control of sexual differentiation, which all takes place post-natally, and discovered some unexpected findings that have caused re-evaluation of accepted dogma. We have described a new alternate androgen synthesis pathway in the developing young that is responsible for virilisation of the prostate and phallus at specific windows of sensitivity. We also overturned the powerful Jost paradigm that sexual differentiation simply depended on hormones secreted by the testis when we demonstrated a number of hormone-independent sexual dimorphisms before the testicular differentiation. We now know that there are many other hormone-independent sexual dimorphisms, not only in mammals but also in birds. In addition, because of the post-natal gonadal development, we have been able to achieve testicular sex reversal after treatment with oestrogen *in vivo* and *in vitro*, and furthermore can induce sex reversal and hypospadias of the penis with oestrogen treatment, and sex reversal of the clitoris with androgen treatment *in vivo*. Marsupials may only be 6% of living mammals, but their unique post-natal sexual differentiation provides novel perspectives for further understanding the evolution and control of sexual differentiation of the internal and external genitalia.Acknowledgements: Supported by the Australian Research Council and the Australian National Health and Medical Research Council.

PL4 - Wednesday June 7, 8:00 am

NASCE Gorbman-Bern Lecturer: Robert Denver (USA)

KRÜPPEL-LIKE FACTORS ARE EFFECTORS OF NUCLEAR RECEPTOR SIGNALING

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Department of Molecular, Cellular and Developmental Biology, The University of Michigan, Ann Arbor, MI 48109 USA

The Krüppel-like family of transcription factors (Klf) is an evolutionarily conserved family of DNA-binding proteins that function in many aspects of animal development and physiology. Several members of this family have been shown to have diverse roles in nuclear hormone receptor (NR) signaling. For example, Klfs can act as accessory transcription factors for NR actions, they regulate expression of NR genes, and they are gene products of primary NR response genes, and thus play key roles in hormone-dependent transcriptional networks. In mouse models, deletion of different Klfs leads to aberrant transcriptional and physiological responses to hormones, underscoring the importance of these proteins in the regulation of hormone signaling. In my presentation I will present a conceptual framework for understanding how Klfs participate in NR signaling. I will highlight our recent findings on how Klfs function in chromatin to regulate gene transcription, how multiple Klfs interact to form a regulatory network in neurons, how they play key roles in promoting and maintaining neuronal differentiation, and novel roles for Klfs in the feed forward modulation of the cellular circadian clock.
Acknowledgements: Original research discussed in this presentation was supported by grants from the National Institute of Neurological Disorders and Stroke (1 R01 NS046690), and from the National Science Foundation (IOS 0922583 and IOS 1456115) to R.J.D.

PL5 - Thursday June 8, 8:00 am

NASCE Gorbman-Bern New Independent Investigator: Valerie Langlois (Canada)

THE INSIDE STORY OF STEROID 5-REDUCTASES IN FROGS

Langlois, V.S. [1,2,3], Bissegger S and DEK Campbell
[1]Department of Chemistry and Chemical Engineering, Royal Military College of Canada, Kingston, ON, Canada; [2] Biology Department, Queen's University, Kingston, ON, Canada; [3]Institut national de recherche scientifique – Eau, Terre, Environment (INRS-ETE), Quebec, QC, Canada (Dr. Langlois' new affiliation as of September 2017)

Amphibian populations are declining worldwide partly due to environmental pollution. Certain chemicals present in our ecosystem have been shown to disrupt the endocrine system in vertebrates by interfering with normal steroidogenesis. Androgens are synthesized from the precursor testosterone (T) by specific enzymes (e.g., steroid 5-reductases) and need to be present in a balanced ratio for an organism to function normally. Three types of 5α-reductases (Srd5α) and one type of 5β-reductase (Srd5β) are involved in the conversion of T into 5α-dihydrotestosterone (5α-DHT) and 5β-dihydrotestosterone (5β-DHT), respectively. In amphibians, the inhibition of the activity of Srd5α2 leads to feminization and decreased spermatogenesis. However, little is known about the biological functions and regulation of Srd5 in this taxon and how endocrine disrupting chemicals affect these enzymes. Hence, the main objective of this research project was to further our understanding on Srd5 in developing and adult *Silurana tropicalis* frogs. First, we confirmed that endocrine disrupting chemicals (i.e.,

plasticizers, pharmaceuticals, and industrial dyes) modify *srd5* mRNA levels during *S. tropicalis* embryogenesis. Then, to gain a better understanding of the tissue distribution of srd5 (*srd5α1, srd5α2, srd5α3*, and *srd5β*), whole mount *in situ* hybridization was performed in embryos and showed a unique expression pattern for all *srd5*. Data suggested that metabolites synthesized by Srd5 are required in the central nervous, sensory, cardiac, respiratory, and detoxifying systems aside from reproduction in early anuran development. Complementary hormonal and specific DNA methylation assays were conducted during anuran embryogenesis and adulthood. Experiments suggested that specific DNA methylation of srd5α1 and srd5α3 is involved in regulating their expression during embryogenesis as well as in mature gonads. In addition, further chemical challenges to T, triiodothyronine, and to a known thyroid hormone inhibitor potassium perchlorate modified *srd5* mRNA levels in gonads in a sex-specific manner demonstrating that androgens and thyroid hormones can also regulate *srd5* transcription. Taken together, these data confirm the importance of Srd5 in biological functions related to anuran reproduction, but also provides evidence that this enzyme family is crucial in other biological functions for proper development.

PL6 - Thursday June 8, 2:40 pm

ICCE18 Plenary Lecturer: Xavier Bellés (Spain)

REGULATION OF INSECT METAMORPHOSIS BY HORMONAL SIGNALING AND MicroRNAs

Bellés X
Institute of Evolutionary Biology, CSIC-Universitat Pompeu Fabra, Passeig Marítim 37, 08003 Barcelona, Spain.

Insect metamorphosis is a process of post-embryonic development, by which immature forms of the insect become morphologically and functionally an adult, able to fly and reproduce. The study of the mechanisms regulating metamorphosis, a complex and fascinating process, has experienced spectacular progress in the last 10 years. The new discoveries have focused on the mode of action of hormones that regulate the metamorphosis, especially juvenile hormone (JH), and on the role of microRNAs. Hormonal patterns and functional genomic experiments show that JH represses adult morphogenesis in pre-adult stages. At the beginning of the last juvenile instar, JH levels dramatically drop, which determines that the next molt will be the adult one. At molecular level, the regulatory axis of the action of JH in metamorphosis is very simple and based on the MEKRE93 pathway (Belles and Santos, 2014): JH binds to *Methoprene-tolerant* (*Met*, which is the JH receptor) and this complex induces the expression of *Kruppel-homolog 1* (*Kr-h1*), the transducer of the antimetamorphic signal of JH. Essentially, the antimetamorphic action is exerted by repressing the expression of *E93*, the master trigger of metamorphosis (Belles and Santos, 2014; Ureña et al., 2014). MicroRNAs (miRNAs) are ca. 22 nucleotides non-coding RNA molecules that block gene expression post-transcriptionally by binding a target mRNA, which cannot be ultimately translated into protein. A number of miRNAs are involved in the regulation of insect metamorphosis, either in the less modified hemimetabolan mode or in the more modified holometabolan mode (Belles, 2017). A special case is the control of miR-2 over *Kr-h1* transcripts, which determines adult morphogenesis in the hemimetabolan metamorphosis (Lozano et al., 2015). This is an elegant example of how a single miRNA can control the entire process of metamorphosis by acting upon a crucial mediator of the hormonal action.

PL7 - Friday June 9, 8:00 am

ISAREN Plenary Lecturer: Taisen Iguchi (Japan)

TRPV4 ASSOCIATES ENVIRONMENTAL TEMPERATURE AND SEX DETERMINATION IN THE AMERICAN ALLIGATOR

Iguchi T(1)
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Prof. Howard A. Bern told me to meet and work together with Lou Guillette since we both found polyovular follicles in neonatally estrogen-exposed mice and American alligators (Alligator mississippiensis) from Lake Apopka. We finally met in Washington, D.C. in 1994. Since then, we enjoyed collaboration cloning steroid receptors from various animal species including alligator. We finally started to tackle temperature-dependent sex determination in alligators and found TRPV4 is a key sensor of male producing temperature. Temperature-dependent sex determination (TSD), commonly found among reptiles, is a sex determination mode in which the incubation temperature during a critical temperature sensitive period (TSP) determines sexual fate of the individual rather than the individual's genotypic background. In the American alligator, eggs incubated during the TSP at 33 °C (male producing temperature: MPT) yields male offspring, whereas incubation temperatures below 30 °C (female producing temperature: FPT) lead to female offspring. However, many of the details of the underlying molecular mechanism remains elusive, and the molecular link between environmental temperature and sex determination pathway is yet to be elucidated. We show the alligator TRPV4 ortholog (AmTRPV4) to be activated at temperatures proximate to the TSD-related temperature in alligators, and using pharmacological exposure, we show that AmTRPV4 channel activity affects gene expression patterns associated with male differentiation. Our characterization of transcriptomes during alligator TSD allowed us to identify novel candidate genes involved in TSD initiation. High-throughput RNA sequencing (RNA-seq) was performed on gonads collected from A. mississippiensis embryos incubated at both MPT and FPT in a time series during sexual development. RNA-seq yielded 375.2 million paired-end reads, which were mapped and assembled, and used to characterize differential gene expression. Gonadal global gene expression kinetics during sex determination has been extensively profiled for the first time in a TSD species. These findings are the first experimental demonstration of a link between a well-described thermo-sensory mechanism, TRPV4 channel, and its potential role in regulation of TSD in vertebrates,

shedding unique new light on the elusive TSD molecular mechanism, and provide insights into the genetic framework underlying TSD, and expand our current understanding of the developmental fate pathways during vertebrate sex determination.

Acknowledgements: Supported by JSPS KAKENHI grant no. 15H04396 and a grant of UK-Japan Research Collaboration from Ministry of the Environment, Ja

State of Art Lectures

Monday June 5

SOA1 Chang, John (Canada)

BIASED SIGNALING IN GONADOTROPIN-RELEASING HORMONE STIMULATION OF GOLDFISH PITUITARY GONADOTROPES AND SOMATOTROPES.

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The goldfish brain and pituitary contain two gonadotropin (GTH)-releasing hormones (GnRHs), chicken GnRH-II (GnRH2) and salmon GnRH (GnRH3); both stimulate luteinizing hormone (LH) and growth hormone (GH) release as well as gene expression through shared G protein-coupled receptors (GnRHRs) on gonadotropes and somatotropes. We have shown that protein kinase C (PKC), extracellular Ca^{2+} entry through voltage-sensitive channels, extracellular signal-regulated protein kinase (ERK), and cyclic GMP/PKG are involved in mediating GnRH2- and GnRH3-induced hormone release; however, differential involvement of intracellular Ca^{2+} stores with distinct pharmacological properties, mitochondrial Ca^{2+} buffering, nitric oxide production, and arachidonic acid are also evident in a GnRH- and cell-type-selective manner. Although PKC activates ERK, ERK-dependent signaling, but not PKC, mediates GnRH-stimulated increases in GTH subunit and GH mRNA levels. Increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) also generally suppress GTH and GH gene expression. Recently, we showed that distinct combinations of the phosphatidylinositol-3,4,5-P_3 (PtdIns(3,4,5)P_3)-generating class I phosphoinositide 3-kinases (PI3Ks), including the classically GPCR-insensitive PI3Kδ, as well as the Gβγ–sensitive PI3Kβ and PI3Kγ isoforms, are selectively involved in GnRH2 and GnRH3 signaling in LH and GH release. The relationship between PI3Ks and GnRH-induced changes in $[Ca^{2+}]_i$ also differs between LH and GH release. Downstream of class I PI3Ks activation, results indicate the recruitment of non-canonical effectors possessing PtdIns(3,4,5)P_3-binding pleckstrin homology domains, as well as demonstrate the selective participation of the canonical PI3K-dependent transduction targets, protein kinase B (Akt) and Bruton's tyrosine kinase (BTK), in GnRH-stimulated hormone release. PI3K- and ERK-dependent signaling also differentially mediate GnRH-selective effects on elevations in cellular LH and GH contents in a time-dependent manner. These results, when taken as a whole, support the hypothesis that biased GnRHR signaling forms the basis for ligand-, function-, and cell-type specific neuroendocrine control of gonadotrope and somatotrope activities by native GnRH peptides. (Supported by NSERC, AIHS and Killiam Trusts).

SOA2 Duan, Cunming (USA)

A NOVEL MECHANISM UNDERLYING CALCIUM DEFICIENCY-INDUCED IIS-PI3K-AKT-TOR SIGNALING AND EPITHELIAL CELL PROLIFERATION

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The evolutionarily ancient insulin-like growth factor (IGF)/insulin (IIS) hormonal pathway plays key roles in regulating development, growth, metabolism, and aging. Aberrant regulation of this pathway is linked to major human diseases, and key components of this pathway are major targets for therapeutic intervention. In many cell types, IGFs and insulin are strong activators of the PI3K-Akt-TOR signaling pathway. The biological actions of IGFs and insulin are mainly mediated through the IGF1 receptor (IGF1R) and insulin receptor (InsR), respectively IGFs but not insulin are bound to a family of IGF binding proteins (IGFBPs) in the extracellular environments. These IGFBPs alter IGF biological activity by modulating their interaction with the IGF1R. Here we report the discovery of a novel mechanism underlying the Ca^{2+} deficiency-induced IIS signaling and epithelial proliferation. Zebrafish embryos uptake Ca^{2+} from the surrounding water using a group of epithelial cells known as NaR cells. NaR cells specifically express Igfbp5a. We have discovered that low $[Ca^{2+}]$ stress results in a robust and sustained activation of the IGF1R-PI3K-Akt-Tor signaling in NaR cells and stimulates their proliferation. Blockage of IGF1R, PI3K, Akt, and Tor abolishes Ca^{2+} deficiency-induced NaR cell proliferation. We have developed a transgenic line, *Tg(igfbp5a:GFP)*, which expresses GFP under the control of the *igfbp5a* promoter. Using this phenotype-based whole organism platform, a chemical biology screen was carried out. Among several hits are several inhibitors of Trpv5/6, a selective Ca^{2+} channel. Further analyses suggest that blockage of Trpv5/6 activity abolishes the low $[Ca^{2+}]$-induced IGF1R-PI3K-Akt-Tor signaling and NaR cell proliferation. Interestingly, blockage or knockout of Trpv5/6 results in elevated IGF1R-PI3K-Akt-Tor signaling and increased NaR cell proliferation under normal $[Ca^{2+}]$. These data suggest that Trpv5/6 is a key regulator of the IGF1R-PI3K-Akt-Tor signaling pathway in NaR cells. Trpv5/6 represses IGF signaling under normal $[Ca^{2+}]$, whereas it strongly activates IGF signaling under low $[Ca^{2+}]$. Importantly, this new signaling mechanism is conserved in human epithelial cells.

SOA3 Tsutsui, Kazuyoshi (Japan)

GNIH CONTROL OF REPRODUCTIVE PHYSIOLOGY AND BEHAVIOR.

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Based on the morphology of hypothalamic neurons that terminate at the median eminence (ME), Harris hypothesized that these hypothalamic neurons may secrete neurohormones from the ME into the hypophysial portal system to regulate the secretion of anterior pituitary hormones. At the beginning of the 1970s, gonadotropin-releasing hormone (GnRH), a hypothalamic neuropeptide known to stimulate the release of gonadotropins from gonadotropes in the anterior pituitary, was discovered in mammals by Schally's and Guillemin's groups. Subsequently, several GnRHs have been identified in other vertebrates. Based on extensive studies of GnRH, it was assumed that GnRH is the only hypothalamic neuropeptide regulating gonadotropin release in mammals and other vertebrates. In 2000, however, Tsutsui and colleagues challenged this notion with the discovery of gonadotropin-inhibitory hormone (GnIH), a hypothalamic neuropeptide that actively inhibits gonadotropin release, in quail. Subsequent studies conducted by Tsutsui and colleagues over the past decade and a half demonstrated that GnIH is highly conserved among vertebrates, from agnathans to humans, acting as a key player regulating reproduction. In addition, recent studies by Tsutsui's group have demonstrated that GnIH has important functions beyond the control of reproduction. Based on these findings, it now appears that GnIH acts on the pituitary and the brain to affect a number of behaviors, including reproductive behavior through changes in neurosteroid, such as neuroestrogen, biosynthesis in the brain. Thus, the following 15 years of GnIH research has permitted a more complete understanding of the neuroendocrine control of reproductive physiology and behavior. Herein I summarizes the discovery of GnIH and the advances made in our understanding of GnIH control of reproductive physiology and behavior.

SOA4 Adams, Gregg (Canada)

ENDOCRINE ROLE OF OVULATION-INDUCING FACTOR/NERVE GROWTH FACTOR (OIF/NGF)

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Ovulation-inducing factor (OIF) was coined over 30 years ago in reference to an undefined substance in the seminal plasma of Bactrian camels thought to be responsible for inducing ovulation in this species. More recent studies, primarily in llamas and alpacas that are also induced ovulators, characterized the biological and chemical properties of OIF and ultimately identified it as beta nerve growth factor (βNGF). Nerve growth factor belongs to a family of neurotrophins, all of which exist in nature as homodimers with a molecular mass of 26-27kDa. Originally discovered in mouse sarcoma, cobra venom, and mandibular salivary glands of adult mice, NGF has been characterized classically by its role in promoting survival and growth of sensory (dorsal root) and sympathetic neurons, and cells of the adrenal medulla. The discovery that OIF = NGF has brought together the disciplines of neurobiology and reproductive biology, and different mechanisms of action of the same factor; i.e., autocrine/paracrine vs endocrine. To preserve this link, we refer to the seminal factor as OIF/NGF. As a highly conserved protein, the implications of discoveries related to OIF/NGF in reproductive tissues extend beyond the camelid species, and results of recent studies show that the presence and function of OIF/NGF in seminal plasma is conserved among species considered to be induced ovulators as well as those considered to be spontaneous ovulators. The abundance of OIF/NGF in seminal plasma and the effects of seminal plasma on ovarian function strongly support the idea of an endocrine mode of action (i.e., systemic distribution with distant target tissues). An overview and update on the progress in our understanding of the nature of OIF/NGF in seminal plasma will provide a better understanding of its effects on reproductive function in the female, including the effects of dose and route of administration, evidence for ovarian effects in other species, tissue sources of OIF/NGF, and early findings related to the mechanism of action of OIF/NGF. *Supported by NSERC Canada*.

SOA5 Takei, Yoshio (Japan)

HORMONAL CONTROL OF WATER AND ION ABSORPTION BY THE DIGESTIVE TRACT OF SEAWATER-ACCLIMATED EELS

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Marine teleost must absorb water by the intestine to compensate for the osmotic water loss. The efficient water absorption is achieved most importantly by the active desalination of imbibed seawater by the esophagus and the vigorous secretion of HCO_3^- by the intestine to precipitate Mg^{2+} and Ca^{2+}, both of which act to decrease the osmolality of luminal seawater to isotonicity for water and ion absorption by the distal digestive tract. Using euryhaline eels, *Anguilla japonica*, as a model, we identified all candidate transporters and transport-related molecules in the esophagus and intestine by the transcriptomic (RNA-seq) analyses. The candidate genes were screened by the following criteria: (1) blockade of the transport by the specific blocker in physiological experiments using Ussing chamber or esophageal/intestinal sac, (2) upregulation of the genes after transfer of eels from fresh water to seawater, (3) expression of the genes in the absorptive epithelial cells by in situ hybridization. These studies revealed transport molecules that are involved in the regulation of water and ion absorption at the subtype level.

As it is most likely that hormones are involved in the regulation of transport activity, we initially identified candidate hormones in the eel. Among them, natriuretic peptides (NP) and vasoactive intestinal peptides (VIP) applied to the serosal side and guanylins (GLN) applied to the mucosal side influence water and ion absorption when examined in the Ussing chamber or sac preparation. NPs act on the epithelial cells from blood while GLN is secreted into the lumen from the goblet cells, which reflects the difference in the minimum effective doses (10^{-10} M for NP and 10^{-7} M for GLN). The target of these hormones are NKCC2b that is a major transporter responsible for water and NaCl

absorption in the seawater eel intestine, but GLN also acts on the CFTR chloride channel to induce Cl^- and water secretion. The target of VIP is under investigation.

SOA6 Markov, Gabriel (France)

ORIGINS AND EVOLUTION OF THE NUCLEAR RECEPTOR / HORMONE COUPLE ILLUSTRATED BY THE CASE OF STEROIDS

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The evolution of the ligand/receptor couple is a question that attracts considerable debate, as it is key to understand the origin and diversification of hormonal systems in living organisms. The study of their origin as well as their parallel variation through co-evolution is a major evolutionary question. On this aspect of coevolution of ligand/receptor pairs the field is sharply cut into two parts given the chemical nature of the ligand. All the ligands that are peptides or proteins, i.e. that are encoded by genes provide conceptually relatively simple cases of ligand/receptor coevolution, with continuous adaptation across time. This situation contrasts with the second case, that of receptors for which the ligand is not a peptide or a protein but rather a small molecule. In such cases the ligand is not a gene product but is derived from a biochemical pathway that starts from an inactive precursor, sometimes derived from an external source such as food, which is transformed into the active molecule. This is the case for some GPCRs but also for many nuclear hormone receptors, for which the ligands are the products of complex biochemical pathways. In this case ligands are simply not supposed to evolve. However, because metabolic pathways are inherited by descent with modification, their structure can be compared using cladistic analysis. Using this approach, we studied the evolution of steroid hormones. We show that side-chain cleavage is common to most vertebrate steroids, whereas aromatization was co-opted for estrogen synthesis from a more ancient pathway. The ancestral products of aromatic activity were aromatized steroids with a side chain, which we named "paraestrols." We synthesized paraestrol A and show that it effectively binds and activates the ancestral steroid receptor. Our study opens the way to comparative studies of biologically active small molecules.

Tuesday June 6

SOA7 Ge, Wei (Macau)

GENETIC ANALYSIS OF EARLY FOLLICULOGENESIS IN THE ZEBRAFISH.

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Ovarian folliculogenesis is one of the most dynamic physiological and developmental processes in vertebrates. Despite numerous studies on this issue in mammals, the molecular mechanisms that control follicle development, especially the early phase of folliculogenesis, still remain poorly understood. Gonadotropins are primary endocrine hormones that control ovarian development and function. In addition, various local paracrine and autocrine factors in the ovary also play important roles in this process. Although the endocrine and paracrine controls of folliculogenesis have been studied in fish, there has been a lack of genetic data on their functional importance, mostly due to unavailability of the gene knockout approach in fish models. In a recent study using the emerging genome editing technology TALEN, we analyzed the impacts of FSH and LH on zebrafish follicle development by disrupting *fshb, lhb, fshr* and *lhcgr* genes. FSH-deficient zebrafish ($fshb^{-/-}$) were surprisingly fertile in females; however, the puberty onset or start of vitellogenic growth was significantly delayed. In contrast, LH-deficient zebrafish ($lhb^{-/-}$) showed normal gonadal growth, but the females failed to spawn and were therefore infertile. In contrast to *fshb* deficiency, the FSH receptor (*fshr*)-deficient females showed a complete failure of follicle activation; however, the deletion of *lhcgr* gene caused no obvious phenotypes. In summary, our results in the present study showed that Fshr was indispensable to folliculogenesis. In contrast, *lhcgr* does not seem to be essential to zebrafish reproduction. Neither Fshr nor Lhcgr deficiency could phenocopy the deficiency of their cognate ligands FSH and LH, which is likely due to the fact that Fshr can be activated by both FSH and LH in the zebrafish. Acknowledgement: This work was funded by grants from University of Macau and The Macau Fund for Development of Science and Technology.

SOA8 Bernier, Nicholas (Canada)

EARLY LIFE ENVIRONMENTAL CHALLENGES AND THE STRESS RESPONSE IN ZEBRAFISH

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Eutrophication and climate change are increasing the incidence of severe hypoxia and high environmental ammonia (HEA) in fish nursery habitats yet the short- and long-term impact of these challenges on stress responsiveness in later life are largely unknown. Therefore, to identify the potential programming effects of these environmental stressors in fish, we explored the consequences of HEA and hypoxia exposure during early life on the stress response of zebrafish. Larval exposure to HEA stimulated the endocrine stress axis, inhibited neuronal differentiation but also increased ammonia tolerance in later life. While early life HEA had little impact on the cortisol stress response to a repeat HEA exposure, it abolished a later life stress response to a novel vortex stressor in both larval and adult fish, suggesting that early-life

HEA exposure can have persistent effects on the stress response. Larval hypoxia exposure also stimulated the endocrine stress axis, inhibited neuronal proliferation and differentiation, and increased hypoxia tolerance in later life. However, in contrast to the effects of HEA, larval hypoxia exposure inhibited the cortisol stress response to a repeat hypoxia exposure in larval fish, but had no effect on the cortisol response to a novel vortex stressor, and no sustained effects on the stress response to hypoxia in adults. Finally, although anoxia exposure in embryos had no effect on the stress response to hypoxia in later life, adults derived from anoxia-exposed embryos exhibited dominance during dyadic interactions and had lower whole body cortisol levels. Anoxia-exposed embryos raised to adults were also more aggressive and had higher whole body testosterone levels. These results suggest that acute embryonic anoxia favors the development of a dominant and aggressive phenotype, and that a disruption in sex steroid production may contribute to the programming effects of environmental hypoxia. Overall, while early life environmental challenges can affect the larval stress response and stress phenotype of adult zebrafish, our results also show that the programming effects of early life environmental stressors are both life stage- and stressor-specific, and dependent on exposure history.
Acknowledgements: Supported by an NSERC Discovery Grant to NJB.

SOA9 Dores, Robert M. (USA)

CARTILAINGOUS FISH COMPARATIVE ENDOCRINOLOGY OVER THE PAST 50 YEARS: RECENT STUDIES ON MELANOCORTIN RECEPTORS

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With the advent of improved biochemical procedures for isolating and characterizing hormones in the 1960s, considerable progress was made in identifying glycoprotein hormone orthologs, growth hormone orthologs, melanocortin-related orthologs, neurohypophyseal hormone orthologs, and hypophysiotropic factor orthologs, as a well as steroid hormones involved in reproduction and osmoregulation from several representative species of cartilaginous fish. In addition, the subsequent utilization of molecular biology procedures in the 90's and in this century led to the identification of genes that code for nearly every other polypeptide hormone family. However, work on the evolution of cartilaginous fish endocrine systems and the physiology of cartilaginous fish endocrine systems had been limited in the early part of this century by the cost of maintaining these creatures, and shifts in areas of emphasis in comparative endocrinology. That said, since 2008, as a result of the characterization of the elephant shark genome, there has been a renewed interest in cartilaginous fish endocrinology. To highlight these recent advances, in this symposium, Professor Akiyoshi Takahashi (Kitasato University, Japan) will speak on the phylogeny of POMC in the subclasses of cartilaginous fishes. Prof. Susumu Hyodo (University of Tokyo, Japan) will present his work on the use of the elephant shark as a model system for studying endocrine networks in cartilaginous fishes. Prof. Yasuhisa Kobayashi (Kindai University, Japan) will speak on the reproductive system of the red stingray (*Dasyatis akajei*), with a focus on oogenesis, sex differentiation and the role of the uterus with respect to development of the embryo. Finally this state-of-the-art lecture will provide recent observations on the pharmacology of the melanocortin receptors for the elephant shark and the red stingray. This presentation was supported in part by the Long Endowment and the Department of Biological Sciences, University of Denver.

SOA10 Denslow, Nancy (USA)

THE IMPACT OF OMICS TECHNOLOGIES ON ENVIRONMENTAL ASSESSMENTS OF CONTAMINATION

Nancy Denslow

OMICS technologies have opened the door to new evaluative procedures for environmental toxicology. In particular, these technologies have provided tools for problems that were not solvable in the past. Evaluating toxicity of emerging contaminants is important for risk assessment, especially when these chemicals target the endocrine systems of environmental organisms, often with off-target effects. Of special concern are chemicals that may be additive (or synergistic) in their molecular activities and which may activate adverse outcome pathways, leading organisms towards decreased growth, decreased reproduction, increased susceptibility to disease or even death. In the past evaluation of toxicity has been accomplished by life cycle toxicity assessments, one chemical at a time. With the myriad of chemicals now identified in surface waters it is important to develop more effective toxicity tests that are high throughput and that link from the molecular initiating event to adverse outcomes in exposed organisms. Global gene expression and proteomics methods have been used to define molecular pathways that lead to adversity and to identify their molecular drivers. This information is being utilized to develop high throughput assays for easier screening and monitoring of contaminants. While still in their infancy, bioanalytical methods can complement traditional analytical chemistry methods to monitor surface waters and eventually may be used in risk assessment.

SOA11 Schreck, Carl (USA)

FISH STRESS RESPONSE: CONSISTENCIES AND INCONSISTENCIES IN ENDOCRINE DATA

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Between animal variation is a cornerstone of evolution. I argue that comparative endocrinologists should embrace this inherent variation, even if it does not fit with their preconceived notions. I will present information from my work, most of which is new and unpublished, that supports the contention that the generalities concerning the endocrine system across the great diversity of fishes is remarkably similar, yet at the level of the individual, population and species, is remarkably dissimilar. I use examples of research on stress and the brain, pituitary,

interrenal axis in this regard. While between study variation can often be explained by differences in analytical methods, biological differences attributable to the animals themselves and their reaction to environmental conditions are more important factors to consider. The main attributes in terms of factors involved in response to stressors are generally the same amongst the fishes, as are the generalities concerning the patterns of the responses. However, marked differences are apparent in the details of such responses, both temporally and in magnitude. It is well established that this variation is driven by the diversity in genetics and environments. However, the variation is also influenced by unsuspected, subtle differences within a population. Such variation is attributable to effects of: ontogenetic stage (e.g., transitional stages are more sensitive to stressors), life history tactics (e.g., fish seemingly sympatric may in actuality not be), energetic status [e.g., how fish cope energetically with stressors (allostasis) is context specific], temperature (e.g., a fraction of a degree decrease in temperature can affect directionality of fish movement), the nature of rearing tanks used in experiments (e.g., minute changes in lighting, flow and density can affect the physiological nature of the fish; a small amount of cover can affect brain development), the social environment (e.g., individually housed fish may be stressed if they are a gregarious species), and diet (e.g., not simply diet quality but also feeding tactics employed directly affect fish quality), and the fact that data may be mulit-modal.

SOA12 Lovejoy, David (Canada)

ROLE OF ELASMOBRANCHS AND HOLOCEPHALANS IN UNDERSTANDING PEPTIDE EVOLUTION IN THE VERTEBRATES: LESSONS LEARNED FROM GONADOTROPIN-RELEASING HORMONE (GNRH) AND CORTICOTROPIN-RELEASING HORMONE (CRH) PHYLOGENIES.

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The cartilaginous fish (Class Chondrichthes) comprise two morphologically distinct subclasses; Elasmobranchii (sharks, skates and rays) and Holocephali (chimaeras). Despite numerous reports arguing that the Holocephali represent a separate vertebrate class, current evidence supports an early divergence of the two subclasses early in their ancestry suggesting monophyly of their lineage. However, given this, such a phylogenetic understanding is not entirely consistent with two highly conserved peptide lineages, GnRH and CRH. The first clear evidence of GnRH in the chordate lineage occurs within the tunicates, a protochordate species, now considered to be part of a direct sister lineage to the chordates. There are two GnRH distinct genes in the tunicate*, Ciona intestinalis* which are thought to be representatives of the progenitor lineages of GnRH-I and –II in chordates. In the Chondricthyes, 4-7 forms of GnRH have been partially described in Elasmobranchs, although only 2 have been characterized. In contrast, only a single form of GnRH (GnRH-II) has been found to exist in the Holocephali. On the other hand, CRH phylogeny in the Chondrichtyes may follow a more consistent pattern with respect to vertebrate evolution. A single form of CRH is present in the *C. intestinalis* genome. Interestingly, three forms are found in the lamprey lineage and appear to represent two direct CRH paralogues and a representative of the urocortin 2/3 lineage. A similar set of CRH-related peptides are found in the holocephalan genome of *Callorhynchus milii* including one associated with urotensin-I (urocortin in tetrapods). These findings are consistent with new studies on the presence of phylogenetically younger and older lineages with respect to the Chondrichtyes. However, a lack of information on CRH in elasmobranches has confounded a clear understanding of CRH evolution in vertebrates. Assuming that the Elasmobranchii and Holocephali are part of a monocladistic clade within the Chondricthyes, then we interpret the findings of GnRH and CRH to be derived conditions of their respective lineages.

Wednesday June 7

SOA13 Ito, Michihiko (Japan)

MAINETANCE AND DECONSTRUCTION OF MASS-IN-LINE STRUCTURES BY SEX STEROIDS OR TGF-β DURING ERALY GONADAL DIFFERENTIATION IN THE AFRICAN CLAWED FROG –IS THE DEFAULT SEX FEMALE OR MALE?

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The African clawed frog *Xenopus laevis* has a female heterogametic ZZ/ZW-type sex-determination system. We previously reported that a W-linked gene *dm-W* can determine female sex. In addition, we discovered a unique "mass-in-line structure" consisting of steroidogenic *cyp17a1*- and/or *cyp19a1*-expressing cells in both the ZZ and ZW gonads just after sex determination, which is maintained for ovarian cavity formation in ZW gonads and disappears from ZZ gonads during early testicular development. However, it remains unclear how "mass-in-line structure" is maintained or destructed in developing ZW or ZZ gonads, respectively. In this study, we found that two nuclear factor genes, *dax1* and *sox9*, and two TGF-β family genes, *inhibin βb* (*inhbb*) and *anti-Müllerian hormone* (*amh*) were much higher expressed in ZZ than in ZW gonads during early sex development. In situ hybridization analysis showed that *inhbb* and *amh* transcripts were expressed in somatic cells on the outer and inner sides of cell masses in the mass-in-line structure, respectively, in the developing ZZ gonads. Importantly, estrogen exposure attenuated disappearance of the mass-in-line structure in developing ZZ tadpoles. These findings suggest that TGFβ signaling might participate in the deconstruction of the mass-in-line structure, which could be maintained by estrogen. Finally, we propose a model for gonadal differentiation mediated through the mass-in-line structure and endocrine systems, and discuss whether the default sex is female or male. Acknowledgements: Supported by: a Grant-in-Aid for Scientific Research.

SOA14 Demeneix, Barbara (France)

SCREENING ENVIRONMENTAL CHEMICALS FOR EFFECTS ON THYROID SIGNALLING AND EARLY BRAIN DEVELOPMENT IN XENOPUS.

Barbara Demeneix
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During vertebrate evolution, thyroid hormone acquired multiple roles in development, especially brain development. Examples include promotion of myelination thereby increasing speed of neuronal transmission, as well as modulation of neuronal differentiation, as exemplified by the exquisite sensitivity of the Purkinje neuron to thyroid hormone deficiency. We have exploited the conservation of thyroid signalling throughout evolution to use transgenic Xenopus as screen for environmental chemicals affecting thyroid hormone signalling, and hence brain development. Our recent data show that a mixture of chemicals present in human amniotic fluid alter thyroid hormone signalling, brain gene expression and behaviour in early Xenopus embryos, prior to thyroid gland formation. Similarly, research in the last 15 years has demonstrated that early development, again prior to thyroid gland formation (the first trimester of pregnancy in humans) is a thyroid-hormone dependent period. In the same time span, we have witnessed an unprecedented increase in Autism Spectrum Disorders (ASD) incidence, correlated in many data sets with loss of IQ. Although, changes in diagnosis and awareness can contribute the ASD increase, many authors consider that environmental factors, possibly exacerbating genetic susceptibilities, are implicated. Numerous lines of evidence argue that interference with thyroid hormone orchestration of brain development could be a major player in the increased incidence of neurodevelopmental disease, such as autism spectrum disorder, as well as in significant IQ loss at a population level.Acknowledgements: Supported by EU grant EDC-Mix-Risk.

SOA15 Unniappian, Suraj (Canada)

BIOLOGY OF NESFATIN-1: A DECADE AND BEYOND

Unniappan S (1)
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Nucleobindins (nucleobindin-1/NUCB1, and nucleobindin-2/NUCB2) are DNA binding proteins. In 2006, nesfatin-1, a satiety and fat influencing peptide was identified from the rat hypothalamus. The past decade witnessed rapid growth in nesfatin-1 research. This talk will review nesfatin-1 biology in rodents, primarily using research from my lab. We discovered that nesfatin-1 is insulinotropic, both *in vivo* in rats and *in vitro* in mice pancreatic islets and islet beta cell lines, and it suppresses glucagon release *in vivo* in rats. Sub-cutaneous infusion of nesfatin-1 resulted in a reduction in food intake, and an increase in energy expenditure and physical activity in rats. Nesfatin-1 co-localized gastric ghrelin, and intestinal glucagon like peptide-1 (GLP-1), and peptide YY (PYY). Nesfatin-1 suppressed ghrelin, while enhanced GLP-1 and PYY *mRNA* expression. Co-administration of nesfatin-1 and ghrelin nullified the effects of both peptides on food intake. Glucose, amino acids, and fatty acids stimulated nesfatin-1 mRNA expression, and/or secretion from a stomach cell line. Nesfatin-1 modulated gonadotropin releasing hormone, and kisspeptin receptor in the hypothalamic neurons of mice, and luteinizing hormone and kisspeptin receptor in mice gonadotropes. Very recently, we identified a nesfatin-1 like peptide (NLP), encoded in NUCB2. Similar to nesfatin-1, NLP inhibits food intake and stimulates glucose dependent insulin secretion. Collectively, nesfatin-1 and NLP are multifunctional orphan ligands, and novel regulators of metabolism in rodents. They are likely integrators of metabolism and reproduction in rodents, and these effects are achieved by its actions on brain-gut hormones. Future research should focus to identify receptors, mechanism of action, and tissue specific effects of nesfatin-1 and NLP.Acknowledgments: We thank the Canadian Institutes of Health Research, Saskatchewan Health Research Foundation, and the Canada Foundation for Innovation for generous funding.

SOA16 Kloas, Werner (Germany) [Replaced]

AMPHIBIANS AS MODELS TO ASSESS ENDOCRINE DISRUPTION AFFECTING REPRODUCTION, DEVELOPMENT, AND BEHAVIOR.

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Anthropogenic pollution has become an emerging concern especially for aquatic wildlife because surface waters are the main sink of various compounds without remarkable acute toxicity but affecting indirectly populations by disruption of endocrine systems.
Amphibians are the classical endocrinological model organisms associated with impacts reaching from reproduction and development, including sex reversal, to metamorphosis. However, also behavior can be used as biomarker for endocrine disruption concerning reproduction. In the past research focused on (anti)estrogenic, (anti)androgenic, and (anti)thyroidal substances and more recently environmental gestagens, e.g. synthetic progestins, which have been identified as potential endocrine disruptors affecting reproduction as well as development. Microplastics have been identified as an emerging issue in surface waters suggesting the potential to interfere with endocrine disruption.
Pure microplastic such as polyethylene (PE) has no impact on sexual differentiation and larval development in X. laevis. However, combined treatments of PE and ethinylestradiol (EE2) or PE spiked with EE2 are able to cause feminization, similarly to pure EE2 at the same concentration. Most research on endocrine disruption has been limited to a few model species such as Xenopus laevis and X. tropicalis, and rarely to Rana catesbeiana. However, in order to extend our knowledge whether the results from model species hold true for amphibians in general, comparative studies have been undertaken to assess vulnerability to the estrogenic compounds, EE2 and bisphenol A, in deeply

diverged anuran lineages (X. laevis, Hyla arborea, and Bufo viridis). Comparing genetic with phenotypic sex proved the xenoestrogens to cause feminization in all species but revealed different sensitivities. Thus model organisms can serve very well as mechanistic indicators but not for an assessment of NOEC (no observed effect concentration) in amphibians in general.

SOA16 - Jean-Francois Baroiller (France)
SEX REVERSALS IN GONOCHORISTIC FISH: INSIGHTS AND CONSEQUENCES FOR WILD POPULATIONS.
Baroiller JF, D'Cotta H

SOA17 Darras, Veerle (Belgium)

TRANSIENT AND PERMANENT SILENCING OF THYROID HORMONE TRANSPORTERS AND DEIODINASES IN CHICKEN AND ZEBRAFISH.
Darras V.M., Vancamp P., Houbrechts A.M.
Laboratory of Comparative Endocrinology, Biology Department, KU Leuven, Belgium

Thyroid hormones (THs) act predominantly through nuclear receptors which are ligand dependent transcription factors. To control intracellular TH availability in a time- and tissue-specific way, cells express two types of regulators: transmembrane transporters facilitating TH influx and efflux and deiodinases catalyzing TH activation and inactivation. To dissect the role of each regulator in this network, gene-specific silencing is a valuable tool. We here elaborate on two examples of this approach: transient knockdown of monocarboxylate transporter 8 (MCT8) in embryonic chicken brain and permanent knockout of deiodinase type 2 (Dio2) in zebrafish. To interfere with neuronal TH uptake in chicken embryos, we use vector-based RNAi to silence the expression of MCT8, the most specific TH transporter. By changing the developmental stage and position of the electrodes during electroporation specific regions/cell populations can be targeted. Electroporation of the cerebellar anlage on embryonic day 3 (E3) transfects Purkinje cell precursors and results in aberrant Purkinje cell development as well as disturbed proliferation and migration of granule cells later on. Electroporation of the mesencephalic neuroepithelium on E3 leads to transfection of the progenitors that give rise to successive neuroblast populations. This reduces the thickness of the optic tectum and disturbs both the first and second migration wave of neuroblasts. These models enabled us to demonstrate that MCT8-mediated TH uptake is needed from early stages onwards to assure correct development of layered brain structures. Using site directed mutagenesis we generated two zebrafish lines completely and permanently lacking activity of the TH-activating enzyme Dio2. The early phenotype confirms the data from our morpholino based research and results in a clear developmental delay, long-term growth retardation and aberrant locomotor activity. Using this knockout model we are also able to study the adult phenotype where the most striking defect relates to reproduction which is disrupted in both male and female mutants. Acknowledgements: Supported by grants from the Research Foundation – Flanders and the Research Fund KU Leuven.

SOA18 Carr, James (USA)

THE VISUAL SYSTEM IS A TARGET FOR PEPTIDES REGULATING FOOD INTAKE.
Carr JA, Prater C, Islam R, Harris BN.
Department of Biological Sciences, Texas Tech University, Lubbock TX USA

The optic tectum (OT) and superior colliculus (SC) rapidly inhibit food intake when a visual threat is present. Work from our laboratory and others indicates that CRF, acting on CRF R1 receptors, and NPY, acting on Y2 receptors, may play a role in tectal inhibition of prey capture. Here we test the hypothesis that **tectal CRF and NPY receptors modulate food intake in juvenile *Xenopus laevis***. We tested several predictions: 1) Does tectal CRF injection decrease food intake? 2) Does a selective CRF R1 antagonist block CRF effects on feeding? 3) Does a selective R1 antagonist block stressor-induced inhibition of feeding? 4) Does tectal NPY injection decrease food intake? 5) Does a selective Y2 receptor antagonist alter food intake after tectal administration? *X. laevis* were administered oCRF or NPY via bilateral tectal injection alone or in combination with respective R1 (NBI27914) or Y2 (BIIE0246) receptor antagonists or vehicle. oCRF significantly reduced food intake compared to sham and vehicle injected juveniles. When injected with both NBI27914 and oCRF, food intake was maintained at baseline levels. Frogs ate significantly less when exposed to a reactive stressor (ether vapors). NBI27914 reversed stressor-induced inhibition of food intake. NPY at three doses failed to statistically alter food intake. Interestingly, BIIE0246 alone increased food intake. Overall, we found support for questions 1-3 and 5 and conclude that activation of the tectal CRF R1 inhibits food intake in frogs. Furthermore, tectal CRF R1 receptors appear to be involved in the reduction of food intake that occurs in response to a reactive stressor. While activation of tectal Y2 receptors did not affect food intake, blocking these receptors dramatically increased food intake. This work was done in partial completion of requirements for the doctoral (C.P.) and M.S. (R.I.) degrees at Texas Tech University.

SOA19 Van Der Kraak, Glen (Canada)

DEFINING THE ENDOCRINE TARGETS THAT MEDIATE THE ENVIRONMENTAL REGULATION OF REPRODUCTION
Glen Van Der Kraak
Author affiliation: Department of Integrative Biology, University of Guelph; Guelph Ontario, Canada

Comparative endocrinologists continue to interested in defining the manner by which the environment influences the endocrine control of reproduction. In recent years much progress has been made as the toolbox has expanded to include genomic, proteomic and metabolomic

approaches and *in vitro* and *in vivo* test methods that are tractable to a wide array of species. Moreover, interest has grown beyond the assessment of the effects of environmental factors such as temperature, photoperiod, or salinity and now includes studies of natural hormones, pharmaceuticals and anthropogenic chemicals that are proving to be pervasive in the environment. Researchers have had a long standing interest in defining the mechanisms and pathways that are impacted by environmental stressors. Progress has been made in toxicological studies by defining Adverse Outcome Pathways (AOP) that describe the manner by which chemical stressors interact at the molecular level (e.g. binding to a receptor, inhibition of an enzyme, or damage to DNA) to initiate changes at increasingly higher levels of biological organization (cell and organ level function through to a biological change considered relevant for risk assessment/regulatory decision making (e.g., impacts on human health/well-being or effects on survival, growth, or reproduction in wildlife). In this presentation I will explore how the AOP approach may represent a useful framework for defining how different environmental factors may affect the endocrine system and reproduction in wildlife. In particular, the framework is useful in moving beyond descriptive science and have utility in determining if the responses detected (e.g. changes in gene and protein expression, hormone levels) are critical to outcomes at the whole animal level. Specifically, selected case studies will be used to define how a hypothesis driven approach can be used to confirm that specific endocrine pathways mediate the effects of environmental factors on reproduction. Acknowledgements: Supported by NSERC Discovery Grant and Strategic Programs

SOA20 Abouheif, Ehab (Canada)

WHAT ANTS TEACH US ABOUT MECHANISMS OF SIZE, GROWTH, AND ALLOMETRY DURING DEVELOPMENT AND EVOLUTION.

Rajakumar R(1), Couture M(1), Fave MJ(1), Chen T(1), Lillico-Ouachour A(1), Koch S(1), and Abouheif E(1)
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Ants show a remarkable diversity in size and allometry both within and between species, so much so that Darwin considered ants a 'climax of the difficulty' for his theory of natural selection. For example, in colonies of the marauder ant *Pheidolgeton diversus*, the smallest worker stands on the head of the largest worker. The developmental mechanisms producing these extreme differences in growth, size, and allometry between workers and how this extreme variation evolved between ant species are questions that have remained largely unknown for over 150 years, puzzling some of the most notable biologists since Darwin, including Sir Julien Huxley, EO Wilson, and others. Over the last 20 years, my lab has made considerable progress in understanding this mystery by uncovering developmental and epigenetic mechanisms that work together to regulate growth, size, and allometry in ants. These mechanisms may be general features of both development and evolution of complex biological systems. Acknowledgements: Supported by: NSERC Discovery Grant and E.W.R. Steacie Fellowship to EA.

SOA21 Satake, Honoo (Japan)

PEPTIDERGIC REGULATION OF FOLLICLE MATURATION AND OVULATION IN A PROTOCHORDATE, CIONA INTESTINALIS.

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Bioorganic Research Institute, Suntory Foundation for Life Sciences, Kyoto, Japan

The critical phylogenetic position of the ascidian, *Ciona intestinalis*, as a protochordate suggested its potential appliciability as a model organism in diverse fields of comparative biology involving neuropeptides and hormones. In our previous study, a combination of mass spectrometric analyses and database-searching detected 35 peptides. These peptides are categorized into three types. i) orthologs of vertebrate peptides including tachykinin, calcitonin, galanin, RF-amide peptides, and neurotensin-like peptides. ii) vasopressin and GnRH-related peptide possessing ascidian-specific molecular forms and/or functions. iii) novel family peptides such as LF peptides and YFL/V peptides. These results proved that ascidians, unlike other invertebrates, possess a variety of homologs and/or prototypes of vertebrate neuropeptides and peptide hormones, and that several ascidian peptides were diverged in ascidian-specific evolutionary lineages. Recent high-resolution imaging MASS demonstrated the localization of multiple neuropeptides in the brain of *C. intestinalis*, which cannot be obtained by conventional immumohistochemical analysis and *in situ* hybridization. Moreover, our machine-learning-based prediction of *Ciona* peptide-receptor pairs followed by validation of specific interaction of these predicted pairs led to the idetification of receptors for novel *Ciona* peptides, none of which was predicted by any BLAST homology-searching. We have substantiated a novel protease-associated follicle growth pathway regulated by tachykinins and colecystokinins using *C. intestinalis*, which is the first functional characterization of these peptides in ovaries of any organisms. Quite recently, unique vasopressinergic and GnRHergic regulatory mechanisms underlying germinal vescicle breakdown and ovulation were also elucidated in *C. intestinalis*. Such remarkable significance of the peptidergic ovarian maturation in *C. intestinalis* will not only lead to the verification of the essential reproductive system in protochordates but also pave the way for the exploration of both conserved and diversified endocrine, neuroendocrine, and nervous systems in the evolutionary lineage of deuterostome invertebrates and/or chordates. Acknowledgements: Supported by: JSPS grants to HS.

SOA22 Capilla, Encarnación (Spain)

GROWTH POTENTIAL IMPROVEMENT IN GILTHEAD SEA BREAM BY RECOMBINANT BOVINE GROWTH HORMONE (rBGH)

Capilla E, Vélez EJ, Perelló M, Azizi Sh, Lutfi E, Navarro I, Blasco J, Fernández-Borràs J, Gutiérrez J
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Growth in fish is a process with multifactorial regulation, among which the growth hormone (GH)/insulin-like growth factors (IGFs) axis plays the most important role. Besides, knowledge on other local regulatory systems involved such as the myogenic regulatory factors (MRFs) has increased recently. Although, efforts to transform the basic information into aquaculture production strategies are still needed. Thus, in the present study we aimed to determine the effects of a sustained-release recombinant-bovine growth hormone (rBGH, Posilac®) treatment in gilthead sea bream fingerlings or juveniles at a dose of 4 or 6 mg/g body weight respectively, in order to know how far we are from optimal growth in this species and how is this regulated. We studied the role of GH/IGF axis but also paid special attention to muscle and bone development analyzing the expression of myogenic- and osteogenic-related genes. The rBGH-treated fish showed significantly higher body weight and specific growth rate with lower subcutaneous or visceral fat without changes in feed intake in comparison to control fish, indicating an enhancement in somatic growth. In fingerlings at 6 weeks post-treatment, the mRNA levels of MRFs were unchanged, whereas those of osteogenic genes, such as the extracellular matrix structural marker type 1 collagen subunit 1a and the mineralization-related molecules osteopontin and osteocalcin, were significantly increased, suggesting improved osteogenesis. In juveniles at the same time of treatment, MRFs genes expression was down-regulated but then recovered at 12 weeks post-injection showing no differences. These data suggested a switch from hypertrophic to hyperplastic muscle growth that was supported by muscle histology. In addition, a texture analysis at week 9 confirmed rBGH-treated juveniles present a firmer fillet. Overall, these results reveal that better growth potential and quality for gilthead sea bream can be achieved, and support the possibility to improve them through the optimization of the culture conditions. Acknowledgements: Thanks to Elanco Animal Health for kindly providing the rBGH. Supported by MINECO (AGL2012-39768, AGL2014-57974-R) and Catalonian Government (2014SGR-01371 and XRAq).

SOA23 Shah, Nirao (USA)

MODULAR GENETIC AND NEURAL CONTROL OF SEXUALLY DIMORPHIC SOCIAL BEHAVIORS

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What mechanisms control instinctual displays of sexually dimorphic behaviors and allow them to adapt to social context and experience? What mechanisms control social attachments? My lab uses mice, flies, and prairie voles to address these questions. Despite their fundamental importance to social interactions in health and neuro-psychiatric disorders, the molecular and neural mechanisms underlying sex-differences in behaviors remain mysterious. To tackle this long-standing problem, we leverage the fact that sex hormones regulate sexual differentiation of the brain during development and adulthood to control sex-typical behaviors. Thus, identifying sex hormone-responsive neurons and genes should allow us to access the underlying mechanisms. We have used this strategy and developed sensitive genetic reagents to make significant discoveries about how sex hormones regulate neural circuits controlling sex-typical behaviors. We have built upon these findings to identify and link sex hormone-responsive genes and neural pathways to specific sexually dimorphic behaviors. My lab has also initiated inquiries into the related issues of control of social interactions during speciation and social attachment behaviors. I will present our findings related to these ongoing research directions.

SOA24 Thomas, Peter (USA)

ROLE OF THE NOVEL MEMBRANE PROGESTIN, ESTROGEN, AND ANDROGEN RECEPTORS (GPER, PAQR7, AND ZIP9) IN THE REGULATION OF FISH OVARIAN FUNCTIONS.

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Departments of Marine Science and Integrative Biology, University of Texas at Austin, Port Aransas, Texas, USA

Although nongenomic, cell surface-initiated steroid actions were first described 40 years ago, the identities of novel membrane receptors mediating these non-classical steroid actions have only been discovered within the past 15 years. Two of these novel receptors, membrane progestin receptor alpha (mPRα, PAQR7), and membrane androgen receptor (ZIP9), were first identified and characterized in fish ovaries, whilst the orphan receptor, GPR30 (now known as GPER), was initially characterized as a membrane estrogen receptor in breast cancer cells. All three receptors are on the cell surface, have 7-8 transmembrane domains, and are coupled to G proteins, but only GPER belongs to the GPCR superfamily. These receptors regulate critical physiological processes in teleost ovaries. Estrogen activation of GPER, which is coupled to a stimulatory G protein, increases intra-oocyte cAMP levels to maintain meiotic arrest. The gonadotropin-induced switch from estrogen to progestin production during the periovulatory period induces the resumption of meiosis through decreasing this GPER inhibitory mechanism, and by activating the mPRα-dependent signaling pathway. The mPRα is coupled to an inhibitory G protein so that progestin activation of the receptor causes intra-oocyte cAMP levels to decline resulting in removal of the inhibitory cAMP mechanism that prevents

completion of oocyte maturation. The membrane androgen receptor, ZIP9, a member of zinc transporter SLC29A family, is highly expressed in granulosa cells and mediates testosterone-induced apoptosis, which suggests it has a role in follicle atresia and ovarian remodeling. These investigations in fish oocyte and granulosa cell models demonstrate the important functions of novel membrane steroid receptors in teleost ovarian physiology.

SOA25 Björnsson, Björn Thrandur (Sweden)

ENVIRONMENTAL ENDOCRINOLOGY OF GROWTH HORMONE IN SALMONIDS

Björnsson BTh
The Fish Endocrinology Laboratory, Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

Growth hormone (GH) is a pluripotent pituitary hormone, being a major driver of several interrelated physiological processes in fish. In tandem with other functions such the development of seawater tolerance during the parr-smolt transformation of anadromous salmonids, it stimulates growth of soft and skeletal tissues directly as well as indirectly.
Although somewhat counter-intuitive, due to changes in receptor densities and turn-over rates, plasma GH levels are normally lower in fast-growing than slow-growing fish. Breeding selection or GH-transgenesis has created growth-enhanced phenotypes for aquaculture production. This has warranted research into the consequences of growth-enhanced fish at various ecosystem levels. The main approach has been to use various GH-treatment protocols to induce fast growth over the short- as well as long-term, and study the effects under controlled laboratory conditions as well as in various field settings. At the level of the individual, GH promotes faster, leaner growth through anabolic effects on muscle and catabolic effects on adipose tissue. The faster growth calls for greater foraging activity and higher feed intake. With conspecifics, this translates into increased aggression and dominance behavior, and in presence of perceived predators, GH-treated fish take greater foraging-related risks. This has led to the hypothesis that fast-growing phenotypes will suffer greater mortality in nature, but most field experiments do not support this. A reason may be that GH-implanted salmonids appear not to increase their feed intake primarily by expanding their foraging range, which would increase predator encounters, but rather by more efficient foraging within the home range. There is also a shift in the trophic level of pray items consumed, which may lead to broader ecosystem effects. Acknowledgements: Supported by Formas and EU grants.

SOA26 Belosevic, Miodrag (Canada)

DEVELOPMENT OF MYELOID CELLS IN BONY FISH

Miodrag (Mike) Belosevic,
Department of Biological Sciences, University of Alberta

Development of progenitor cells into myeloid cells (i.e. macrophages and neutrophils) is critical to the survival of metazoans for maintenance of homeostasis and defense against pathogens. While much is known about myeloid cell development in mammals, less is known about this process in fish. Myelopoiesis in bony fish is controlled by a number of different growth factors (M-CSF, G-CSF, granulin) and a number of different transcription factors, that act at specific junctures of myelopoietic development. The mechanisms of myelopoiesis in the goldfish were examined by cloning, expressing and functionally characterizing major growth factors at both gene and protein levels. Evidence will be presented supporting both stimulatory and inhibitory roles for growth factors that profoundly influence the development of myeloid cells in bony fish.

SOA27 Nelson, Erik (USA)

A CHOLESTEROL METABOLITE PROMOTES BREAST CANCER PROGRESSION; NEW PERSPECTIVES ON SELECTIVE ESTROGEN RECEPTOR PHARMACOLOGY.

Erik R. Nelson
(1) Department of Molecular and Integrative Physiology and the University of Illinois Cancer Center, University of Illinois, Urbana, IL, USA.

Breast cancer remains the second leading cause of cancer death in women, highlighting the continuing need for novel strategies to treat this disease. In this regard, patients with elevated circulating cholesterol have a poor prognosis while patients taking inhibitors of HMGCoA-reductase (statins) experience a significantly increased recurrence free survival time. Therefore, we evaluated the causality of the relationship between cholesterol and breast cancer progression. Using murine models we have found that cholesterol promotes the growth and metastasis breast cancer. Importantly, the stimulatory effects of cholesterol require the activity of CYP27A1, an enzyme that converts cholesterol to its primary metabolite, 27-hydroxycholesterol (27HC). In subsequent work, we demonstrated that 27HC behaves as a Selective Estrogen Receptor Modulator (SERM) to promote cellular proliferation. On the other hand, the cancer cell-intrinsic expression of the estrogen receptor (ER) is dispensable for its pro-metastatic properties, suggesting that the metastatic effects of 27HC may be mediated by host cells within the microenvironment. In support of this notion, are our findings that the metastatic effects of 27HC are due to its actions on immune cells. Specifically, 27HC has a unique ability to modulate both the activity of the ER and liver X receptors (LXRs) within myeloid-immune cells, thereby allowing cancer cells to escape immune surveillance. Thus, 27HC behaves as a SERM in the traditional cell-intrinsic sense, but also by (1) concomitantly engaging the LXRs and subsequent crosstalk with the ERs, and (2) promoting communication between different cell

types, providing a new level of complexity for the well described divergent activities of certain ER ligands. In summary, our data strongly suggests that through its selective nuclear receptor modulating activities, 27HC is the biochemical mediator of the effects of cholesterol on breast cancer metastasis. Our results provide strong support for the development of therapeutics targeting this axis. Furthermore, this updated model of multi-layered nuclear receptor pharmacology should be considered when evaluating the biology of nuclear receptor ligands including endocrine disrupting chemicals. Supported by: NIH-NCI R00CA172357 and Susan G. Komen PDF16377624.

Friday June 9

SOA28 Tyler, Charles (UK)

THE FEMINIZATION OF NATURE – CHEMICALS, MECHANISMS AND CONSEQUENCES

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It is now well established that various chemicals discharged into the environment can alter hormone systems in exposed wildlife (and humans) affecting developmental processes, including reproduction. More than 1000 chemicals have now been shown to be capable of interacting with hormone receptors or affect enzyme systems involved with hormone biosynthesis or metabolism, illustrating the scale of the issue faced. Chemicals with estrogenic activity (more than 200) are perhaps the best known endocrine disrupting chemicals (EDCs) and they have been associated with feminised responses in male fish, frogs, reptiles, birds and mammals. Their effects include altered sexual behaviours, reduced sperm production and quality, and intersex. Some studies have shown reduced reproductive fitness in males, but the population level consequences for wild animal populations are still poorly understood. Nevertheless, global concern over the adverse health effects of some estrogenic chemicals has led to their removal from the market place. This lecture will provide a critical analysis on the effects of environmental estrogens on aquatic wildlife and wildlife populations, with a principal focus on fish. It will illustrate some of the mechanisms (and complexities in those mechanisms) through which environmental estrogens disrupt endocrine signalling, including showing how transgenic fish models are helping us to understand some of those mechanisms, as they occur in real time in exposed organisms. Of course, wildlife populations are exposed to complex mixtures of chemicals and subject to other stressors that may be interactive in their effects, which is rarely considered in the possible risk of EDCs. Equally however, what is also rarely considered is the fact that in natural systems effects of EDCs may be buffered in some situations reducing their impact. The final part of this presentation will present a few thoughts to provoke discussion(s) on these points. This lecture is dedicated to Professor Louis J. Guillette, Jr., who was a pioneer in his work on endocrine disruption in wildlife and for whom there is a session dedicated at this conference: Endocrine Disruption in Aquatic Vertebrates - Lessons Learned and Future Prospects. A Tribute to Professor Louis J. Guillette, Jr.

SOA29 Korf, Horst-Werner (Germany)

IMPACT OF ENDOGENOUS MELATONIN ON CIRCADIAN ORGANIZATION

Korf HW (1,2), Wicht H (2), Pfeffer M (2)
(1) Dr. Senckenbergisches Chronomedizinisches Institut; (2) Dr. Senckenbergische Anatomie II, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany

The hormone melatonin is synthesized at night by the pineal organ, acts upon specific G-protein-coupled receptors (MT1 and MT2) and represents an important output signal of the circadian system. While exogenous melatonin is known to act as a chronobiotic and to entrain circadian rhythms in physiology and behavior, the impact of endogenous melatonin on circadian and diurnal rhythms is still poorly understood. To address this problem we have investigated the role of endogenous melatonin by comparing various mouse strains with an intact or a defective melatoninergic system. Locomotor activity rhythms were analyzed in melatonin-deficient (C57BL) and melatonin-proficient (C3H) mice as well as in melatonin-proficient mice with targeted deletion of MT1 and/or MT2 under entrained- and free-running conditions as well as after experimental jet lag. Chronotype and rhythm stability were determined with methods recently established in our laboratory. We found that melatonin deficient mice (C57Bl) and melatonin proficient C3H mice that lack MT 1 and 2 reproduce their diurnal locomotor rhythms with significantly less accuracy than mice with an intact melatoninergic system. Their respective chronotypes, however, remained unaltered. After jet lag experiments, re-entrainment after the phase advance was significantly slower in melatonin-deficient mice and in mice lacking functional MT2 receptors as compared to melatonin-proficient animals with intact MT2 receptors. The endogenous melatonin signal facilitates re-entrainment of the circadian system by acting upon MT2 receptors, whereas for the stability of rhythmic diurnal behavior both melatonin receptors are necessary. These results show that the endogenous melatoninergic stabilizes internal rhythms under conditions of a steady entrainment, while it has no effects on the chronotype. Further studies are needed to unravel the biological role of a stable vs. a sloppy circadian rhythm.

SOA30 Kriegsfeld, Lance (USA)

THE ROLES OF KISSPEPTIN AND GONADOTROPIN-INHIBITORY HORMONE IN THE SEASONAL CONTROL OF REPRODUCTION

Kriegsfeld, LJ
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Animals inhabiting temperate and boreal latitudes experience marked seasonal changes in the quality of their environments and maximize reproductive success by phasing breeding activities with the most favorable time of year. Whereas the specific neuroendocrine mechanisms driving seasonal changes in reproductive function vary across species, converging lines of evidence point to a key role for two, complementary RFamide (Arg-Phe-NH_2) peptides in guiding this seasonal adaptation. Across mammalian species, kisspeptin and the mammalian ortholog of avian gonadotropin-inhibitory hormone, RFamide-related peptide-3 (RFRP-3), are pronounced positive and negative regulators of the reproductive axis, respectively. In addition to anticipating environmental change through transduction of photoperiodic information and modifying reproductive state accordingly, kisspeptin and RFRP-3 are also positioned to regulate acute changes in reproductive status should unpredictable conditions manifest throughout the year. This overview will summarize our findings on the role of kisspeptin and RFRP-3 in mammalian seasonal breeding while considering commonalities and disparities that have emerged from broad investigations across reproductively photoperiodic species. Supported by NIH grant HD-050470 and the France-Berkeley Fund.

Symposia

Monday AM S1

SIGNALING AND NEUROENDOCRINE CONTROL
Chair: John Chang and Anderson Wong

S1-1) Wong, Anderson (Hong Kong)

INSULIN AS A FUNCTIONAL LINK BETWEEN FOOD INTAKE AND SPEXIN EXPRESSION: RECENT PROGRESS ON SPEXIN AS A SATIETY FACTOR IN FISH MODEL.
Ma A, He M, Bai J, Wong MKH, Ko WKW, Wong AOL
Endocrinology Division, School of Biological Sciences, University of Hong Kong, Hong Kong, China

Spexin, a neuropeptide discovered by bioinformatics approach, has been identified as a satiety factor in fish model. However, the functional link between feeding and spexin expression as well as the signal transduction for spexin regulation is still unknown. Here we used goldfish as a model to examine the functional role of insulin as a postprandial signal for spexin regulation in bony fish. In goldfish, feeding could elevate plasma levels of glucose, insulin and spexin with concurrent rises in insulin and spexin mRNA levels in the liver. Similar elevation in spexin mRNA level was also observed in the liver and brain areas involved in appetite control in goldfish after intraperitoneal injection of glucose and insulin, respectively. By parallel experiments with goldfish hepatocytes and brain cell culture, hepatic insulin expression induced by glucose was shown to exert dual role in spexin regulation, namely (i) acting as autocrine/paracrine signal to trigger spexin mRNA expression in the liver, and (ii) as endocrine signal to induce spexin gene expression in the brain. Apparently, the peripheral (in the liver) and central actions of insulin (in the brain) on spexin gene expression were mediated by insulin receptor (to lesser extent by IGF1 receptor) coupled to $MKK_{3/6}/P_{38}$ ^{MAPK} and PI3K/Akt/mTOR but not $MEK_{1/2}/ERK_{1/2}$ cascades. Our findings indicate that an insulin component inducible by glucose is present in the liver of fish model and may act as the postprandial signal linking food intake with spexin expression both in the central as well as at the hepatic level. Acknowledgements: Supported by GRF grants and NSFC/RGC joint grant, Research Grant Council (HK).

S1-2) Son, You Lee (Japan)

MOLECULAR MECHANISM OF GONADOTROPIN-INHIBITORY HORMONE ACTION ON REPRODICTION
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(1) Department of Biology and Center for Medical Life Science, Waseda University, Tokyo, Japan

Since GnIH was discovered in 2000 as the first hypothalamic neuropeptide actively inhibiting gonadotropin release, it has been demonstrated that GnIH acts as a pronounced negative regulator of reproduction. Inhibitory effect of GnIH on reproduction is mainly accomplished at hypothalamic-pituitary levels; GnRH neurons and gonadotrpoes are major targets of GnIH action based on the morphological interaction with GnIH neuronal fibers and the distribution of GnIH receptor (GnIH-R). We have demonstrated the molecular mechanism of GnIH action by investigating the signaling pathways of GnIH-R occurring in these target cells. For the mechanistic study of target cell-specific action, we used *in vitro* models, GnRH neural GT1-7 and gonadotrope LβT2 cells. We examined GnIH-mediated second messenger pathways by GPCR reporter assay, then analyzed the change in downstream MAPK phosphorylation. GnIH specifically inhibited the AC/cAMP/PKA-mediated ERK and/or p38 pathways in GnRH neurons as well as gonadotropes. The physiological relevance of the inhibitory effect of GnIH on each target cell was indicated by the reduction of GnRH or gonadotropin levels by GnIH treatment. GnIH effectively suppressed the stimulatory effect of VIP and kisspeptin on GnRH release, and GnRH-induced LH release was also decreased by GnIH. Our results indicate that GnIH may govern the hypothalamic neuronal activities of GnRH by inhibiting the action of VIP and kisspeptin, and eventually reduce pituitary gonadotropin secretion. We have also demonstrated how imbalance between endocrine systems affect reproduction *via* GnIH regulation. Activation of adrenal system by stress stimuli increased GnIH expression through the direct binding of GR to its promoter, and abnormal thyroid status altered GnIH expression with chromatin modification changes. Together, our findings indicate the significance of GnIH as a key hypothalamic inhibitor to regulate reproduction.

S1-3) Orchard, Ian (Canada)

NEUROENDOCRINE SIGNALLING: DISCOVERIES FROM THE BLOOD-GORGING KISSING BUG, *RHODNIUS PROLIXUS*
Orchard I and Lange A B
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

Rhodnius prolixus, the kissing bug, is an obligatory blood-feeding insect and a major vector for the parasite *Trypanosoma cruzi* that causes Chagas disease in humans. Interest in *R. prolixus* as a model medically-important insect has been reinvigorated by the sequencing of its genome. *R. prolixus* takes a massive blood meal (up to 10 times its initial body weight) once in each instar, which is followed by the rapid post-feeding elimination of excess water and salts. Here we examine the neuroendocrine signalling involved in this diuresis.
Serotonin is a diuretic hormone (DH) in *R. prolixus* and works in concert with a member of the CRF-related family of insect neuropeptides, via G-protein-coupled receptors (GPCRs). Serotonin and the *R. prolixus* CRF-related DH (Rhopr-CRF/DH) are potent neuroendocrine signallers on tissues associated with diuresis (anterior midgut and Malpighian tubules), and act synergistically during rapid post-feeding diuresis.
An anti-diuretic hormone (ADH) Rhopr-CAPA-2, and its cognate GPCR are important mediators involved in the cessation of rapid post-feeding diuresis in *R. prolixus*, inhibiting serotonin-stimulated secretion by Malpighian tubules, but not Rhopr-CRF/DH-stimulated secretion. Rhopr-CAPA-2 appears to prevent the synergism between serotonin and Rhopr-CRF/DH.
The interplay between DHs and ADHs in *R. prolixus* results in a remarkable diuresis; a diuresis that has evolved to eliminate excess water and salts from a massive blood meal in a very rapid way. The parasite, *T. cruzi*, is transmitted via the excreted fluid during this diuresis, and therefore these DHs and ADHs ultimately control the transmission of Chagas disease. Acknowledgements: Supported by: NSERC Canada Discovery Grants to IO and ABL.

S1-4) Michalec, Ola (Canada)

TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP), AN ANCESTRAL PEPTIDE AND ITS EFFECT ON CALCIUM ION FLUX, A MECHANISM OF CELL SIGNALING IN ASTROCYTES.
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The teneurin C-terminal associated peptides (TCAPs) are a family of four 40-41 amino acid peptides located at the C-terminus of each of the teneurin type-II transmembrane proteins. The teneurins share structural similarity to bacterial toxins, suggesting that the teneurin gene arose as a result of a horizontal gene transfer event that involved a choanoflagellate engulfing a prokaryote. Also, the TCAPs share sequence similarity to the Secretin GPCR ligands, a less ancient family that includes corticotropin-releasing factor and calcitonin. Its phylogenetic position in relation to the Secretin ligands places TCAP as a putative progenitor of the Secretin family, further supporting its ancient origin. As maintaining ion homeostasis was crucial to ensuring the survival of the earliest organisms and calcium ion signaling appears to have evolved prior to the emergence of early metazoans, investigating the roles of TCAP on calcium signaling in astrocytes has been of particular interest. Astrocytes communicate by undergoing localized increases in intracellular calcium, a signal that can be passed on to neighboring astrocytes through gap junctions. TCAP exerts its effects by binding to the adhesion G-protein coupled receptor latrophilin (ADGRL) and administering TCAP-1 to astrocyte networks in vitro increases intracellular calcium levels and induces calcium oscillations, an effect not observed with vehicle treatment. Current studies are also investigating the effect of TCAP-1 on astrocyte-neuron co-cultures to better understand its effect on signaling between the two cell types. Acknowledgements: Supported by: NSERC CGSM.

S1-5) Rocco, David (Canada)

NOVEL INSIGHTS ON THE FUNCTIONAL ROLE OF THE GLYCOPROTEIN HORMONE GPA2/GPB5 AND ITS RECEPTOR IN THE ADULT MOSQUITO, *AEDES AEGYPTI*.
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(1) Department of Biology, York University, Toronto, ON, Canada

GPA2/GPB5 is an evolutionarily-ancient glycoprotein hormone found in most bilateral metazoans including the mosquito, *Aedes aegypti*. Unlike the classic glycoprotein hormones, the function of GPA2/GPB5 in both vertebrates and invertebrates remains unknown. In insects, transcript expression of the GPA2/GPB5 subunits and its receptor, the leucine-rich repeat-containing G protein-coupled receptor 1 (LGR1), indicate a role in hydromineral balance as well as development. To unravel additional roles of GPA2/GPB5 in adult mosquito, we aimed to identify physiological targets by examining the expression profile of its receptor, LGR1. Prior to mapping LGR1 protein expression in adult mosquitoes, a heterologous system expressing *A. aegypti* LGR1 was used to characterize an *A. aegypti* LGR1 custom antibody, from which immunoblot analyses confirmed a 112 kDa band associated with membrane-protein fractions. A second heterologous system using HEK 293T cells stably expressing a fusion construct of *A. aegypti* LGR1-EGFP (LGR1: 105 kDa + EGFP: 27 kDa) yielded a 139 kDa band that also associated with membrane-protein fractions, and upon deglycosylation, migrated to the predicted fusion-protein molecular weight of 132 kDa. To verify specificity of the custom antibody, immunocytochemical analysis of HEK 293T cells stably expressing LGR1-EGFP confirmed EGFP fluorescence and LGR1-like staining colocalized to the plasma membrane. In adult mosquitoes, immunohistochemical analyses revealed LGR1-like staining localizes to basolateral surfaces of epithelia associated with various regions of the gut, suggesting a possible role in feeding processes and/or hydromineral balance for GPA2/GPB5. Interestingly, modest levels of LGR1 transcript and strong immunostaining was also identified in reproductive tissues. Specifically, strict regionalization of LGR1 to distal regions of the testes suggests a potential role related to spermatogenesis in adult males. Furthermore, in the ovaries of non-blood fed females, LGR1-like staining implies a potential role associated with previtellogenesis, a reproductive

stage whereby females prepare for engorgement of a blood meal. Acknowledgements: Research supported through an NSERC Discovery Grant to JPP and Queen Elizabeth II Graduate Scholarship in Science and Technology to DR.

S1-6) Hollander Cohen, Lian (Israel)

FUNCTIONAL INVESTIGATION OF GNRH, FSH AND LH IN ZEBRA
Hollander Cohen L(1), Revah O(2), Golan M(3), Mollard P(3), Gutnick M(2). Levavi Sivan B(1),

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Hormonal regulation of the reproductive system involves hormones from the hypothalamus, pituitary, and gonads (the HPG axis). The most crucial hypothalamic hormone - gonadotropin-releasing hormone (GnRH) stimulates the release and synthesis of FSH which stimulates the growth of ovarian follicles in females and spermatogenesis in males, and LH which induces ovulation in females and androgen secretion in males. In fish, FSH and LH are secreted by different cells; as opposed to mammals where both hormones are secreted from the same cell. Using transgenic zebrafish lines that bear genetically-encoded calcium indicators (RCaMP2) in their gonadotropes and GnRH neurons we are able to characterize the activity of GnRH, FSH and LH and the mechanisms controlling those hormones. By measuring RCaMP2 activity in whole brain and pituitary tissue, we found that LH and FSH release seems to be pulsatory, rather than persistent. In response to GnRH stimulation, Ca^{2+} events in the gonadotropes always showed a rapid rise and were correlated in time across cells, suggesting that the cells share information about the timing of secretion. By employing this advanced live-imaging techniques in fish we are able to visualize in vitro the activity of those cell and to better understand the mechanisms controlling LH and FSH release

Monday AM S2

INSULIN AND INSULIN-LIKE PEPTIDES, VERTEBRATE AND INVERTEBRATE
Chair: Angela Lange and Cunming Duan

S2-1) Brown, Mark (USA)

OF THE INSULIN-LIKE PEPTIDES IN MOSQUITOES: ILP3 PREVAILS
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Multiple insulin-like peptides (ILPs) are encoded in the *Aedes*, *Culex* and *Anopheles* mosquitoes that are important vectors of human and animal disease pathogens. Most ILPs, including ILP3, are centrally expressed in neurosecretory cells clustered in the brain of all mosquito life stages or distributed throughout the nervous system. The diverse functions of *Aedes* ILP3 are best known, because it is most similar to vertebrate insulin, binds with high affinity to the native insulin receptor, and activates the insulin signaling pathway. It exhibits insulin-like activity in sugar-fed females and is a pleiotropic gonadotropin in blood fed females. This ILP also directly promotes proliferation of circulating hemocytes and follicle cells in the ovary of blood fed females. Such a mitogenic action suggests ILP3 has a role similar to that of vertebrate insulin-like growth factors. Comparative studies show that the gonadotropic action of ILP3 is conserved across *Culex* and *Anopheles* species. Work in progress seeks to determine the degree of functional redundancy or specificity of the other ILPs and their signaling pathways in female mosquitoes. Research support from the National Institutes of Health (RO1AI33108) and the Georgia Agricultural Experiment Station.

S2-2) Defferrari, Marina (Canada)

INSULIN-LIKE PEPTIDES IN *RHODNIUS PROLIXUS*, THE VECTOR OF CHAGAS DISEASE
Defferrari MS, Orchard I, Lange AB
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Insulin-like peptides (ILPs) are functional analogs of insulin and have been identified in many insect species. The insulin/insulin-like growth factor (IGF) pathway is a conserved regulator of metabolism, and in insects, as well as in other animals, it can modulate physiological functions associated with growth, development and metabolism of lipids and carbohydrates. Although the signaling components of the pathway are well conserved throughout evolution, the number of peptides and receptors differ considerably between species. The presence of insulin-like

immunoreactivity in neurosecretory cells in the brain of *Rhodnius prolixus* has been previously reported and a link between ILPs and the release of ecdysteroids was suggested. In the present study, we have identified one ILP and one IGF and investigated their involvement in *R. prolixus* metabolism and growth. We have identified the peptides within the *R. prolixus* genome and have cloned their cDNA sequences. Expression profile analyses showed that the ILP transcript is predominantly present in the brain while the IGF is distributed among a variety of tissues, mostly in the fat body, the dorsal vessel and the central nervous system. Using RNAi, we have knocked-down the expression of both transcripts separately and examined the effects on metabolism and growth. We observed that the absence of the ILP transcript increased the levels of lipids and carbohydrates in the hemolymph, while the lipid content in the fat body was increased. At the same time, the carbohydrate level was decreased in the fat body and the leg muscles, indicating that this peptide is involved in energy homeostasis. The absence of the IGF transcript resulted in defective molting of fifth instars into adults. Compared to the control, insects lacking IGF display abnormal morphological features such as smaller wings and reduced body size. Further experiments are being conducted on the physiological significance and downstream signaling of both peptides. This work was supported by NSERC.

S2-3) Kamei, Hiroyasu (Japan)

ROLES OF INSULIN RECEPTOR SUBSTRATES IN CONTROLLING EMBRYONIC GROWTH IN RESPONSE TO CHANGING ENVIRONMENTAL OXYGEN TENSION.

Kamei H
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Oxygen tension is a common environmental cue regulating embryonic growth and development. In most cases, animal embryos retard growth in low oxygen tension or hypoxia; however, upon its return to the normal oxygen condition, they often show quick growth restoration. This phenomenon has been known as "*catch-up*" growth, and in human, recent epidemiological data have shown that the catch-up growth following intrauterine growth restriction (IUGR) often associates with increased risks of adult-onset disorders. Colleagues and I have studied the role of insulin/insulin-like growth factor signaling (IIS) in this unique growth acceleration phenomenon. By using zebrafish embryo, a quick and easy catch-up growth model was developed, and it was found that the expressions of insulin receptor substrate genes (*irs1* and *irs2*) were greatly increased under the hypoxic period. The loss of *irs1* expression severely hampered the catch-up growth, indicating that the *irs1* was highly required for the rapid growth restoration during the reoxygenation period. Further analysis revealed that the loss of *irs*-mediated IIS in early embryogenesis led to a decreased viability of neural crest cells (NCCs) under prolonged hypoxia. The NCCs are multi-potent stem cells emerged at the early embryogenesis to give rise to a diverse cell lineage such as craniofacial cartilage and bone, smooth muscle, and even subsets of neurons and glia. Subsequent chemical and genetic ablation of NCCs clearly blunted the growth acceleration during reoxygenation period. These results have delineated the importance of *irs*-mediated IIS and the stem cells in the hypoxia/reoxygenation-induced catch-up growth. Since the fine-tuning of IIS under various environmental or physiological conditions are center for normal growth and metabolism, and since the NCCs differentiate into various types of cells, this finding would provide an important clue for understanding the previously unknown causal nexus between IUGR and its prognosis in human. Acknowledgements: Supported by Japan Society for the Promotion of Science, Grant-in-Aid for Young Scientists to HK

S2-4) Bogerd, Jan (Netherlands)

Igf3 AND Amh, TWO Fsh-RESPONSIVE GROWTH FACTORS, REGULATE SPERMATOGONIAL DIFFERENTIATION IN A CONCERTED MANNER.

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Follicle-stimulating hormone (Fsh) regulates fish spermatogenesis in a steroid-dependent and a steroid-independent manner. The latter involves differential expression of growth factors that in turn regulate spermatogonial proliferation and differentiation. Here, we find that two of these Fsh-responsive growth factors, anti-Müllerian hormone (Amh) and insulin-like growth factor 3 (Igf3), influence each other's effects on zebrafish spermatogonia. Fsh-time and -dose response experiments *ex vivo* demonstrate that *igf3* transcript levels are rapidly up-regulated, remain elevated, and respond to lower Fsh concentrations than are required to decrease *amh* mRNA levels. Immunofluorescence studies suggest that Fsh also decreases Amh protein levels while increases in Igf3 protein levels were comparatively small. Zebrafish Amh compromised Igf3-induced proliferation of type A undifferentiated (A_{und}) and type A differentiating (A_{diff}) spermatogonia. Proliferation of Sertoli cells associated with type A_{und} spermatogonia was also reduced by Amh. To better understand the inhibitory effects of Amh on germ cell development, we investigated Amh-induced changes in zebrafish testicular gene expression by RNAseq. Analyzing the differentially expressed genes demonstrates that several signaling pathways are potentially involved in mediating the inhibitory activity of Amh. The majority of the genes identified were down-regulated, such as the transcript levels of *igf3*, *insl3* and of steroidogenesis-related genes, all three stimulating germ cell differentiation. At the same time, Amh increased the expression of inhibitory signals, such as *inha* and *id3* transcript levels. Altogether, our results provide new information on how Fsh regulates zebrafish spermatogenesis by orchestrating the actions of different growth factors and other paracrine signaling systems, including the control of the multiple inhibitory effects of Amh.

S2-5) Jia Yudong (PRC)

DEVELOPMENTAL EXPRESSION PATTERNS OF INSULIN-LIKE GROWTH FACTORS DURING TURBOT METAMORPHOSIS

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Insulin-like growth factors I and II (IGF-I and IGF-II) are important regulators of vertebrate growth and development. This study characterized the mRNA expressions of *igf-i* and *igf-ii* during turbot (*Scophthalmus maximus*) metamorphosis to elucidate the possible regulatory role of the IGF system in flatfish metamorphosis. Results showed that the mRNA levels of *igf-i* significantly increased at the early-metamorphosis stage and then gradually decreased until metamorphosis was completed. By contrast, mRNA levels of *igf-ii* significantly increased at the pre-metamorphosis stage and then substantially decreased during metamorphosis. Meanwhile, the whole-body thyroxine (T4) levels varied during larval metamorphosis, and the highest value was observed in the climax-metamorphosis. The mRNA levels of *igf-i* significantly increased and decreased by T4 and thiourea (TU, inhibitor of endogenous thyroid hormone) during metamorphosis, respectively. Conversely, the mRNA levels of *igf-ii* remained unchanged. Furthermore, TU significantly inhibited the T4-induced mRNA up-regulation of *igf-i* during metamorphosis. The whole-body thyroxine (T4) levels were significantly increased and decreased by T4 and TU during metamorphosis, respectively. These results suggested that *igf-i* and *igf-ii* may play different functional roles in larval development stages, and *igf-i* may have a crucial function in regulating the early metamorphic development of turbot. These findings may enhance our understanding of the potential roles of the IGF system to control flatfish metamorphosis and contribute to the improvement of broodstock management for larvae.

Acknowledgements: Supported by: China Agriculture Research System (CRAS-50) and National Natural Science Foundation of China (31302205).

S2-6) Vahkal, Brett (Canada)

POTENTIAL ROLE OF INSL5 AND RXFP4 IN THE IMMUNE SYSTEM

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INSL5 is a peptide hormone with primary sites of expression in human in the distal colon and hypothalamus, with secondary sites of expression in the thymus, leukocytes and reproductive organs (testis and ovary). Its cognate receptor, relaxin family peptide receptor 4 (RXFP4), is a G-protein coupled receptor (GPCR) closely related to other small-peptide hormone receptors involved in neuroendocrine functions. RXFP4 has a similar, but wider expression profile than INSL5, including sites such as colon, vagus nerve, thymus and leukocytes. A variety of recent studies show that INSL5 influences glucose homeostasis and may play roles in appetite, satiety, glucose metabolism and/or hepatic gluconeogenesis. INSL5 and RXFP4 could also be markers for rectal neuroendocrine tumors and influence cell proliferation and survival. We hypothesize that INSL5/RXPF4 plays a role in the development (proliferation and survival) of naive T-cells in the thymus; and that INSL5 may affect peripheral immune system through RXFP4 on the vagus nerve in response to feeding status in the colon. To investigate the hypotheses, INSL5 and RXFP4 RNA expression and protein presence in immune system tissues were measured in a cohort of C57BL/6 mice. Localization of INSL5 was confirmed in colon by immunohistochemistry. Flow cytometry was used to detect RXFP4 on mature T-cells. To assess effects of INSL5 *in vivo,* nine C57BL/6 mice were injected with 30ug/kg of INSL5 following fasting and the effect on cytokines and metabolic peptide levels assayed. INSL5 is expressed at low levels in thymus, spleen and bone marrow, but high levels in the colon. We found that RXFP4 is highly expressed in thymus, spleen and bone marrow, but lower levels in colon. Western blotting exhibited a similar pattern of protein presence. Immunohistochemistry confirmed INSL5 presence in epithelial cells (L-cells) in the colon. No RXFP4 was detectable on mature T-cells. Preliminary data from cytokine assay showed decreases in anti-inflammatory cytokines following INSL5 injection. Metabolic assay showed a significant correlation between INSL5, insulin and pre-insulin C-peptide, indicating INSL5 might potentiate insulin release. Pending data from in vivo experiments, this research could uncover a novel feedback pathway between gut-immune axis relating to feeding and immune system status and relate to the research of various chronic diseases, such as diabetes, inflammatory bowel disease and Crohn's.

Monday AM S3

NEUROCHEMICAL REGULATION OF INSTINCTIVE BEHAVIOR
Chair: Kazuyoshi Tsutsui and Takayoshi Ubuka

S3-1) Ubuka, Takayoshi (Malaysia)

GNIH GENE KNOCKOUT REDUCES PAIN, ANXIETY, AND ENABLES INTENSIVE EXERCISE IN MICE.

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Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic neuropeptide that inhibits gonadotropin secretion in birds, mammals and fish. Orthologous peptides of GnIH that have an LPXRFamide (X = L or Q) motif at its C-terminal are conserved in vertebrates. GnIH orthologous peptides are also named RFamide-related peptide (RFRP) in mammals. It was shown in many vertebrate species that GnIH peptides play a role as a suppressor of the reproductive axis. However, GnIH may have multiple functions such as the regulation of behavior beyond the control of reproductive functions because GnIH neuronal fibers and its receptor GPR147 are widely expressed in the brain. Here, we created GnIH gene knockout (GnIH-KO) mice to establish the role and functional significance of GnIH in the regulation of pain, anxiety, and physical activity. GnIH-KO mice reduced sensitivity to pain and anxiety as shown by hot plate and elevated plus maze tests, respectively. On the other hand, central administration of GnIH increased sensitivity to pain. Spatial working memory was not improved in GnIH-KO mice as shown by Y-maze test. Finally, spontaneous physical activity was measured by wheel rotating exercise. GnIH-KO mice showed clearer circadian activity and performed intensive exercise soon after light off compared with wild type mice. These results indicate that endogenous GnIH increases sensitivity to pain, anxiety, and limits physical activity. Previously, it has been demonstrated that GnIH expression and the activity of GnIH neurons significantly increase in stressful conditions of the animal. Taken together, GnIH may not only play a role as a suppressor of the reproductive axis but also suppress physical activity and behavior in stressful conditions.

S3-2) Ando, Hironori (Japan)

PERIODIC CONTROL OF KISSPEPTIN AND ITS RECEPTOR GENE EXPRESSION BY PHOTIC AND NON-PHOTIC ENVIRONMENTAL CUES IN THE GRASS PUFFER, A SEMILUNAR SPAWNER.

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Many organisms use a combination of photic (e.g. daylight and moonlight) and non-photic (e.g. temperature) environmental cues to synchronize their maturation and spawning to specific seasons, to a particular moon phase, and/or to particular times of the day. These cues are integrated to regulate the activity of hypothalamic neuropeptides including kisspeptin, GnIH and GnRH. However, molecular mechanisms of periodic control of these neuropeptides are little understood. Grass puffer, *Takifugu niphobles*, provides a suitable model to assess this question because it spawns in semilunar cycles during spring tide in early summer and spawning occurs several hours before high tide. The mRNA levels of *kiss2* and its receptor gene (*kiss2r*) significantly increased during several months over the spawning period. The administration of Kiss2 stimulated the expression of *kiss2r* and *gnrh1* in the diencephalon and *fshb* and *lhb* in the pituitary. In the diencephalon, *kiss2* and *kiss2r* are synchronously expressed with daily and circadian variations under light/dark and constant dark conditions, respectively, and these changes were consistent with the changes in expression of melatonin receptor genes. The administration of melatonin significantly stimulated the expression of these genes. Furthermore, in the spawning season, *kiss2* and *kiss2r* showed specific changes in expression in a lunar month with a peak at full moon night. On the other hand, the levels of *kiss2* and *kiss2r* mRNAs were decreased in the fish exposed to high temperature (28°C) when compared to normal temperature (21°C), concomitant with the decrease in expression of *gnrh1*, *fshb* and *lhb*. The water temperature at the end of the spawning period rises up to 28°C in the field. The transitional increase in temperature to 28°C may provide an environmental signal to terminate reproduction. The present results indicate that periodic expression of *kiss2* and *kiss2r* may be important in the seasonal, semilunar, and precisely-timed spawning of the grass puffer through the control by circadian clock, melatonin and water temperature.

S3-3) Soga, Tomoko (Malaysia)

SEROTONERGIC REGULATION OF GONADOTROPIN-INHIBITORY HORMONE (GNIH) NEURONS IN SOCIAL STRESS

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Early-life social isolation impairs brain serotonin system during adulthood. Reproductive decline, influenced by early-life social isolation, remains unknown. Gonadotropin-inhibitory hormone (GnIH) neurons in the dorsomedial hypothalamic nucleus (DMN) have an inhibitory effect on the reproductive system. Our recent study shows GnIH is up-regulated by citalopram, a selective serotonin reuptake inhibitor. In this study, we investigated the role of GnIH neurons in early-life social isolation using transgenic Enhanced Green Fluorescent Protein (EGFP) tagged-GnIH promoter rat line. First, to study GnIH neuronal activity, we determined cFos and cellular transcription factor, beta-catenin expression in EGFP-GnIH neurons by immunohistochemistry (ICC) and EGFP intensity in GnIH neurons in adult male rats at the age of 9 weeks, after a post-weaning six weeks isolation or group-housing. Next, we observed serotonergic fiber appositions to EGFP-GnIH neurons in control and socially isolated male rats. The immunochemical study revealed markedly lower co-localization of cFos and EGFP intensity in GnIH neurons. However, higher levels of beta-catenin in GnIH neurons were observed in socially isolated male rats. Furthermore, the levels of serotonin related genes, *serotonin transporter* and *tryptophan hydroxylase2* mRNA expression in the dorsal raphe were lower in socially isolated rats. Importantly, serotonin fiber appositions to EGFP-GnIH neurons in the DMN were reduced by social isolation. These results suggest serotonergic regulation of GnIH neurons under social isolation by demonstrating that lower serotonin levels caused by post-weaning social isolation reduce GnIH neuronal activity in the DMN. Acknowledgements: Supported by: e-Science from the Ministry of Science, Technology and Innovation Malaysia (02-02-10-SF0164-164) to TS and ISP

S3-4) Takeuchi, Hideaki (Japan)

MOLECULAR BASIS UNDERLYING SOCIAL COMPETENCE IN MEDAKA FISH

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Oryzias latipes (Medaka) is an established vertebrate model for studying developmental genetics, genomics, and evolutionary biology. The physiology, embryology, and genetics of this species have been extensively investigated for centuries. Medaka fish recently attracted attention in the field of social neuroscience. I will introduce recent advances in medaka behavioral studies, focusing on female mating preferences and male mate-guarding behaviors. The medaka female has the ability to discriminate male individuals and prefers to mate with socially familiar males (female mating preference). In triadic relationships (two males and one female), the dominant male remains closer to the female and repels the other male (mate-guarding). Interestingly, mate-guarding blocks female social familiarization of the rival male, which can increase the mating success of the dominant male. Importantly, behavioral analyses using a series of medaka mutants revealed critical roles of neuropeptide neuromodulatory systems in regulating their social behaviors. The extra-hypothalamic gonadotropin releasing hormone system has a central role in activating female mating preference. The arginine-vasotocin system is required for the emergence of mate-guarding behavior. Acknowledgements: Supported by: JSPS KAKENHI Grant Numbers 26290003; a Grant-in-Aid for Scientific Research on Innovative Areas "Memory dynamism" (26115508) from the Ministry of Education, Culture, Sports, Science, and Technology, Brain Science Foundation; and the Yamada Science Foundation.

S3-5) Jarvis, Erich (USA)

DISSECTING THE MOLECULAR MECHANISMS OF VOCAL LEARNING AND SPOKEN LANGUAGE

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Our long-term goal is to decipher the molecular mechanisms that construct, modify, and maintain neural circuits for complex behavioral traits. One such trait is vocal learning, which is critical for song in song-learning birds and spoken-language in humans. Remarkably, although all are distantly related, song-learning birds (songbirds, parrots, and hummingbirds) and humans have convergent forebrain pathways that control the acquisition and production of learned sounds. This convergent anatomy and behavior is associated with convergent changes in multiple genes that control neural connectivity and brain development, of which some when mutated are associated with speech deficits. Non-human primates and vocal non-learning birds have limited or no such forebrain vocal pathways, but yet possess forebrain pathways for learning and production of other motor behaviors. Also interestingly, there are sex differences in brain and behavior in all vocal learning species, and these appear to be modulated in part by sex hormones. To explain these findings, I propose a motor theory of vocal learning origin, in which brain pathways for vocal learning evolved by brain pathway duplication of an ancestral motor learning pathway. Once a vocal learning circuit is established, it functions similarly as the adjacent motor learning circuits, but with some divergences in neural connectivity. I further argue that vocal learning was at first a sexually selected trait, that then become used for other purposes. In some species, such as some songbirds, sexual dimorphism increased further such that estrogen become a major molecular that modulates development of the song learning systems, and testosterone activates it. We are now conducting experiments to determine where sex hormones are modulating neuroanatomy and genes involved in the development of vocal learning circuits.

S3-6) Knapp, Rosemary (USA)

BRAIN TRANSCRIPTIONAL PROFILES OF MALE ALTERNATIVE REPRODUCTIVE TACTICS IN BLUEGILL SUNFISH WITH A FOCUS ON ENDOCRINE CANDIDATE GENES

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Understanding the molecular mechanisms influencing variation in behavior can provide insight into how different behavioral phenotypes are mediated. One type of behavioral variation that has not yet received much attention in this respect are the distinct phenotypes that comprise male alternative reproductive tactics (ARTs), which are found in a wide array of taxa, especially among fishes. One of the classic systems for studying male ARTs are bluegill sunfish, *Lepomis macrochirus* (Centrarchidae). This species has two distinct life histories: parental and cuckolder, encompassing three reproductive tactics, parental, satellite, and sneaker. Parental males provide sole parental care to offspring, including any sired by the cuckolder males, who provide no parental care. We used RNAseq to characterize the brain transcriptome of each male tactic during spawning to identify gene categories associated with each tactic and identify potential candidate genes, including endocrine genes, influencing their different spawning behaviors. We found that sneakers had higher levels of differential gene expression compared to the other tactics, suggesting that life history is not the main factor driving differential gene expression. Sneakers had high expression in ionotropic glutamate receptor genes, specifically AMPA receptors, which may be important for increased working spatial memory while attempting to cuckold nests on bluegill colonies. We also found significant expression differences in several candidate genes involved in ARTs that were previously identified in other species of fish. Several endocrine pathways were prevalent among the differentially expressed

genes between two or more male phenotypes. These genes included several in the glucocorticoid and thyroid hormone signaling pathways, genes involved in the control of feeding, and brain aromatase and several other steroidogenic enzymes. We will also discuss how these differences correlate with previously documented differences in plasma steroid hormone levels during spawning. The results of this study open new avenues of research into the neuroendocrinology underlying profound within-sex differences in reproductive behavior. Support from NSERC to BDN and Univ. Oklahoma Dept. Biology to RK.

Monday PM S4

TACKLING ENDOCRINE MYSTERIES IN DOMESTIC ANIMALS
(Sponsored by the University of Calgary School of Veterinary Medicine and University of Saskatchewan Western College of Veterinary Medicine)

Chair: Daniel J. MacPhee and Prasanth Chelikani,

S4-1) Singh, Jaswant (Canada)

EFFECT OF KISSPEPTIN ON BOVINE FOLLICULAR FUNCTION.

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Kisspeptin effects gonadotropin release via activation of GPR-54 receptors on GnRH neurons. We tested the hypothesis that the human kisspeptin-10 (Kp) increases LH release and dominant follicle growth in a dose- and duration-dependent manner in cattle. In the Experiment 1, beef cows were treated 5 days after ovulation with GnRH im (100 µg, n=9), saline (n=5), or Kp iv (10 mg, n=5 or 15 mg, n=5). Plasma samples were collected at -15, 0, 15, 30, 60, 120, 180 min for LH measurements. The ovaries were examined daily by ultrasonography. Peak plasma LH concentrations were higher in cows given 15 mg than 10 mg Kp (1.8±0.44 ng/ml vs. 0.9± 0.06 ng/ml; p=0.01). Plasma LH concentrations were higher ($p<0.0001$) at 30 and 60 min in cows given 15 mg Kp than in those given 10 mg Kp or saline. The GnRH group had higher ($p<0.001$) plasma LH concentrations (maximum of 7.4±1.2 ng/ml at 30 min) than other groups. Only cows in GnRH group ovulated (9/9). The diameter profile of the dominant follicle was greater (p=0.04) in the Kp groups (groups combined) than in the saline group. In Experiments 2 and 3, wave emergence (Day 0) was synchronized by follicular ablation, a progesterone releasing device (CIDR) was placed in vagina from Day 0 to 12, and a luteolytic dose of prostaglandin was given on Day 3.5 and 4 to create low plasma progesterone milieu during subsequent treatments. In Experiment 2, 2/10 heifers treated with 45 mg Kp iv on Day 6 ovulated vs none (0/10) in the saline group. In the Experiment 3, heifers were treated with multiple iv doses of Kp over a 2-hour period on Day 6: 9 doses of 5 mg Kp at 15-minute intervals (K9x5, n=6), 5 doses of 9 mg Kp at 30-minute intervals (K5x9, n=6), 3 doses of 15 mg Kp at 1 hour intervals (K3x15, n=6), or a single iv dose of 100 µg GnRH. The ovaries were examined by ultrasonography every 12 hourly. Multiple doses of Kp induced ovulation in >60% of heifers (11/18), with no difference among groups (K9x5: 3/6, K5x9: 4/6, K3x15: 4/6, GnRH: 5/6; p=0.71). All ovulations occurred between 36-48 hours of treatment. In summary, single iv dose of human kisspeptin 10 increased LH plasma concentrations and dominant follicle diameter in a dose-dependent manner, but resulted in low rate of ovulation induction. Multiple iv doses of kisspeptin 10 over a 2-hour period induced ovulation at a rate similar to that of GnRH. *Supported by NSERC Discovery grants to JS and GPA*

S4-2) McMillan, Chantal (Canada)

INCRETIN HORMONES IN CATS

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Glucagon–Like Peptide-1 (7-36) amide (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are enteroendocrine hormones secreted by L and K cells, respectively. Both play an important role in glucose homeostasis by amplifying insulin secretion following an oral glucose load - the 'incretin' effect. GLP-1 also inhibits glucagon secretion, delays gastric emptying, decreases eating and has been used therapeutically in humans with Type 2 Diabetes Mellitus. However, little is known about these hormones or the distribution of their receptors in the feline species. We evaluated fasting and postprandial plasma concentrations of GLP-1 and GIP in client-owned diabetic cats (DC) compared to healthy lean (LC) and overweight cats (OC) (n=10-11/group). The LC and OC were fed a standardized commercially available high carbohydrate diet exclusively for the first 2 wks of the study. After 2 weeks, they were exclusively fed a standardized high protein, low carbohydrate diet commonly prescribed to diabetic patients for 4 weeks. DC were transitioned to this high protein, low carbohydrate diet following diagnosis of diabetes and were fed this exclusively during the study. Blood sampling was performed pre- and post- prandially in all groups at baseline, 2 and 4 weeks. Our results revealed that plasma GLP-1 concentrations were significantly greater in DC when compared to LC and OC at baseline and were greater than LC at 2 and 4 weeks. Further, DC had greater GIP concentrations than LC and OC at baseline and 4 weeks and were greater than LC at 2 weeks. In addition, we also assessed the tissue distribution of transcripts for incretins, and their receptors, in non diabetic cats (n=4) undergoing euthanasia. We detected transcripts for proglucagon in the ileum, as well as GLP-1 and GIP receptors in the pancreas. Together our results demonstrate that feline gut expresses incretins and their receptors, and that circulating GLP-1 and GIP concentrations are increased in newly diagnosed diabetic compared to lean and overweight cats. It is unknown whether increased

circulating incretin concentrations are secondary to a potential signaling or receptor dysfunction in the diabetic state. Acknowledgements: University of Calgary CRF to CJM, NSERC Discovery Grant to PKC, and University of Saskatchewan ECS

S4-3) Snead, Elisabeth (Canada)

SAFETY AND EFFICACY ASSESSMENT OF A GLP-1 MIMETIC: INSULIN GLARGINE COMBINATION FOR TREATMENT OF FELINE DIABETES MELLITUS

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New therapeutic strategies for diabetes mellitus involve the use of synthetic incretin hormones, including exenatide. The primary objective of this study was to evaluate the safety and efficacy of a combination of short-acting exenatide with insulin versus insulin and placebo for the treatment of diabetes mellitus in cats. In addition, the effect of short- acting exenatide on body weight and serum concentrations of specific hormones that play a role in glycemic regulation (insulin and glucagon) or in altering insulin sensitivity and resistance (leptin and adiponectin) was evaluated. A double-blind, randomized, placebo-controlled, crossover study was performed.

Treatment with exenatide was well tolerated. During the study five cats experienced asymptomatic hypoglycemia (Blood glucose (BG) < 3.5 mmol/L) documented during a 12-hour glucose curve; one while receiving exenatide, three while receiving placebo, and in one cat during both treatment arms. Two cats (25%) went into diabetic remission while receiving exenatide and none while receiving placebo. Four cats had a decrease in the total daily dose of insulin administered while on exenatide compared to only one cat on placebo. The average change in the daily exogenous insulin dose was significant (β= -0.56 U/kg, 95% CI: -0.96 - -0.15, p= 0.007) and the dose of insulin administered was lower during exenatide treatment compared to during placebo treatment. Change in serum fructosamine from the beginning to the end of each treatment arm was not significantly different between exenatide and placebo (β= -23 µmol/L, 95% CI: -52- 5.3, p= 0.11). Six cats lost weight on exenatide (median: -0.72 kg, range: -1.4- -0.3 kg) while only 3 cats lost weight on placebo (median: -0.26 kg, range: -0.57- -0.03 kg). The average weight loss experienced on exenatide was significantly higher than on placebo (β= 0.65 kg**,** 95% CI 0.09-1.21, p= 0.02). There was no significant difference in any of the hormone concentrations evaluated for cats on exenatide versus placebo treatments. In this study, treatment of diabetic cats with insulin and a fixed dose of exenatide was safe. The weight loss and decreased exogenous insulin requirement experienced with exenatide treatment could be a significant benefit for overweight diabetic cats and warrants further evaluation.

S4-4) Thundathil, Jacob (Canada)

NUTRITIONAL MODULATION OF HORMONE PRODUCTION AND REPRODUCTIVE DEVELOPMENT IN BULLS

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Holstein breeding bulls that reach puberty early and produce large numbers of normal sperm are highly desirable. The objective was to determine effects of early-life nutrition on post-pubertal reproductive performance of dairy bull calves. Twenty-six bull calves were randomly allotted to 3 groups and fed approximately 70, 100, or 130% of National Research Council's recommendations for both energy and protein from 2-31 wk; thereafter, all were fed a 100% diet. There were several significant differences between bulls on a high- versus low-nutrition diet, including: larger testes (~20%), younger at puberty (~45 d), an earlier rise in luteinizing hormone (LH; ~1 month), greater magnitude of LH secretion (~5 ng/mL over 10 h), an earlier increase in testosterone concentrations (~1 month), and higher Insulin-Like Growth Factor-I concentrations (~5x higher at each age) throughout the differential feeding period. There was no effect of diet on semen characteristics (motility and morphology) or on seminal plasma proteins. Semen from a subset of bulls in each nutritional group was used to perform in vitro fertilization. A total of 1249 bovine oocytes were used; there were no significant differences among groups in fertilization percentage (mean ±SEM of 68.0±8.7, 77.1±3.5 and 68.7±4.5% for Low, Medium and High, respectively) or blastocyst yield (31.5±5.6, 41.4±4.9 and 33.7±4.6%).
In conclusion, underfeeding young bulls delayed puberty and reduced testis size, whereas better early-life nutrition resulted in earlier puberty, larger testes and potential for more sperm to be produced, with no significant reduction in semen quality. Therefore, maintaining future breeding bulls at a higher plane of nutrition during their early life could be used as a management strategy to improve future reproductive potential.

S4-5) Danks, Janine (Australia)

PARATHYROID HORMONE RECEPTOR 1 AS A PROGNOSTIC INDICATOR IN CANINE OSTEOSARCOMA

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Osteosarcoma (OS) is the most common type of malignant primary bone tumour in dogs. In addition to their critical roles in bone formation and remodelling, parathyroid hormone-related protein (PTHrP) and its receptor (PTHR1) are involved in progression and metastasis of many

types of tumours in humans. The aims of this study were to determine the localisation and expression levels of PTHrP and PTHR1 in canine OS tissues using immunohistochemistry and to investigate if this expression is correlated with survival time. Formalin-fixed, paraffin-embedded tissue samples from 51 dogs with known survival time that had been diagnosed with primary osteosarcoma were analysed for localisation of PTHrP and PTHR1. Findings showed that both PTHrP and PTHR1 were present in all OS samples. The dogs with high level of PTHR1 protein (16%) had decreased survival time ($P<0.05$) compared to dogs with less PTHR1 protein. PTHrP levels did not correlate with survival time ($P>0.05$). The results of this study indicate that the PTHR1 is expressed differently in canine OS tissues and this may be correlated with poor prognosis. This may mean that PTHR1 may be useful as a prognostic indicator in canine OS and could represent a good therapeutic target in OS.

S4-6) Mo, Chunheng (PRC)

CHARACTERIZATION OF CHICKEN GRP, NMB AND THEIR RECEPTORS: DISCOVERY OF THE ENDOGENOUS LIGAND FOR ORPHAN RECEPTOR BRS3 AND EVIDENCE FOR GRP BEING A NOVEL POTENTIAL PITUITARY HORMONE IN CHICKENS

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The two structurally and functionally related peptides, gastrin-releasing peptide (GRP) and neuromedin B (NMB) play critical roles in many physiological/pathological processes in mammals. However, the information regarding the expression and functionality of avian NMB, GRP, and their receptors is limited. Here, we characterized cGRP, cNMB, and their receptors (cGRPR, cNMBR, cBRS3) in chickens. Our results showed that: 1) cNMBR and cGRPR expressed in CHO cells could be potently activated by cNMB and cGRP respectively, as monitored by cell-based luciferase reporter assays, indicating that cNMBR and cGRPR are cNMB- and cGRP-specific receptors; Strikingly, BRS3 of chickens (/spotted gars), which is orthologous to mouse bombesin receptor subtype-3 (BRS3), could be potently activated by GRP and NMB, demonstrating that both peptides are the endogenous ligands for chicken(/spotted gar) BRS3; 2) Quantitative real-time PCR revealed that cGRPR is widely expressed in chicken tissues with abundant expression in the ovary, pancreas, proventriculus, spinal cord and brain, whereas cNMB, cNMBR and cBRS3 are mainly expressed in the brain and testes; 3) Interestingly, qPCR, Western blot, and immuno-staining revealed that cGRP is predominantly expressed in the anterior pituitary and mainly localized to LH-cells, suggesting that cGRP is likely a novel pituitary hormone in chickens. In summary, our data helps to uncover the roles of GRP, NMB and their receptors in birds, and provides the first persuasive evidence from an evolutionary prospective that in vertebrates, GRP and NMB are the endogenous ligands for BRS3, an orphan receptor which has puzzled endocrinologists for more than two decades. Acknowledgements: This work was supported by grants from the National Natural Science Foundation of China (31272436, 31572391, 31271325) and the National High Technology Research and Development Program of China (2013AA102501).

Monday PM S5

THE HORMONAL CONTROL OF OSMOREGULATION IN VERTEBRATES
Chair: Jason P. Breves and Steve Perry

S5-1) McCormick, Steve (USA)

THE ROLE OF CORTICOSTEROIDS IN OSMOREGULATION OF BASAL VERTEBRATES.

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There is abundant information that corticosteroids are important for regulation of salt secretory mechanisms in advanced (teleost) fish, but almost no information on their osmoregulatory role in basal vertebrates. We investigated the role of cortisosteroids in regulating seawater tolerance and its underlying mechanisms in a chondrostean (Atlantic sturgeon) and agnathan (sea lamprey). Transfer of Atlantic sturgeon from freshwater to seawater resulted in transient increases in plasma chloride, cortisol and glucose levels and long term increases in the abundance of gill Na,K,2Cl cotransporter (NKCC) which plays a critical role in salt secretion. Treatment of Atlantic sturgeon in freshwater with exogenous cortisol implants resulted in dose dependent increases in cortisol, glucose and gill NKCC. Sea lamprey metamorphosis, which precedes downstream migration and seawater entry, is accompanied by increased salinity tolerance, gill and intestinal Na/K-ATPase activity. Treatment of metamorphosing sea lamprey with 11-deoxycortisol implants resulted in dose dependent increases in salinity tolerance, gill and intestinal Na/K-ATPase (NKA) activity, and NKA and NKCC mRNA levels. Our results indicate that corticosteroids have an important role in regulating salt secretion in sturgeon and lampreys. Corticosteroid regulation of osmoregulation is thus a common feature of basal vertebrates that have adopted an osmoregulatory strategy.

S5-2) Breves, Jason (USA)

MULTIFACTORIAL CONTROL OF BRANCHIAL CLC-2C GENE EXPRESSION IN TILAPIA: EFFECTS OF SALINITY, PROLACTIN AND EXTRACELLULAR OSMOLALITY.

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In order to sustain hydromineral balance, teleosts inhabiting fresh water (FW) rely upon the activities of ion-absorptive ionocytes to counteract diffusive ion losses to the external environment. A member of the Clc Cl^- channel family, Clc-2c, was recently identified as a conduit for basolateral Cl^- transport by Na^+/Cl^- cotransporter (Ncc)-expressing ionocytes in stenohaline zebrafish. It is unresolved whether Clc-2c is expressed in gill of euryhaline species and how extrinsic (salinity) and/or intrinsic (hormones) factors modulate *clc-2c* mRNA levels. The present study investigated whether environmental salinity, prolactin (Prl) and local osmotic conditions modulate *clc-2c* mRNA levels in euryhaline Mozambique tilapia (*Oreochromis mossambicus*). Branchial *clc-2c* and *ncc* gene expression was enhanced in tilapia transferred from seawater to FW, whereas *clc-2c* and *ncc* expression was attenuated upon transfer from FW to seawater. To probe the endocrine control of *clc-2c* expression, we injected hypophysectomized tilapia with ovine prolactin (oPrl). oPrl induced a >8000-fold increase in *clc-2c* expression from saline-injected controls. To then assess whether Prl regulates *clc-2c* expression in a gill-autonomous fashion, we incubated gill filaments in the presence of tilapia Prls ($tPrl_{177}$ and $tPrl_{188}$). By 24 h, $tPrl_{188}$ stimulated *clc-2c* expression ~5-fold from controls. Lastly, filaments incubated in media ranging from 280 to 450 mOsm/kg for 3 and 6 h revealed that extracellular osmolality exerts a local effect on *clc-2c* expression. Resembling patterns previously observed for *ncc*, *clc-2c* showed higher expression with decreasing media osmolalities. Collectively, our results suggest that both hormonal and osmotic control of branchial *clc-2c* contributes to the FW adaptability of Mozambique tilapia.

S5-3) Gilmour, Kathleen (Canada)

REGULATION OF CARBONIC ANHYDRASE IN IONOCYTES OF THE FISH GILL.

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Ionic/osmotic and acid-base regulation in freshwater teleosts are tightly coupled through the ion-transporting cells or ionocytes of the gill that take up Na^+ and Cl^- in exchange for H^+ and HCO_3^-, respectively. The H^+ and HCO_3^- are supplied by intracellular hydration of CO_2 catalyzed by cytosolic carbonic anhydrase (CAc). Cortisol is well-known for its involvement in ionic and osmotic regulation in fish, but has received less attention as a possible regulator of acid-base balance. In rainbow trout (*Oncyrhynchus mykiss*), two main ionocyte types have been identified; the peanut lectin agglutinin-positive (PNA^+) ionocyte which has been proposed to be a base-secreting cell, and the acid-secreting PNA^- ionocyte. Cortisol treatment increased the abundance of both ionocyte types and caused a significant increase in the proportion of PNA^+ ionocytes that expressed CAc. The proportion of PNA^+ ionocytes that expressed CAc, which was unexpectedly low under control conditions, also was increased in response to systemic alkalosis. Thus, cortisol may play a role not only in regulating osmotic and ionic balance in freshwater fish, but also in regulating ionocyte abundance and the expression of CA in response to acid-base disturbances..
Acknowledgements: Supported by: NSERC of Canada Discovery and Research Tools and Instruments grants.

S5-4) Seale, Andre (USA)

OSMOTIC REGULATION OF PROLACTIN 188 AND PROLACTIN 177 AND THEIR RECEPTORS IN TWO TILAPIA CONGENERS WITH DISTINCT SALINITY TOLERANCES.

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Osmoregulation in vertebrates is largely controlled by the neuroendocrine system. Prolactin (PRL) is critical for the survival of euryhaline teleosts in fresh water (FW) due to its ability to conserve ions, reduce water influx, and promote ion uptake. In the euryhaline Mozambique tilapia (*Oreochromis mossambicus*), PRL cells from the *rostral pars distalis* of the pituitary release two PRL isoforms, PRL_{188} and PRL_{177}, in response to hyposmotic stimulation. Both PRLs function via two PRL receptors (PRLRs), PRLR1 and PRLR2, that are expressed in multiple osmoregulatory tissues, including gill. We conducted a comparative study using the Nile tilapia (*O. niloticus*), a congener of Mozambique tilapia that is less tolerant to increases in environmental salinity, to investigate the regulation of PRLs and PRLRs upon acute hyperosmotic challenges *in vivo* and *in vitro*. When transferred from FW to brackish water (BW; 20‰), plasma osmolality of Mozambique tilapia remained below 400 mOsm/Kg, while that of Nile tilapia reached 600 mOsm/Kg by 24 h. Furthermore, upon transfer from FW to BW, Nile tilapia decreased circulating PRL_{177} to a greater extent than Mozambique tilapia. In dispersed PRL cell incubations, the release of both PRLs was less sensitive to variations in medium osmolality in Nile tilapia than in Mozambique tilapia. By contrast, increases in pituitary and branchial *prlr2* gene expression in response to a rise in extracellular osmolality were more pronounced in Nile tilapia relative to its congener, *in vitro* and *in vivo*. Together, these results support the conclusion that inter-specific differences in salinity tolerance between Mozambique and Nile tilapia are linked with the distinct responses of both PRLs and their receptors to osmotic stimuli. Acknowledgements: Supported by grants from the NIH, USDA, and Sea Grant to APS, and NSF to DTL, and EGG.

S5-5) Shaughnessy, Ciaran (USA)

11-DEOXYCORTISOL PROMOTES SEAWATER TOLERANCE IN METAMORPHOSING SEA LAMPREY (*PETROMYZON MARINUS*)

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Lampreys (Petromyzontiformes) are the most basal extant osmoregulating vertebrates. The sea lamprey (*Petromyzon marinus*) is an anadromous species that makes a freshwater-to-seawater (SW) migration after metamorphosis. Although the basic osmoregulatory mechanisms in the lamprey appear to be similar to those of teleosts, utilizing active and secondary ion transport across branchial and intestinal epithelia, very little is known about the hormonal control of osmoregulation in lamprey. In teleosts, cortisol is known to have both glucocorticoid and mineralocorticoid actions controlling stress and osmoregulatory responses. Lamprey lack 11β-hydroxylase (the enzyme that converts 11-deoxycortisol (11-DOC) to cortisol) and it has been shown that 11-DOC is the likely corticosteriod of lamprey. The present study examined organismal-, protein-, and transcriptional-level osmoregulatory responses in the sea lamprey throughout metamorphosis and to *in vivo* and *in vitro* 11-DOC treatment. Gill Na^+/K^+-ATPase (NKA) activity in the sea lamprey increased 15-fold throughout the six-month metamorphosis from larvae to juvenile and was highest in downstream-migrating juveniles; these changes in NKA activity appeared to track with the ontogeny of SW tolerance over this time. Late-stage metamorphic individuals administered 11-DOC *in vivo* (10 or 50 µg/g implant) had higher gill NKA activity, higher *nka* and $Na^+/K^+/2Cl^-$-cotransporter (*nkcc*) mRNA expression, and greater SW tolerance than controls. Likewise, excised gill tissue incubated *in vitro* for 24 h with varying doses of 11-DOC exhibited dose-dependent increases in *nka* and Cl^- channel (*cftr*) mRNA expression. This work is the first to show that 11-DOC treatment improves SW tolerance in sea lamprey and further substantiates the hypothesized role of 11-DOC as a mineralocorticoid in these basal vertebrates fishes.

S5-6) Hwang, Pung-Pung (Taiwan)

MOLECULAR PHYSIOLOGY OF HORMONAL ACTIONS ON BODY FLUID IONIC AND ACID-BASE HOMEOSTASIS: USING ZEBRAFISH AS A MODEL

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Fish, a group of aquatic vertebrates, have developed sophisticated mechanisms of ionic and acid-base regulation for maintaining body fluid homeostasis as mammals do. Many hormones have been proposed to control the ionic and acid-base regulation mechanisms in fish; however, most of the proposed actions lack convincing cellular and/or molecular evidence. Given the advantages of genetic database availability and manipulation, zebrafish is an emerging model for research into regulatory and integrative physiology. Different types of ionocytes were found to transport ions through various sets of ion transporters, and the molecular mechanisms of ionocyte proliferation and differentiation have also been dissected. These provided a competent platform to precisely study the ion transport pathways and ionocytes targeted by hormones. Isotocin, prolactin, cortisol, stanniocalcin-1, calcitonin, endothelin-1, vitamin D, parathyorid hormone 1, catecholamines, the renin-angiotensin-system, estrogen-related receptor α, calcitonin gene-related peptide, etc. have been demonstrated to positively or negatively regulate ion transport through specific receptors at different molecular levels (transcriptional, translational, or posttranslational) or at different developmental stages of ionocytes (proliferation or differentiation). The knowledge obtained using zebrafish answered many long-term contentious or unknown issues in the field of fish iono-/osmoregulation. The homology of ion transport pathways and hormone systems also means that the zebrafish model informs studies on mammals or other animal species, thereby providing insights into related fields.

Monday PM S6

INVERTEBRATE SEX STEROIDS AND PEPTIDES
Chair: Pei-San Tsai and David Norris

S6-1) Tsai, Pei-San (USA)

GONADOTROPIN-RELEASING HORMONE-RELATED PEPTIDES AND THEIR RECEPTORS IN A GASTROPOD MOLLUSK

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Gonadotropin-releasing hormone (GnRH) belongs to a large peptide family consisting of vertebrate GnRH, protostome GnRH, adipokinetic hormone (AKH), corazonin (CRZ), and AKH/CRZ-related peptide (ACP). An ancestral peptide to the GnRH family likely arose in a basal bilaterian 600 million years ago and evolved among the protostome and deuterostome lineages with a complicated history of gene duplication and loss. The goal of the present study was to examine a snapshot in the evolutionary trajectory of GnRH-related peptides and their receptors

using a model organism, the gastropod *Aplysia californica*. Although GnRH among vertebrates is known for its ability to activate reproduction, our results reveal that GnRH in *A. californica* (named ap-GnRH) plays no role in reproduction. Rather, it controls several motor functions implicated in metamorphosis and feeding behavior. Similarly, AKH in *A. californica* (named ap-AKH) plays no role in reproduction, but is instead a regulator of digestive, osmoregulatory, and metabolic functions. The expression pattern of the receptors for ap-GnRH and ap-AKH in *A. californica* is relatively diverse and consisted of variants derived possibly from multiple translational start sites and differential splicing. In sum, our results from a model mollusk suggest that the evolutionary history of the GnRH-related peptides is diverse, and the function of protostome GnRH and related peptides should not be pigeonholed into a specific category. Rather, the function of these peptides may be highly variable and taxon-specific.
Acknowledgments: Supported by National Science Foundation Grant IOS 1352944 to PST.

S6-2) Alexander, Scott (UK)

VERTEBRATE STEROIDS PRESENT IN MOLLUSK TISSUES ARE MORE LIKELY TO BE OF EXOGENOUS THAN ENDOGENOUS ORIGIN

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Many consider that the widespread presence of vertebrate steroids such as testosterone (T) and 17β-estradiol (E_2) in mollusks is evidence that they are of endogenous origin and are thus likely to be involved in the control of reproduction (as they are in vertebrates). This has led to many papers over the past 50 years in which the authors have tried to show that these steroids regulate processes as diverse as egg yolk protein induction in bivalves and penis development in snails However, very little, if any, of this evidence is incontrovertible (i.e. apparently 'positive' findings have alternate explanations). What has become increasingly clear is that mollusks (at least those that have been studied) have an ability to absorb and, crucially, to store, steroids such as E_2 and T that they encounter in their environment. When mussels (*Mytilus* spp.) are exposed to tritiated E_2 and T in the water, radioactivity decreases rapidly and exponentially. The rate of uptake of the radiolabels cannot be saturated by concentrations as high as 25 μg L^{-1} of non-radiolabeled steroids (vastly in excess of typical environmental concentrations of these steroids). Most of the radioactivity in the tissues (between 25% to 35% of what had been added to the water) was extractable in the form of fatty acid esters. Following alkaline hydrolysis, this yielded, in the case of the animals exposed to E_2, intact (i.e. non-metabolized) E_2; and in the case of animals exposed to T, mainly 5α-dihydrotestosterone (5α-DHT) and 5α-androstane-3β,17β-diol. Depuration experiments determined that E_2-related radioactivity had a half-life of ca. 12 days, but T-related radioactivity was unchanged after 10 days, confirming that mussels can hold on to exogenously-derived vertebrate steroids for periods of weeks (and, in the case of T metabolites, possibly months). These findings, coupled with the fact that vertebrate steroids are ubiquitous 'contaminants' in the environment, suggest that there is very little merit in measuring either E_2 or T in mollusks in the hope that their concentrations in tissue will in some way be indicative of the reproductive status of the animal as, firstly, it is impossible to know with any certainty how much is of exogenous and how much of endogenous origin and, secondly, how much is of recent and how much of historic origin.

S6-3) Thitiphuree, Tongchai (Japan)

GAMETOGENESIS AND MRNA EXPRESSION OF STEROIDOGENIC ENZYME GENES DURING REPRODUCTIVE CYCLE IN JAPANESE SCALLOP

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Japanese scallop, *Patinopecten yessoensis*, has an annual reproductive cycle and cultured nearby coast of North Pacific areas in Japan. The presence of vertebrate-like steroids has been proposed in mollusks including bivalves. To examine the steroidogenesis of *P. yessoensis*, *in silico* survey of steroidogenic enzyme genes was carried out. Ten putative genes were identified, namely, *cyp17a1*, *hsd17b8*, *hsd17b10*, *hsd17b11*, *hsd17b14*, *hsd3b1*, *hsd3b2*, *srd5a1*, *star3* and *star9*. Since candidate genes were predicted from contigs of transcripstome library by blasting, it is thus important to clarify cDNA sequence of steroidogenic enzyme genes in scallops. In this study, the scallops were collected at three development stages; early differentiating, growing and mature stages. Their maturational status were determined by morphology, gonad index (GI) and histology. Then, gonads of scallops were collected for RNA extraction and subjected to RACE-PCR analysis to obtain the full-length cDNA. Finally, qPCR analysis was performed for mRNA expression in gonad tissues. In early differentiating stage, gonad morphology showed transparent and lower GI (7.72±0.47 %). At mature stage, it became larger, thick and maximum GI (17.42±0.73 %). By histological observation, we found a few germ cells and thin acinar wall at the early differentiating stage. In mature stage, several types of germ cells presented in the acinar lumen and acinar wall became thicker. At this point, we can identify steroidogenic enzyme genes (42-99% of sequence identity with other bivalve) which included in the steroidogenic pathway from our *in silico* mining. We obtained full-length cDNA of *cyp17a1*, *hsd17b14* and *srd5a1* by RACE-PCR. The mRNA expression was analyzed for ten putative genes. These finding showed that mRNA levels of *hsd17b14*, *cyp17a1*, *srd5a1*, *star3* and *star9* expressed at high level in early differentiating stage. In mature stage, the high expression showed in *hsd17b8*, *hsd17b10*, *hsd17b11*, *hsd3b1* and *hsd3b2*. In addition, the mRNA expression of *hsd3b1* and *hsd17b11* showed the relation with gonadal maturation which increased from early differentiating until mature stages. These findings indicate that both *hsd3b1* and *hsd17b11* play an important role on steroidogenesis of this scallop.

S6-4) Mita, Masatoshi (Japan)

HORMONAL ACTION OF RELAXIN-LIKE GONAD-STIMULATING PEPTIDE: REGULATORY MECHANISM OF STARFISH OOCYTE MATURATION AND OVULATION.

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Relaxin-like gonad-stimulating peptide (RGP) of starfish *Patiria pectinifera* is the first identified invertebrate gonadotropin to trigger final gamete maturation. Although RGP is the primary mediator of oocyte maturation and ovulation, its effect on oocyte maturation and ovulation is indirect. Resumption of meiosis in immature oocytes and release from the ovary are induced by a second mediator, maturation-inducing hormone, identified as 1-methyladenine (1-MeAde) in starfish. Thus, RGP plays an important role in 1-MeAde production in ovarian follicle cells. Oocyte release from ovaries of *P. pectinifera* occurs after germinal vesicle breakdown (GVBD) and follicular envelope breakdown (FEBD) when gonads are incubated *ex vivo* with either RGP or 1-MeAde. On the other hand, L-glutamic acid blocked this spawning phenotype, causing the mature oocytes to remain within the ovaries. Neither RGP-induced 1-MeAde production in ovarian follicle cells nor 1-MeAde-induced GVBD and FEBD was affected by L-glutamic acid. Application of acetylcholine (ACH) to ovaries under inhibitory conditions with L-glutamic acid, however, brought about spawning, possibly by inducing contraction of the ovarian wall to discharge mature oocytes from the ovaries concurrently with GVBD and FEBD. Thus, starfish spawning induced by RGP can thus be understood as follows: RGP acts on ovarian follicle cells to produce 1-MeAde. 1-MeAde stimulates oocytes to produce maturation-promoting factor (MPF), which is a direct trigger for maturation and dissociation of the follicular envelope. After FEBD, the denuded mature oocytes become free within the ovary. The defolliculated egg jelly contacts the ovarian wall, stimulating ACH release from adjacent nerve terminals. ACH-mediated contraction of the ovarian wall leads to spawning of the loose, mature oocytes. L-Glutamic acid may inhibit ACH secretion from gonadal nerve cells in the ovary. Acknowledgements: Supported by JSPS KAKENHI Grant number 16K07417.

S6-5) Paluzzi, Jean-Paul (Canada)

CAPA NEUROPEPTIDES IN THE DISEASE VECTOR, AEDES AEGYPTI: HORMONAL ACTIONS AND FUNCTIONAL ANALYSIS OF MULTIPLE RECEPTOR VARIANTS.

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Several hormones control hydromineral balance in insects by regulating the excretory system comprised of the Malpighian tubules (MTs) and hindgut. Several diuretic factors have been identified in insects and multiple factors are often active in a single insect species. Comparably, knowledge of anti-diuretic factors, which may inhibit secretion by the MTs or increase reabsorption by the hindgut, are not as extensively described.

Members of the CAPA neuropeptide family share a highly conserved C-terminal sequence (FPRV-NH_2) and have been identified in all studied insects and arachnids. Interestingly, CAPA peptides are diuretic in some insect species, while in others, they elicit anti-diuretic actions. In the mosquito *Aedes aegypti*, an important vector of various human diseases, the endogenous CAPA peptides are known; however, the physiological roles of these neuropeptides, particularly in the adult stage mosquito, remains ambiguous.

Utilizing an integrative approach, we determined actions of CAPA neuropeptides in adult stage *A. aegypti*. Firstly, we mapped the neurosecretory cells producing CAPA peptides within the nervous system combining immunohistochemistry and fluorescent *in situ* hybridization. Secondly, we used both *in vitro* and *in vivo* bioassays to determine the function of CAPA peptides in adult stage insects in combination with select diuretic factors. Thirdly, we identified transcripts encoding the CAPA neuropeptide receptor, including two receptor isoforms that may elicit distinct intracellular signalling cascades through unique G protein coupling. Using a heterologous receptor functional assay, we determined the *A. aegypti* CAPA receptor is activated by subnanomolar doses of endogenous CAPA peptides, but is only weakly responsive to structurally-related pyrokinin peptides. Finally, we examined expression of the CAPA receptor transcripts in post-embryonic developmental stages and in tissues of adult-stage insects. These findings advance our knowledge on the physiological functions of CAPA neuropeptides in the mosquito and help resolve the interplay between the various diuretic and anti-diuretic hormones in insects.Acknowledgements: Research supported by an NSERC Discovery Grant and Petro Canada Young Innovator Award to JPP.

S6-6) Liutkeviciute, Zita (Austria)

OXYTOCIN/VASOPRESSIN SIGNALLING IN ANTS

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The oxytocin/vasopressin signalling system comprises G protein-coupled receptors and their endogenous peptide ligands and appears to be important for water homeostasis, reproduction, learning, memory and behaviour [1]. To date, its biological function in insects has only been studied in the beetle Tribolium castaneum where it has been implicated in water retention in Malpighian tubules [2; 3]. The first evidence that an oxytocin/vasopressin-like signalling system exists in social insects came from ant genome sequencing [4] indicating the presence of one receptor and one neuropeptide precursors protein in all sequenced ant species [5]. For our study we have chosen two ant species of the genus Lasius that are closely related genetically, but significantly differ in their ecology and colony structure [6]. Following pharmacological characterization of the ligand-receptor pair in vitro, our aim was to determine the distribution and expression level of both the receptor and

precursor in different parts of the body and developmental stages in ants using qPCR and immunostaining. Next we were able to generate a knock-down of the precursor in Lasius ants and performed in vivo behavioural experiments comparing the frequency of common activities between knock-down and control animals. Our qPCR results indicate that this signalling system can be involved in male reproduction (high expression of receptor in male heads and reproductive organs) similar to other invertebrate species. On the other hand, the expression of receptor in Malpighian tubules is very low which contradict earlier findings in beetles [2]. Additionally, high expression of receptor in some parts of digestive system and fat body indicate that oxytocin/vasopressin signalling in ants may be involved in digestion and fat metabolism.
Acknowledgements: The project has been funded by the Vienna Science and Technology Fund (WWTF) through project grant LS13-017.

Tuesday AM S7

FISH SEXUAL DEVELOPMENT
Chair: Bon-chu Chung and Chun Peng

S7-1) Tanaka, Minoru (Japan)

CHARACTERIZATION OF A FEMINIZING POWER BY GERM CELLS DURING SEX DIFFERENTIATION IN MEDAKA
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In genetically sex-determined fish, sex of germ cells obeys the direction by surrounding somatic cells under natural conditions – if the somatic cells become male by the expression of sex determination gene, the somatic cells send signals to make germ cells fated to spermatogenesis. Medaka is one of the genetically sex-determined fish. However, we have been revealing a core mechanism in medaka that can determine the sex independent of the direction of sex determination gene. Artificial modification of the core mechanism could develop a tool to manipulate the sex according to our desire.
In the core mechanism, germ cells have a critical function for feminization of the gonad (ovarian formation). Our studies indicate that the enough number of germ cells is necessary and sufficient for forming ovary. However, we did not know if the feminizing power of germ cells is germ cell-developmental stage or not. Here I show, using several medaka mutants, that it is not developmental stage-dependent, but can be attributed to an original and inherent nature of germ cells. Interestingly our results also suggest that a fate decision of germ cells towards eggs or sperm by foxl3 (sex determination gene of germ cell) can be distinct from the inherent nature of germ cells.

S7-2) Canário, Adelino (Portugal)

THE TIMING OF PUBERTY IN FISH: NOVEL INSIGHTS FROM TRANSCRIPTOMICS.
Rute S. T. Martins (1), Patrícia Pinto (1), Manuel Carrillo (2), Silvia Zanuy (2), Bruno Louro (1), Ana Gomez (2), Tine, M.(3), Machado, R.(1), Reinhardt (3), R. and Adelino V. M. Canário (1)*
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Puberty is a developmental period that covers the transition of the reproductive system from an immature juvenile to a mature adult capable of producing viable gametes. This process requires activation of the brain-pituitary-gonadal (BPG) axis, possibly linked to internal signals for somatic growth, and external factors such as photoperiod or temperature. Although the role of gonadotropins and kisspeptins in the activation of gonadal maturation has been characterized in several teleost fish species, the internal signals factors responsible for the activation of BPG axis are still largely unknown. Recent research combining high throughput transcriptomics and environmental control have shed significant new information on the gene networks likely to be involved in signaling the puberty onset. These studies also highlighted the importance of metabolic physiology in the decision as to whether fish should proceed to puberty.
Acknowledgements: Supported by: European Commission Lifecycle project and the Foundation for Science and Technology of Portugal (FCT) through projects PEst-C/MAR/LA0015/2011 and UID/Multi/04326/2013.

S7-3) Wang, Deshou (PRC)

ADVANCES IN ENDOCRINE CONTROL OF TILAPIA SEX DIFFERENTIATION.
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In vertebrates, sex is determined either genetically (genetic sex determination) or environmentally (environmental sex determination), or a combination of both. In non-eutherian vertebrates including fish, environmental factors, especially steroid hormones, play a critical role in the sex determination. Estrogen is produced via the conversion of androgen by aromatase, which is encoded by *cyp19a1a/b* in fish. Gonadal

transcriptome analysis showed that almost all steroidogenic enzyme genes, including *cyp19a1a*, were up-regulated in XX tilapia gonads at 5 days after hatching (dah); but in XY gonads these enzyme genes, including *cyp11b2*, were significantly up-regulated until ~45 dah, indicating that, at the time critical to sex determination, the XX fish produced estrogen and the XY fish were unable to produce neither estrogen nor androgen. Administration of aromatase inhibitor in XX fish was able to induce primary sex reversal when applied before sex differentiation and secondary sex reversal when applied after sex differentiation in teleosts. Simultaneous treatment with estrogen and androgen before sexual differentiation resulted in all females in both XX and XY fish. Homozygous mutation of *cyp19a1a* in XX fish by CRISPR/Cas9 resulted in female to male sex reversal, which could be rescued by exogenous estrogen treatment. In contrast, homozygous mutation of *cyp19a1b* in XX fish resulted in no defects in ovary development, indicating its redundancy for ovarian differentiation. Homozygous mutation of *foxl2* or heterozygous mutation of *sf-1*, the two major positive regulators of *cyp19a1a* transcription, also significantly decreased aromatase expression and serum estrogen level, and resulted in female to male sex reversal. On the other hand, administration of exogenous estrogen alone can reverse XY fish to phenotypic females when applied during the narrow window of sex differentiation. Blockage of androgen synthesis and simultaneous administration of estrogen in XY fish can even induce the transdifferentiation of differentiated testis into functional ovary. Taken together, these results strongly emphasize the sexual plasticity of fish, the critical roles of endogenous estrogen in female sex differentiation and maintenance and endogenous androgen in male sex maintenance.

S7-4) Chun, Bon-chu (Taiwan)

Sexually dimorphic gene expression during early zebrafish gonadal development

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In zebrafish, little is known about the mechanism of early gonadal development and sex determination. There is a hypothesis that zebrafish testes may develop from ovaries based on the observation of ovaries and ovotestes before the appearance of testes. However, dimorphic size of gonad is observed from 12 days post fertilization (dpf) when female gonads become bigger than male gonads. To examine germ cell growth at different stages of gonadal development, we counted the number of *vasa*-positive germ cells. Zebrafish germ cells started to increase in numbers after 8 dpf, therefore the expression of genes involved in gonadal development was examined from 8 dpf to 14 dpf by *in situ* hybridization. Two somatic genes, *gsdf* and *dmrt1a*, were detected in presumptive female gonads at higher amounts than presumptive male gonads at 10 dpf and 12 dpf, respectively. However, male somatic genes such as *sox9a* and *ar* remained the same in all gonads until 3 weeks of age when higher levels of *sox9a* was detected in presumptive male gonads. These data indicated that female gonads started to differentiate early at 8-14 dpf while male gonads remained undifferentiated at this early stage.
To dissect the function of *gsdf*, we used TALEN-based knockout technology to generate three independent *gsdf*$^{-/-}$ lines, which had 25-bp or 11-bp deletion, or 4-bp insertion, resulting in frame-shift mutations and truncated proteins. The *gsdf*$^{-/-}$ adults had normal sex ratio and their gonocytes grew normally. However, *gsdf*$^{-/-}$ females had reduced fecundity at the age of 6 months. The H&E staining result indicated that *gsdf*$^{-/-}$ ovary had more stage I oocytes. The failure of early stage oocytes to enter later stages might be the cause of infertility in older female *gsdf*$^{-/-}$ zebrafish. Thus *gsdf* may function in oocyte maturation in the ovary. Taken together, both increasing growth of germ cells and higher expression of somatic genes were detected in presumptive female gonads at 8-14 dpf. These indicated that at this early stage, development of male and female gonads were already in a dimorphic manner rather than in an obligatory hermaphroditic pathway. Acknowledgement: Supported by National Health Research Institutes (NHRI- EX106-10506SI), Taiwan.

S7-5) Piferrer, Francesc (Spain)

THERMAL INFLUENCES ON FISH SEXUAL DEVELOPMENT

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Temperature is the main abiotic factor that affects multiple biological functions at different organization levels by changing the rates of physiological processes and chemical reactions. This is especially relevant for poikilothermic animals such as fish and of particular interest in processes like the sexual development. Sex in fish is very plastic since fish exhibit several types of reproduction, including gonochorism, various forms of hermaphroditism, and unisexuality. Further, sex determination can range from genetic sex determination (GSD) to environmental sex determination (ESD). Temperature-dependent sex determination (TSD) has been identified in several species but now is recognized that even species with GSD may have populations with sex ratio response to temperature which, under certain environmental conditions, can produce skewed sex ratios. Here, we used data from many species to show type of reproduction-related differences in thermal preferences and, using a cold water species (turbot, *Scophthalmus maximus*), a temperate water species (sea bass, *Dicentrarchus labrax*) and a tropical species (zebrafish, *Danio rerio*), we investigated common patterns of gene expression in response to heat. We focus not only in the analysis of genes and signaling pathways related to the endocrinology of testis and ovarian differentiation, but also consider genes related to the stress response and to epigenetic regulatory mechanisms. Further, we compared effects of temperature during early development at both the time of sex differentiation and in juveniles and adults. We describe the appearance of new phenotypes (neomales and pseudofemales) as a result of elevated temperature, suggest the existence of transgenerational transmission of epigenetic marks related to sexual development and discuss the possible consequences for natural populations in a global warming scenario. Acknowledgements: Supported by: MINECO AGL2013-41047-R and 2016-78710-R grants to FP. DA and AV were the recipients of MINECO FPI scholarships.

S7-6) Rafael, Nobrega (Brazil)

IDENTIFICATION OF PLURIPOTENCY GENES IN ZEBRAFISH TESTIS AND EFFECTS OF FSH AND GDNF ON SPERMATOGONIAL FATE FROM A SERTOLI CELL PESPECTIVE

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Stem cells are slow-dividing cells found in multicellular organisms with potential to self-renew or differentiate into a specialized cell type. In the testis, spermatogonial stem cells (SSCs) are the male germline stem cells supporting spermatogenesis and male fertility. SSCs reside within specific regions, the so-called spermatogonial niche, in which stem cell activity is regulated by interactions with the surrounding somatic cells. The aim of this study is to characterize pluripotency genes in zebrafish testis. Further evaluation of the Fsh (follicle stimulating hormone) and Gdnf (glial-derived neurotrophic factor) effects in the spermatogonial activity were carried out by morphometrical analysis, BrdU incorporation and qPCR using a tissue culture system. Expression analysis showed expression of *gfrα1b*, *geminin*, *pou5f1*, *nanog*, *sox2*, *tert* and *nanos3* in the zebrafish testis. *gfrα1b*, *pou5f1*, *nanog* and *nanos3* were differentially expressed in spermatogonia belonging to cysts of 1, 2 or 4 cells. Fsh and Gdnf increased the mitotic index and cyst proportion of type A undifferentiated spermatogonia (A_{und}). However only Gdnf increased type A differentiated spermatogonia (A_{diff}) with respect to proliferation and cyst proportion. Interestingly, Fsh and Gdnf stimulated Sertoli cell proliferation in association with BrdU-positive germ cells. Gene expression analysis showed elevated transcript levels of *pou5f1*, *nanog*, *nanos3*, *igf3* while *gdnfα* was down-regulated in the presence of Fsh. Gdnf did not change any pluripotency gene expression. Our results suggested that Fsh increased the number of A_{und} with *stemness* properties (self-renewal), and at the same time, new producing-Sertoli cells were generated to produce new cysts. On the other hand, Gdnf increased A_{und} without *stemness* and A_{diff} (differentiation). Since Gfrα1 is expressed in germ cells, Gdnf may act primarily in A_{und}/A_{diff}. By increasing the number of A_{und}/A_{diff}, it is suggested a germ cell paracrine regulation on Sertoli cell proliferation. Acknowledgements: FAPESP (14/07620-7)

Tuesday AM S8

EARLY LIFE ADVERSITY AND THE STRESS RESPONSE/STRESS COPING MECHANISMS
Chair: Nick Bernier, Patrick Prunet, Kathleen Gilmour

S8-1) MacDougall-Shackleton, Scott (Canada)

SEX-SPECIFIC EFFECTS OF DEVELOPMENTAL STRESS ON SONGBIRDS

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Developmental stressors are thought to have two kinds of effects. First, stressors may program development, preparing an animal for a future adverse environment. Second, stressors may impair development, resulting in a less competitive phenotype in adulthood. The costs and benefits of coping with developmental stressors, and the payoff of developmental programming, likely vary depending on life history traits, and may differ between the sexes. I will review a program of research examining the effects of developmental stress on development in three species of songbirds. Almost all effects of stress involve sex-specific components. In the free-living birds, birdsong variation is correlated to HPA axis regulation, suggesting that both are affected by developmental stressors. In captive experiments, developmental stressors (food restriction or corticosterone treatment) have a variety of sex-specific effects. In song sparrows early corticosterone treatment increased mass in males, but decreased mass and increased metabolic rate in females. Developmental food restriction in zebra finches also had sex-specific effects. Adult sex steroid regulation was also affected by developmental stressors. Stressors increased testosterone in male, but decreased estradiol in female sparrows. In starlings juvenile food restriction affected androgen levels in males, but not females. Developmental stressors affects adrenal sensitivity to ACTH in both male and female sparrows. Finally, developmental stressors also had sex-specific effects on immune function. These results indicate that the effects of developmental stressors likely depend on trade-offs that differ between males and females.

S8-2) Crespi, Erica (USA)

EFFECTS OF EARLY-LIFE ADVERSITY ON POST-METAMORPHIC PHYSIOLOGY AND BEHAVIOR IN AMPHIBIANS: PATTERNS AND MECHANISMS

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Early environmental effects on the development of the brain and neuroendocrine systems generates phenotypic variation in physiology and behavior, but much is not known about the specific mechanisms that drive this plasticity or the adaptive value of these effects. Studies conducted in amphibians have shown that experiencing adverse conditions during the larval stage (e.g., pond drying, high competition, predation threat or salinity, can affect post-metamorphic traits such as growth rate (i.e., catch-up growth), fat allocation, behavior, and activity of the hypothalamo-pituitary-interrenal axis, with evidence supporting the hypothesis that elevated glucocorticoids program such responses, at least in some contexts. In some contexts, adverse early conditions can elevate resting corticosterone levels after metamorphosis, and this is associated a reduction in negative feedback. In other contexts, resting corticosterone levels are not altered but the magnitude of the corticosterone response to stressors is blunted after experiencing adverse conditions during larval development. Findings from ACTH-response experiments suggest that a reduction in adrenal sensitivity or corticosteroid synthesis capacity is involved in producing this effect. However, post-metamorphic effects of experiencing adverse conditions during larval development vary within and between populations. Thus, factors such as social interactions or variation in nutritional state, as well as genetics, likely affect the long-term phenotypic outcomes of early life stress, and local adaption can shape these outcomes as well. Much work is needed to determine whether these post-metamorphic outcomes are adaptive, thereby enhancing the ability of animals to cope with future stressors predicted by conditions during the larval stage (i.e., matching hypothesis), or pathological (i.e., silver spoon hypothesis), which can depend on the post-metamorphic environment. It is becoming clear that adverse early environments can have transgenerational effects that may or may not be adaptive. Acknowledgements: Supported by NSF awards IOS-0818212 and BCS-1134687 to EJC.

S8-3) Brunton, Paula (UK)

PROGRAMMING OF THE STRESS AXIS AND BEHAVIOUR BY PRENATAL STRESS: MALADAPTIVE AND ADAPTIVE STRESS COPING RESPONSES

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Stress exposure during pregnancy can 'programme' the offspring's brain leading to profound alterations in neuroendocrine function and behaviour in later life. The phenomenon of 'fetal programming' is often considered detrimental to the offspring, increasing vulnerability to stress in later life. Indeed we have shown in both rats and pigs that repeated social stress exposure during pregnancy results in hyperactive hypothalamo-pituitary-adrenal (HPA) axis stress responses, heightened anxiety behaviour and abnormal social behaviours in the offspring in later life. Using a rat model of prenatal stress (PNS), I will present evidence supporting a role for altered steroid signalling in the brain in underpinning HPA axis dysregulation in PNS rats.
There is an alternate view that phenotypes programmed during fetal life are adaptive, preparing the offspring to cope in a sub-optimal post-natal environment. Hence, signalling to the foetuses *in utero* that they are to be born into a stressful environment induces adaptations to promote survival, which are beneficial in evolutionary terms. For example, heightened anxiety and stress reactivity could be expected to increase vigilance and risk aversion. I will present data demonstrating that when there is a 'match' between the predicted and actual post-natal environment (i.e. prenatal and adulthood stress), PNS rats cope better than controls, indicating an adaptive role for fetal programming. For example, social memory is impaired in PNS rats compared with controls when tested under non-stress conditions in adulthood. However, under stress conditions, both social and spatial memory are significantly impaired in adult control offspring, but facilitated in PNS rats, suggesting an adaptive role for fetal programming in aiding PNS offspring to cope with stressful situations in later life. Hence both control and PNS rats show cognitive deficits when there is a 'mis-match' between the prenatal and post-natal environment.
Thus, phenotypic adaptations triggered by environmental signals during prenatal development can be maladaptive and result in adverse effects on future health; however, these are likely to be more pronounced when there is a mis-match between the predicted (based on *in utero* signals) and the actual post-natal environment. [Funding: BBSRC and BSN, UK]

S8-4) Prunet, Patrick (France)

EARLY LIFE ENVIRONMENTAL STRESS MODIFIES PHYSIOLOGY AND ABILITY TO COPE WITH ACUTE STRESS IN JUVENILE RAINBOW TROUT.

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During the life cycle, early stages are the most variable and also sensitive to environmental stress. Recent studies have illustrated not only impact of chronic stress on fish larvae stages but also effects on performances during juvenile life, particularly on brain functions (Fokos et al., 2017; Vindas et al., 2016). These effects in juveniles can be interpreted as long term consequences of early stress exposure but also in term of programming to adaptively cope with this chronic stress. Such ability to change individual's phenotype is also observed in response to environmental cues in order to modify its fitness in a given harsh environment. In order to see whether such programming for change in environmental coping ability may be acquired during early life stages, we develop a study where rainbow trout at larvae stage were exposed to chronic low water quality condition (mostly hypoxia). Thus, one month after first feeding, trout larvae were exposed or not to these stressful conditions during 4 weeks and thereafter reared in normal water. Long-term effects of this early life chronic stress were analyzed in juveniles 4 months later. These juveniles were exposed or not to acute environmental stress, i.e. 24h in poor water conditions. Ability to cope with stressful environment was assessed in these different groups through study of growth and important physiological functions involved in welfare and health, i.e. related to stress reactivity (corticotrope axis activity) acclimation (gill functions including osmoregulation) and immunity. We analyzed various blood parameters (ions, cortisol, hematocrit, gene expressions) but also biochemical parameters and gene expressions (candidate genes

and/or microarray) in various tissues (brain, pituitary, gills, interrenal). Altogether, our data showed that early life environmental stress experience modifies later several acute responses to poor water quality stress as indicated by several blood parameters and also gene expression in various analyzed tissues. These results will be discussed in term of interrelated physiological functions possibly leading or not to more effective coping ability and compared with similar approaches developed recently in fish with other type of stressors. Acknowledgements: Supported by: INRA Métaprogrammes 'Integrated management of animal health'.

S8-5) Whitehouse, Lindy (Canada)

THE ONTOGENY OF THE HPI-AXIS AND ITS RESPONSE TO STRESS IN EMBRYONIC AND POST HATCH LAKE WHITEFISH (COREGONUS CLUPEAFORMIS).

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The activation of the hypothalamus-pituitary-interrenal (HPI) axis is a fundamental component of the stress response in teleost fishes. Embryos and post-hatch fry represent sensitive life stages, so understanding when the HPI-axis response develops can help elucidate when these early life stages are able to respond to environmental and other stressors. We assessed the ontogeny of the HPI axis in lake whitefish (*Coregonus clupeaformis*) (LWF) by measuring mRNA levels of key HPI genes and whole body cortisol levels following exposure to 9.5 mg/L dissolved oxygen (DO) (normoxia), 4.5 mg/L DO and 2 mg/L DO at 9 different time points, from 21 days post fertilization (dpf) to 4 weeks post hatch (wph). Initially we have targeted the following mRNA transcripts; corticotropin-releasing hormone (CRH), corticotropin-releasing hormone binding protein (CRHBP), cytochrome P-45011beta hydroxylase (CYP11beta) and phosphoenolpyruvate carboxykinase (PEPCK). Results indicate that cortisol levels appear to increase and decrease throughout the early life stages; periods of high whole body cortisol levels (21, 83, 103 dpf, and 3 and 4 wph) were separated by periods of low levels (38, 63 dpf and 1 and 2 wph). PEPCK mRNA levels were initially high at 21dpf before decreasing at 38dpf and remaining stable. mRNA levels of CRHBP are stable throughout embryogenesis, and gradually increase between 1 and 4 wph. The response to hypoxia exposure seemed variable with some stages (38 dpf and 1 wph) showing increases in whole body cortisol and PEPCK mRNA but most others showing no response. CRHBP mRNA levels are down-regulated at 38, 63 and 83 dpf in response to hypoxia. Data on CRH and CYP11beta mRNA levels will also be presented and discussed, in order to provide a complete picture on the development of the HPI axis in LWF. Understanding when the HPI-axis matures and when it can be activated by stress will enable us to decipher which developmental stages are most at risk from changes in the external environment.

S8-6) Herron, Crystal (USA)

SPLENIC OXIDATIVE BURST ACTIVITY IN JUVENILE CHINOOK SALMON IS INCREASED AFTER FISH ARE EXPOSED TO A STRESSOR.

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Routine handling and processing procedures of fishes have been verified to be stressful through increases in circulating cortisol concentrations. Days after these same procedures, there are often large mortality events in the handled fish due to disease. We assessed juvenile Chinook salmon (*Oncorhynchu tshawytscha*) splenic leukocyte oxidative burst activity before, 3 and 20 h after fish had been subjected to a stressor. Within the battery of the innate immune system, oxidative burst is an effective defense measure against pathogens through the creation of reactive oxidative species (ROS). Flow cytometry was used to measure splenic ROS. In fish that had been stressed, oxidative burst was higher in splenic leukocytes after a 3 h stressor and was still elevated over unstressed fish 20 h after the end of the stressor. Physiologic stress was confirmed by the elevation of plasma cortisol. While it still not fully understood why fishes become sick after stressors, we hypothesize that an initial upregulation of the innate immune system in response to stress ultimately leaves the fishes more vulnerable to subsequent diseases due to an eventually exhausted immune system. Cortisol receptors on leukocytes are upregulated during stress in a time frame comparable to that affecting ROS; we are thus testing the hypothesis that elevated cortisol may play a key role in the enhancement of ROS.

Tuesday AM S9

RECENT PROGRESS IN CARTILAGINOUS FISH ENDOCRINOLOGY (NASCE PRESIDENT'S SYMPOSIUM)

Chair: Robert M. Dores and Akiyoshi Takahashi

S9-1) Takahashi, Akiyoshi (Japan)

MELANOCORTIN SYSTEMS OF STINGRAY, A CARTILAGINOUS FISH

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Melanocortin (MC) systems are composed of MC receptors (MCRs) and MC peptides, derived from proopiomelanocortin (POMC), such as adrenocorticotropic hormone (ACTH) and several molecular forms of melanocyte-stimulating hormone (MSH). POMC of cartilaginous fish (e.g., rays, sharks, and ratfishes) include four types of MSH, whereas that of bony fish includes two or three types of MSH. In the present study, we demonstrated the presence of functional MC systems in cartilaginous fish. Using red stingray, *Dasyatis akajei*, we identified five subtypes of MCR gene (*mc1r—mc5r*) as in the case of teleost and tetrapod species. To our knowledge, this is the first evidence of the presence of the full repertoire of MCRs in a single species of cartilaginous fish. Moreover, stingray peptides, synthesized on the basis of our previous studies, were used to evaluate pharmacological characteristics of MC peptides. Expression of respective stingray *mcr* cDNAs in Chinese hamster ovary cells revealed that Des-acetyl-α-MSH exhibited cAMP-producing activity, which was similar to or slightly greater than that of ACTH(1-24) on MCRs. One of the roles of MC system resides in regulation of the pituitary-interrenal (PI) axis, a homolog of tetrapod pituitary-adrenal axis, in which the major players are believed to be ACTH, MC2R, and melanocortin receptor-2 accessory protein 1 (MRAP1). In stingray, interrenal tissues were shown to express *mc2r* and *mc5r* as major MCR genes. Moreover, ACTH(1-24) stimulated the release of corticosteroid from dissected interrenal tissues *in vitro*. These results established the presence of functional PI axis in stingray at the molecular and functional levels. Notably, in contrast to other vertebrates, *mc2r* did not require coexpression with an *mrap1* cDNA for functional expression. So far, *mrap1* has not been identified in stingray. Thus, the fact that the sensitivity of MC5R to Des-acetyl-α-MSH and ACTH(1-24) was two times higher than that of MC2R without coexpression with MRAP1 suggested that MC5R could play a more important role than MC2R in transmitting signals conveyed by ACTH and MSHs.

S9-2) Hyodo, Susumu (Japan)

MOLECULAR AND FUNCTIONAL EVOLUTION OF NEUROHYPOPHYSIAL HORMONE SYSTEM: WITH SPECIAL REFERENCE TO A POSSIBLE FUNCTION OF NEWLY DISCOVERED V2B RECEPTOR IN CATSHARK.

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Vasopressin/vasotocin (VT) and oxytocin (OT) family peptides are secreted from the neurohypophysis and exert various actions including salt and water homeostasis and reproduction through distinct G protein-coupled receptors. From teleosts to mammals, four neurohypophysial hormone receptors (V1aR, V1bR, V2R, and OTR) had been identified, and we recently found and characterized a fifth neurohypophysial hormone receptor (V2bR) from the holocephalan elephant fish, *Callorhinchus milii*. This receptor is similar to conventional V2 receptor (V2aR) in sequence, but induced Ca^{2+} signaling in response to VT, which is typical signaling of V1-type receptors. On the other hand, we could not find conventional V2aR from the elephant fish. The same case was evidenced in the elasmobranch catshark, *Scyliorhinus torazame*; V1aR, V2bR and OTR have been identified. Although the timing of diversification of V2aR and V2bR still remains to be clarified, the cartilaginous fish V2bR, which uses Ca^{2+} as an intracellular second messenger, most likely resembles the ancestral V2aR/V2bR. In the catshark, V1aR mRNA was intensely expressed in the rostral pars distalis of pituitary, and colocalized with the mRNA signal of proopiomelanocortin, implying that VT controls the secretion of adrenocorticotropic hormone via V1aR. Meanwhile, intense expression of V2bR was observed in the posterior part of oviduct (PPO). *In vitro* administration of VT caused contraction of the PPO in a dose-dependent manner, while VT treatment had no contractile effect on the anterior part of oviduct, where V2bR is not expressed. Considering that the chicken V2bR, published as a VT1 receptor, is also expressed in the oviduct (Tan et al., 2000), our observation strongly suggests that V2bR contributes to egg laying process throughout vertebrates. In this talk, molecular and functional evolution of neurohypophysial hormone system will be discussed by focusing on the newly identified V2bR.

S9-3) Kobayashi, Yasuhisa (Japan)

TRANSITION FROM OOCYTES TO THE ESTROGEN-PRODUCING CELLS: ANALYSIS OF THEOVARY IN THE RED STINGRAY, *DASYATISAKAJEI.*

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Although various modes of reproductive strategies are documented in cartilaginous fishes, little is known of the reproductive mechanism in these fishes than in other vertebrate. Therefore, we used ovoviviparous red stingray, a common species in our Inland Sea, as an experimental model to investigatethe reproduction, sexual differentiation, role of uterus function and so on. Here the goal was to studythe unclearovarian structure andfolliculogenesis in red stingray. The ovary of adult stingray is single and directly invested in the left epigonal organ. Histological observation revealed that oogonia and previtellogenic oocytes lied in the cortex of ovary. After germinal vesicle breakdown, oocytes migrate into the medulla of ovary to incorporate yolk protein (oocyte diameter > 2.5 mm). Interestingly, the medulla contained few follicles and a large number of corpora lutea-like follicles (CLF), although these round-shaped exterior features appeared to be identical. Follicles were simple yolk-filled oocytes covered by three cell layers (theca, granulosa and follicular epithelium). In CLF, however, the follicular epithelium cell layer elongatedandelaborately invaginatedinto the interior of oocytes. To examine the unknown function of CLF, the expression of steroidogenic enzymes (scc: cholesterol side-chain cleavage, arom: P450 aromatase) were analyzed. The mRNA expressions of both enzymes

were significantly higher in CLF than in other cells. The immunohistochemical observation revealed that localization of scc and arom were inner follicular epithelium cells and granulosa cells, respectively. These results indicate that the stingray CLF was involved in estrogen production. Taken together with the results of our other studies, we presume that physiological/endocrinological systems of cartilaginous fishes areevolving in a unique way but partially reflecting those appeared in teleostsandtetrapods.Acknowledgements: Supported byKyousei Science for Life and Nature, Nara Women's University, through General Collaboration (Project No. 2016-02) to YK.

S9-4) Wheaton, Catharine (USA)

CHALLENGES, PITFALLS AND SURPRISES: MEASURING STRESS, REPRODUCTIVE AND THYROID HORMONES IN ELASMOBRANCHS - Challenges, pitfalls and surprises: measuring stress, reproductive and thyroid hormones in elasmobranchs

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Sharks and rays are popular species used in wildlife ecotourism and exhibits in aquariums to educate the public on the behavior, ecology and conservation challenges of elasmobranchs. To best understand long-term reproductive and physiological health and welfare under varying social and husbandry conditions, we developed and validated in-house and commercial enzyme immunoassays (EIA) to measure stress/ionoregulatory, metabolic, and reproductive hormones in managed and semi-wild male and female southern rays (*Dasyatis americana*). Banked samples (serum, plasma, body fluids, organs and other tissues) from approximately 30 male and female rays managed at Disney's The Seas with Nemo and Friends® and Castaway Cay were used in laboratory and biological validations of sample treatment, processing and extraction protocols to optimize measurement of 1α-hydroxycorticosterone (1αOH-B)), estradiol, progesterone, testosterone, triiodothyronine (T3), and thyroxine (T4) hormones. To improve measurement of 1αOH-B, we developed a monoclonal antibody using a synthesized 1αOH-B derivative for testing in a double-antibody EIA system. Reproductive steroids were measured using in-house EIAs, and thyroid hormones were measured using commercially available T3 and T4 EIA kits. Several experiments utilizing various sample treatments, solvent partitioning and EIA manipulations were undertaken to overcome challenges in extraction and EIA validation. Results were used to guide best practices for steroid measurements in *D. americana,* and may prove useful for extrapolation to other elasmobranch species.
Improved measurement of stress, reproductive and metabolic hormones in sharks and rays will be important for many aspects of collection, transport, medical treatment, and aquaria and conservation management of these charismatic and ecologically important species.

S9-5) Nozu, Ryo (Japan)

SEASONAL CHANGES IN SEX STEROID HORMONES AND FOLLICLE SIZE IN THE ZEBRA SHARK, *Stegostoma fasciatum*.

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The zebra shark, *Stegostoma fasciatum*, is listed in the IUCN Red List as "Endangered." This species is kept in many aquariums, where its reproduction in captivity is undertaken. In order to achieve efficient reproduction, it is essential to acquire fundamental information on their reproductive endocrinology. In this study, we investigated the association between annual variations in sex steroid hormones and follicle size in mature females kept in the Okinawa Churaumi Aquarium. In order to monitor sex steroid hormones and follicle size, we collected blood samples and took ultrasonographic images of the ovaries and uteri of individuals once a month. The ultrasonographic images revealed that follicle size increased from November to January and maximum sizes were maintained until June. Subsequently, follicles rapidly regressed to a minimum size in July when the average water temperature was 28°C. This result might indicate that the water temperature of 28°C was a key factor in terminating the reproductive season. An enzyme-linked immunosorbent assay (ELISA) revealed that plasma estradiol 17-ß (E2) concentrations exhibited definitive seasonal changes, which were negatively correlated with the monthly average water temperatures. The rising trend in E2 concentrations started prior to the increases in follicle size. The E2 dynamics enable us to predict the development of follicles. Additionally, testosterone (T) reached high levels after the follicles attained their maximum size, and then dropped to a basal level in July. The duration for which T was maintained at high levels coincided with the reproductive season. From these results, we can conclude that sex steroid hormones could be indicators of the reproductive status of zebra sharks in captivity. These indicators would be useful in selecting individuals suitable for reproduction in captivity.
Acknowledgements: Supported by: JSPS KAKENHI Grant Number 16K21717 to RN

S9-6) Davis, Perry (USA)

HPI AXIS OF THE ELEPHANT SHARK: DETECTION OF AN MRAP1 ORTHOLOG AND THE PHARMACOLOGICAL INTERACTIONS OF THIS ACCESSORY PROTEIN WITH ELEPHANT SHARK, MC2R AND MC5R.

Perry Davis (1), Megan Deyarmond (1), Michael R. Dores (2), Ayuke Iki (3), Susumu Hyodo (3), and Robert M. Dores (1).
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A striking feature of the mammalian HPA axis is the obligatory interaction between the melanocortin-2 receptor (MC2R) and the accessory protein, MRAP1. This interaction facilitates trafficking of the receptor from the ER to the PM of adrenal cortex cells, and allows for the exclusive activation of MC2R by ACTH, but not by any MSH-sized ligand. While the same obligatory interaction between MC2R and MRAP1 is observed for other bony vertebrates (i.e., bony fishes, amphibians, reptiles, and birds), the same cannot be said for cartilaginous fishes. Recent studies on MC2R orthologs from the elephant shark (es) and the Japanese stingray showed that these receptors could be functionally expressed in CHO cells in the absence of MRAP1, and activated by either ACTH or various cartilaginous fish MSH-related

peptides. These observations led to the assumption that the *mrap1* gene evolved after the divergence of the ancestral cartilaginous fishes and bony fishes. However, the recent discovery of an MRAP1 ortholog in the genome of the elephant shark indicates that this gene evolved prior to the divergence of cartilaginous and bony fishes. To test whether esMRAP1 has an effect on either ligand sensitivity or ligand selectivity, *esmrap1* and *esmc2r* constructs were transiently transfected in CHO cells and stimulated with either cartilaginous fish ACTH (1-24) or ACTH(1-13)NH_2.In these experiments, co-expression with esMRAP1 increased sensitivity for ACTH(1-24) 10 fold, and sensitivity for ACTH(1-13)NH_2 4 fold. rtPCR analysis indicated that *mc2r*, *mrap1*, as well as *mc3r*, and *mc5r* mRNAs are present in the interrenal gland, the glucocorticoid-producing tissue of the elephant shark. In this regard, co-expression of *esmc5r* and *esmrap1* in CHO cells resulted in a 100 fold increase in sensitivity for ACTH(1-24). Immunocytochemical analysis indicated that esMC2R and esMRAP1 are co-localized on the surface of the CHO cells, and Cell Surface ELISA analysis indicates that esMRAP1 does not effect the trafficking of esMC2R. Alanine substitutions in the N-terminal domain esMRAP1 followed by co-expression of the mutant esMRAP1 with wild type esMC2R revealed the importance of residues E^{38} and Y^{39} for activation. These residues are located in a site analogous to the activation motif for mouse MRAP1. Collectively these results indicate that the interaction between MRAP1 and MC2R arose early in the evolution of the jawed vertebrates. In addition, multiple melanocortin receptors are expressed in glucocorticoid cells of the elephant shark, and regulation of glucocorticoid release could be mediated by separate hypothalamus/anterior pituitary, and hypothalamus/intermediate pituitary axes. Finally studies on cartilaginous fish MC2R and MRAP1 orthologs provide a reference point for understanding the unique co-evolution of this receptor and this accessory protein in modern bony fishes and tetrapods. Funding was provided by the Long Endowment (RMD).

Wednesday AM S10

ISAREN: ENVIRONMENTAL AND GENETIC INFLUENCES ON AMPHIBIAN AND REPTILIAN ENDOCRINE SYSTEMS

Chair: Caren Helbing and Werner Kloas

S10-1) Clulow, John (Australia)

BOYS ARE EASY, GIRLS ARE HARD – OBTAINING GAMETES FROM AUSTRALIAN TEMPERATE FROGS BY HORMONAL INDUCTION.

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Reproduction in most Australian frogs has adapted to seasonally unpredictable rainfall and mild winters. Spawning in many temperate species occurs over extended periods during spring/summer/autumn (summer breeders) and autumn/winter/spring (winter breeders). Reproductively mature animals may carry gametes for extended periods, and only release these in spawning events when appropriate environmental cues (e.g., rain) occur. This poses significant challenges for induced spawning, or the collection of gametes for IVF and cryopreservation for numerous threatened and endangered species. We have investigated the collection of gametes from hylid tree frogs, which represent ~50% of all Australian species. Induction of gamete release is more readily achieved from males than females. Four species (*Litoria caerulea*, *L. fallax, L. chloris, L. aurea*) readily release sperm into urine over 6-24 hours following hCG administration (5-10 IU/g). For female *L. fallax*, injection of GnRH-A (0.1-4 µg/g) alone or in combination with the dopamine receptor antagonist metoclopramide (10 µg/g) did not induce ovulation in mature pre-spawning females collected near calling males. Similarly, hCG (3-10 IU/g) did not induce ovulation, whether administered in single or multiple doses, or in combination with GnRH-A. Ovulation has only been achieved in gravid female *L. fallax* with injection of pituitary gland extracts from various hylid species. Gaining control over spermiation and ovulation are critical goals in reproductive endocrinology for assisted reproductive technologies. While hCG protocols are effective for males, we anticipate there is much research required to regulate female ovulatory processes. Currently, there is no evidence of fundamental differences in the endocrine regulation of reproduction in Australian species, but this remains to be rigorously investigated. Acknowledgements. Supported by: U of Newcastle, ARC Australia; NSERC Canada and U of Ottawa grants to VLT.

S10-2) Woodley, Sarah (USA)

TESTING HYPOTHESES ABOUT INDIVIDUAL VARIATION IN PLASMA CORTICOSTERONE IN FREE-LIVING SALAMANDERS

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Plasma glucocorticoid hormones (GCs) mediate many responses to stress as well as homeostatic maintenance of basal function. Interestingly, both baseline and stress-induced levels of GCs are typically variable among individuals. We examined the contribution of several

physiological factors to individual variation in plasma corticosterone (CORT) and the number of corticotropin-releasing hormone (CRH) neurons in the magnocellular preoptic area of the brain in free-living Allegheny Mountain dusky salamanders. We addressed three hypotheses: the current-condition hypothesis, the facilitation hypothesis, and the trade-off hypothesis. Differential white blood cell counts were identified as strong contributors to individual variation in baseline CORT, stress-induced CORT, and the number of CRH neurons. In contrast, we found no relationship between corticosterone (or CRH) and body condition, energy stores, or reproductive investment, providing no support for the current-condition hypothesis or the trade-off hypothesis involving reproduction. Due to the difficulties of interpreting the functional consequences of differences in white blood cell differentials, we were unable to distinguish between the facilitation hypothesis or the trade-off hypothesis related to immune function. However, the strong association between white blood cell differentials and GC and CRH suggests that a more thorough examination of immune profiles is critical to understanding individual variation in hypothalamic-pituitary adrenal/interrenal activation.

S10-3) Gabor, Caitlin (USA)

THE ROLE ANTHROPOGENIC STRESSORS PLAY IN MEDIATING STRESS AND DISEASE IN AMPHIBIANS

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Adverse effects of anthropogenic changes on biodiversity might be mediated by their impacts on glucocorticoid stress hormones in amphibians. To test this hypothesis we crossed exposure to metyrapone, a inhibitor of the stress hormone corticosterone, with exposure to the herbicide atrazine and the fungal pathogen Batrachochytrium dendrobatidis (Bd) to assess whether the effects of these stressors on tadpoles and postmetamorphic frogs were mediated by corticosterone. Metyrapone countered atrazine- and Bd-induced corticosterone elevations. However, atrazine- and Bd-induced reductions in body size were not mediated by corticosterone because they persisted despite metyrapone exposure. Atrazine lowered Bd abundance without metyrapone but increased Bd abundance with metyrapone for tadpoles and frogs. In contrast, atrazine reduced tolerance of Bd infections because frogs exposed to atrazine as tadpoles had reduced growth with Bd compared to solvent controls; this effect was not countered by metyrapone. Our results suggest that the adverse effects of atrazine and Bd on amphibians are not mediated primarily by corticosterone. Instead, these effects are likely a function of energy lost from atrazine detoxification, defense against Bd, or repair from damage caused by atrazine and Bd. We discuss additional lines of research for further understanding the role of anthropogenic stressors on amphibian declines. Supported by: C.G. was funded by a REP grant from Texas State University. J.R. was funded by the National Science Foundation

S10-4) Miura, Ikuo (Japan)

EVOLUTIONARY CHANGE OF GONADAL SEX-REVERSAL SENSITIVITY TO SEX STEROIDS AND ITS RELATION TO TURNOVER OF SEX CHROMOSOMES IN A FROG

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Sex steroids can reverse phenotypic sex of gonad in vertebrates, and its sensitivity is varied widely among species. However, evolutionary reasons for the sensitivity variation are unclear. The Japanese frog *Glandirana rugosa* is unique in the sex determination and sex chromosome differentiation. Five geographic groups are identified to date. The West-Japan and East-Japan groups are ancestral forms with homomorphic sex chromosomes under male heterogametic sex determination, while the other three groups of XY, ZW and Neo-ZW are derived forms with XY and ZW heteromorphic sex chromosomes, respectively. The sex chromosomes of the latter three groups are homologous to each other (chromosomes 7 out of 13 haploid set), while the sex chromosomes in the former two ancestral groups are not identified yet (not linked with chromosomes 7). In this study, we examined sex reversal with sex steroids in the four groups (except Neo-ZW), and found that the sensitivity was different among the groups: it is sensitive in the ancestral two groups while is resistant in the derived two groups. It is evident that the sex reversal sensitivity to sex steroids has been changed from sensitive to resistant during its evolution. Based on our previous studies, hybridization between the two ancestral groups evolved the two heteromorphic XY and ZW sex chromosome groups through sex ratio bias followed by turnover of the sex chromosomes. Focused on sexually dimorphic expression of sex differentiation related genes, the onset timing during development was shifted to earlier stages in the derived forms, and together the window period for sex reversal was shortened or lost. One of the genes examined and showing the earliest expression was *SOX3*, which was strongly related to ovary differentiation because its knockdown induced testis differentiation. Thus, we conclude that hybridization between genetically distinct populations was a trigger for the change of gonadal sex reversal sensitivity: sex ratio was skewed and the following turnover of sex chromosomes (sex determining gene) shifted the sex determining timing to earlier stages, and which shortened or lost the original window period for sex reversal.

S10-5) Orton, Frances (UK)

REPRODUCTIVE BIOLOGY IN *XENOPUS TROPICALIS*

Frances Orton*[1], Moa Säfholm[2], Erika Janson[2], Ylva Carlsson[2], Andreas Eriksson[2], Jerker Fick[3], Verbruggen B[4], Economou T[4], Uren-Webster, T[5], Cecilia Berg[2] and Charles R. Tyler[4].
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Worldwide, many amphibian populations are declining at rapid rates. There is evidence that chemicals may be contributing to some of these declines including via effects on reproduction. Chemical effects on frogs in the wild are hard to quantify and thus most chemical effects studied have been conducted using a laboratory maintained 'model 'species, such as *Xenopus tropicalis.* Despite this, information on many aspects of the basic reproductive biology of *X. tropicalis* are poorly understood. We investigated interrelationships between molecular, morphological and behavioural endpoints in non-exposed organisms (tadpoles, metamorphs and adults) associated with reproductive success. We further investigated the effects of larval exposure to anti-androgenic chemicals (flutamide (181 nM) or linuron (32 or 181 nM)) on these life stages, including impacts on reproductive development and output. Endpoints analysed included: gene expression (*dmrt1, cyp17, amh, ar, cyp19, foxl2*) in tadpole gonad/brain, metamorph brain and adult brain/gonad/arm; gonadal histomorphology in tadpoles, metamorphs and adults; morphology and histomorphology of secondary sexual characteristics (nuptial pad, forelimb) and hormone profiles (plasma testosterone/corticosterone) in adult males; breeding behaviour and reproductive outcomes in adult males and females. The data we will present are likely to significantly contribute to a knowledge gap on the reproductive biology in *X. tropicalis* and effects of chemicals on some of the most important developmental features relating to reproduction

S10-6) Koide, Emily (Canada)

VISUALIZING THE EFFECTS OF THYROID HORMONE: IDENTIFICATION OF AFFECTED METABOLITES USING MATRIX-ASSISTED LASER DESORPTION/IONIZATION-MASS SPECTROMETRY IMAGING (MALDI-MSI) IN BULLFROG TADPOLES.

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The drastic morphological changes that occur in frog metamorphosis are one of the most dramatic demonstrations of thyroid hormone (TH) importance in vertebrate development. TH acts genomically to initiate the massive remodelling of a tadpole into a frog. Due to TH's mode of action, most research has focused on the genomics of metamorphosis. Few studies, however, delve into the resulting molecular phenotypic, or metabolomic, changes that occur. Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is a powerful technique that allows for accurate metabolomic data including spatial distribution. Reconstructed ion maps can be compared to morphology to determine anatomical features for each tadpole. In this study, premetamorphic bullfrog tadpoles were exposed to exogenous TH and then analyzed by (+/-)MALDI-MSI to study how and where metabolomic profiles change in tadpoles induced to undergo metamorphosis. An average of 1000 metabolites were detected for each tadpole in both positive and negative ionization mode. Over 250 of these metabolites were significantly different (α=0.05) between TH treated and control tadpoles. Multivariate analysis of principal component analysis paired with hierarchal clustering was used to create unbiased sections which could be related back to important anatomical features including the eye, brain, tail muscle, lung, liver and notochord. The separation of organ-related spectra allowed for analysis of how metabolomic changes occur within individual organs. Approximately 100 metabolites were significantly altered (α=0.05) in each organ in response to TH exposure. Many of these were found in multiple organs. Further analysis is currently being performed to identify the metabolites detected by MALDI-MSI. The results from this experiment provide fundamental insights into the metabolomic changes caused by TH action, both in individual organs and throughout the whole tadpole, indicating critical pathways that are affected in the initiation of this life stage transformation. To our knowledge, this is the first MALDI-MSI analysis on whole tadpoles, paving the way for potential methods to study the metabolomic results of endocrine disrupting compounds that disrupt TH action. Acknowledgements: Supported by: NSERC Canada operating grants to CCH.

Wednesday AM S11

OLD QUESTIONS, NEW TECHNOLOGICAL APPROACHES IN THYROID HORMONE SIGNALING AND FUNCTION
Chair: Aurea Orozco and Deborah Powers

S11-1) Power, Deborah (Portugal)

FLATFISH EVOLUTION: TILTING THE BALANCE OF THYROID AND RETINOIC ACID SYSTEM SIGNALLING

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The flatfishes are unique among the teleosts as they are asymmetrical and this is most obvious in relation to their pigmentation and the fact they bear both eyes on the same side of the head. The transformation or metamorphosis to a flat, benthic and asymmetric fish occurs in the larvae that initially start life as a symmetric, pelagic animal. The importance of the thyroid hormones in modulating the transformations occurring during metamorphosis was first reported in the Japanese founder (*Paralichthys olivaceus*, Inui & Miwa, 1985) but is now known to apply to all other flatfish studied to date. The means by which the thyroid axis provokes asymmetry has still not been resolved but the use of "*omics*" technologies is starting to unravel this question. Whole genome sequencing and transcriptome analysis of several flatfish together with experimentation has generated a unique resource with which to dissect out the mechanisms by which asymmetry occurs in flatfish. Comparative genome analysis has revealed gene family expansion and contraction that is unique to the flatfish and also positively selected genes involved in the physiological and morphological changes accompanying flatfish metamorphosis. Differential gene expression (DEG) analysis established by comparison of different stages during metamorphosis in the Japanese flounder and Chinese tongue sole (*Cynoglossus semilaevis*) revealed 2,307 orthologs of which 146 genes had common expression patterns in the two species. The enriched genes with a consistent expression pattern during metamorphosis in the two flatfish included members of the TH and retinoic acid (RA) signaling pathway but also the phototransduction pathway. The recent identification of asymmetric expression in the Atlantic halibut of elements of TH signaling raises questions in relation to the factors underpinning signaling pathway asymmetry. We hypothesis that light plays a role in generating a RA gradient that through receptor heterodimerization (*Trb* and *Rar*) cross-talks with the TH system and establishes the basis for asymmetry creation. Acknowledgements: Supported by EU-FP7 (LIFECYCLE No. 222719), FCT (Portugal, CCMAR/Multi/04326/2013) and National Natural Science Foundation and the State 863 High Technology R&D Projects of China.

S11-2) Buchholz, Daniel (USA)

INSIGHTS INTO THYROID HORMONE RECEPTOR FUNCTION FROM GENE KNOCKOUT STUDIES

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Thyroid hormone (TH) and TH receptor (TR) interaction is critical for normal development in all vertebrates. Two genes encode TRs, TRα and TRβ, each with distinct expression patterns during development. How these two genes work together or independently to orchestrate developmental changes induced by TH throughout the body is not well understood. Here, we use TRα knockout (TRαKO) tadpoles to examine the impact of TRα on growth, development, and intestinal remodeling during frog metamorphosis. Surprisingly, TRαKO tadpoles developed faster throughout premetamorphosis when TH is low or absent, and despite their decreased responsivity to exogenous TH, TRαKO tadpoles not only were able to complete TH-dependent metamorphosis but also did so earlier than WT tadpoles. Disrupted TRα had little effect on growth during the larval period, but after metamorphosis TRαKO juveniles grew more slowly than wild-type (WT) juveniles. In contrast to external morphology, larval epithelial cell apoptosis and adult cell proliferation of intestinal remodeling were delayed in TRαKO tadpoles. Also, TRαKO intestines did not shrink in length to the full extent, and fewer intestinal folds into the lumen were present in TRαKO compared to WT juveniles. Such delayed remodeling occurred despite higher premetamorphic expression levels of TH target genes important for metamorphic progression, namely TRβ, Krüppel-like factor 9, and stromelysin 3. Furthermore, the decreased TH-dependent intestinal shrinkage was consistent with reduced TH-response gene expression during natural and TH-induced metamorphosis. As in the TRα null mouse model, TRαKO frogs had significant but surprisingly mild growth and development phenotypes with normal survival and fertility.

S11-3) Orozco, Aurea (Mexico)

IN THYROID HORMONE-MEDIATED GENE TRANSCRIPTION, 3,5-T2 HAS A SAYING.

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3,5-T2 (T2) is an alternative ligand for the thyroid hormone receptor β1 (TRβ1), at least in teleosts. Indeed, binding and transactivation studies showed that T2 is as bioactive as T3 and its effects are mediated by a long (L-)TRβ1 isoform that contains a 9-amino-acid insert in its ligand-binding domain (LDB), whereas T3 binds to and activates preferentially the short (S-)TRβ1 isoform that lacks this insert. In concert, T3 and T2 differentially regulate the expression of S- and L-TRβ1, respectively, and expression of L-TRβ1 is 10^6-fold higher than that of S-TRβ1, reflecting the functional relevance of this signaling pathway. Structure-function studies showed that the N-terminus of TRβ1 is only essential for T2-mediated transactivation, suggesting a functional interaction between the N-terminus and the insertion in the LDB that results in a specific TRβ1 conformation when ligated to T2 or to T3, exposing different interaction coregulator surfaces. Indeed, experimental evidence suggests that the complex L-TRβ1+T2 promotes the recruitment of a coregulator population different from that recruited by S-TRβ1+T3. Yeast 2-hybrid screening identified Jab1 as a binding partner of L-TRβ1+T2 and pull-down assays and transactivation studies showed that Jab1 is a specific co-activator of L-TRβ1+T2 and a co-repressor of the S-TRβ1+T3, suggesting that TH divergent biological functions could be mediated by isoform- and ligand-specific TRβ1 partner protein interactions that result in opposite functional outcomes. These results raised the question of whether T3 and T2 differentially regulated gene expression. Transcriptome analysis showed that both, hepatic and cerebellar gene expression are differentially regulated by the two bioactive thyroid hormones. Furthermore, pathway enrichment analysis showed that T2 and T3 regulate genes involved in unique, tissue-specific processes, consequently suggesting divergent roles at least in cerebellum and liver thyroid hormone dependent homeostasis. Taken together, our results prompted a reevaluation of the role and mechanism of action of thyroid hormone metabolites previously believed to be inactive. Specifically, we propose that T2 acts as an alternative ligand playing a role in the tissue-specific action of receptors.
Supported by PAPIIT IN204517-2 and CONACYT 219833

S11-4) Olvera Vidal, Aurora Maria (Mexico)

DIFFERENTIAL TRANSCRIPTOME REGULATION IN TILAPIA *Orechromis niloticus* BRAIN AND LIVER BY 3,5-T2 AND 3',3,5-T3

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Thyroid hormones (THs) are endocrine messengers that exert a key role in a vast diversity of biological processes in vertebrate physiology. They act primarily regulating gene expression through their nuclear receptors (TRs). T3 is considered the primary bioactive TH because of its high affinity for TRs. However, results from our group in teleosts have shown that 3,5-T2 (T2) can also regulate gene expression as well as promote the recruitment of a different transcription factor population to the TR bound to the TH-response elements. In these vertebrates, the effects of T2 are mediated by a long (L-) TRβ1 isoform that contains a 9 amino acid insert in its ligand-binding domain. In contrast, the short (S-) TRβ1 lacks this insert and is only activated by T3. In concert, T3 and T2 differentially regulate the expression of S- and L-TRβ1, respectively *in vivo*. To determine the functional relevance of these unique receptor-mediated signalling pathways, RNA-seq analysis was conducted in cerebellum, thalamus-pituitary and liver of tilapia treated with equimolar doses of T2 or T3 (25nM per 12 h). We identified a total of 169, 154 and 2863 genes that were TH-regulated (FDR < 0.05) in tilapia cerebellum, thalamus-pituitary and liver, respectively. Among those, 130, 96 and 349 genes were uniquely regulated by T3, whereas 22, 40 and 929 were only regulated by T2 in the cerebellum, thalamus-pituitary and liver. Cluster analysis of gene response revealed that most gene populations are being regulated in the same direction by both hormones, but some responded with different intensity to each hormone. Interestingly a few genes presented opposite regulation by T2 and T3. Pathway enrichment analysis showed that T2 and T3 regulate genes involved in unique processes and this regulation is tissue-specific. These findings highlight the importance of thyroidal systems for vertebrate homeostasis and support the relevance of T2 action as an important transcriptional regulator. Acknowledgements: Supported by: CONACYT 219833, PAPIIT IN204517-2.

S11-5) Tamura, Kei (Japan)

THYROID HORMONE NEGATIVELY REGULATES MYOGENIC DIFFERENTIATION IN TADPOLE TAIL-DERIVED MYOBLAST CELLS IN *XENOPUS LAEVIS*

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Thyroid hormone (TH) is believed to induce metamorphosis of anuran amphibians. In addition, glucocorticoids (GCs) could encourage the TH-induced metamorphosis. We previously established a myoblastic cell line, XL-B4, from *Xenopus laevis* tadpole tail at a premetamorphic stage, and demonstrated that triiodothyronine (T_3) could not only induce apoptosis, but also attenuate Myogenin-driven myogenesis in the cells. In this study, to elucidate how T_3 could regulate myogenesis during metamorphosis, we focused on cell shape change induced by T_3 in XL-B4 cells. Generally, myogenic differentiation was accompanied with cell elongation. Interestingly, treatment with T_3, which can repress myogenic differentiation, enhanced cell spreading. In XL-B4 cells expressing a dominant negative form of TH receptor α, cell spreading rate was reduced by the T_3 treatment. Addition of hydrocortisone, one of GCs, with T_3 promoted the cell spreading compared to the case of only treatment with T_3. We also found that the T_3-treatment increased or decreased the amount of *myoD* or *myogenin* mRNA, respectively. Then, we investigated an effect by T_3 on overexpression of MyoD in XL-B4 cells, indicating that MyoD enhanced myogenesis with cell elongation in T_3-treated cells. Next, we examined whether small G proteins are involved in the morphological changes in XL-B4 cells. Overexpression of a dominant negative form of RhoA suppressed cell spreading observed in the T_3-treated cells. Moreover, inhibition of Rho-associated protein kinase (ROCK), an effector of RhoA, blocked cell spreading induced by T_3, and increased *myogenin* mRNA even in the presence of T_3. These results suggested that T_3 could negatively regulate myogenesis, which was mediated through RhoA-ROCK signaling in *Xenopus* metamorphosis. Acknowledgements: Supported by: Kitasato University Research Grant for Young Researchers (K.T.), and JSPS KAKENHI Grant.

S11-6) Subash, Peter (India)

UNDERSTANDING THE THYROID HORMONE-DRIVEN INTEGRATIVE AND DIFFERENTIAL NA+ SIGNALING IN FISH IONOCYTES

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Osmoregulatory epithelia of air-breathing fish possess varied ionocytes, which act as target for many hormones including thyroid hormones (THs). Thyroid hormones, besides having independent osmotic and ion regulatory actions, are also known for its interaction with cortisol, a prominent stress hormone that coordinates osmotic and ionic regulation in fishes particularly during stress acclimation. It is not certain whether THs as part of its integrative action demands any ion signaling in fish. We, therefore, focused on Na^+/K^+-ATPase (NKA), the key transporter that maintains Na^+ and K^+ gradients across plasma membrane. The direct action of THs on NKA functions was then tested on the ionocytes in gills, kidney and intestine of air-breathing fish kept either in stressed or non-stressed condition. Molecular analyses of nkaα isoforms (α1a, α1b and α1c) mRNA expression in these ionocytes utilizing qRT-PCR and *in situ* hybridization techniques showed differential regulation, indicating an integrator role of THs in Na^+ signaling. Immunocytochemical localization of nka protein in gill ionocytes further showed that THs could modify its distribution pattern in the gill epithelia of fish. Likewise, immunoblotting of nka protein abundance in the ionocytes also showed spatial and temporal responses to THs challenge. Collectively, evidence is presented that THs exert both transcriptomic and post-translational actions on Na transporter functions that drive its integrative and differential Na^+ signaling in varied fish ionocytes (supported by grants from DST project on Fish, UGC-SAP DRS II, Govt of Kerala and UoK).

Wednesday AM S12

INTEGRATING FACTORS OF APPETITE, ENERGY BALANCE AND GROWTH
Chair: Oliana Carnevalli, Suraj Unnappian, Encarnacion Capilla

sS12-1) Carnevali, Oliana (Italy)

EFFECTS OF GUT MICROBIOTA VARIATION ON HOST'S ENERGY BALANCE AND DEVELOPMENT IN FISH MODEL
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The gut microbiota has been identify as an environmental factor that may plays an important role in host's energy balance and development. We investigated the effects of probiotic *Lactobacillus rhamnosus* administration on gut microbiota composition and its ability to modulate lipid and glucose metabolism and appetite during the early stage of larval development, and in juveniles. Metagenomic results showed that *L. rhamnosus* administration shifted the overall gut microbiota composition and such variations induced transcriptional down-regulation of genes involved in cholesterol and triglycerides metabolism. Besides, the probiotic treated larvae showed lower total body cholesterol and triglyceride content and higher fatty acid level. *L. rhamnosus* also decreased lipid droplet size, increased microvilli and enterocyte lengths in the intestinal epithelium. Moreover, gut microbiota changes during early larval development, up-regulated *nucb2a, glp-1* and *insulin* gene transcription, whose hormonal product reduce glucose level. Besides, microbiota composition changing were associated with a downregulation of orexigenic genes and the upregulation of the anorexigenic ones. We also studied the effect of lipid content in the diet on the microbiome of adult zebrafish. Diets containing three different lipid levels (high [HFD], medium [MFD], and low [LFD]) were administered with or without the supplementation of *Lactobacillus rhamnosus* (P) to adult zebrafish in order to explore how dietary lipid content may influence the adult gut microbiota. Results indicated that the different fat percentage in the diets shifted the gut microbiota composition. The addition of *L. rhamnosus* in the diets, induced transcriptional reduction of orexigenic genes and upregulation of anorexigenic ones, in addition to a transcriptional decrease of genes involved in cholesterol and triglyceride (TAG) metabolism, concomitantly with lower cholesterol and TAG content. The probiotic attenuated weight gain in HFD-P and MFD-P fed zebrafish and enhanced nesfatin-1 peptide production in the gut. In conclusion, both probiotic and different dietary fat content were associated with distinct gut microbiota composition, which in turn modulated a set of genes involved in lipid and glucose metabolism and appetite control, thus attenuating obesity.
Acknowledgements: Supported by: OC was the recipient of PRIN 2010–2011 prot 2010W87LBJ, EU COST AQUAGAMETS 2012 FA12025. SU was the recipient of Natural Sciences and Engineering Research Council of Canada (NSERC). AH receiver post-doctoral fellowships from the CIHR and SHRF

S12-2) Sheridan, Mark (USA)

INTEGRATION OF FEEDING, GROWTH, AND METABOLISM: INSIGHTS FROM STUDIES IN FISH
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The flow of energy from the uptake of nutrients to its partitioning is tightly regulated. Fish, which have evolved elegant life history patterns to adjust to the diversity of aquatic ecosystems they inhabit, have provided important information about the regulation of energy allocation. In particular, it is now clear that the pattern of energy allocation is not fixed in an animal's life history and that patterns are strongly influenced by environmental factors such as temperature, photoperiod, and salinity. The coordination and integration of feeding, growth, and metabolism results from linkages between and among many hormonal systems. For example, the stimulation of GH secretion by ghrelin from the orexigenic center of the brain and from the nutrient-stimulated gut and subsequent nutrient- and GH-stimulated insulin (INS) secretion assures the assimilation of nutrients into cells for storage and growth. Feedback from insulin and leptin works in concert with the anorexigenic center to suppress feeding. Somatostatin (SS) meters anabolic actions by inhibiting INS as well as the GH-IGF system at many levels; SS also has direct catabolic actions by mobilizing stored energy reserves when food is not available. Lastly, periods of food deprivation also switch GH away from its growth-promoting role to a lipid catabolic role by alteration receptor-signal pathways linkages. Additional systems of integration will be discussed. Supported by NSF grant 1558037

S12-3) Lange, Angela (Canada)

ADIPOKINETC HORMONE AND INSULIN SIGNALLING PATHWAYS IN THE BLOOD-GORGING DISEASE VECTOR, *RHODNIUS PROLIXUS*

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The regulation of energy storage and mobilization is crucial for the functioning of any organism. In insects, neurohormones such as adipokinetic hormone (AKH) and insulin-like peptides (ILPs) are responsible for the mobilization and storage of energy obtained from the diet. We have isolated the cDNA sequences encoding AKH (Rhopr-AKH) and its receptor (Rhopr-AKHR) in the blood gorging disease vector *Rhodnius prolixus*. The Rhopr-AKH transcript is only expressed in neurosecretory cells (NSCs) of the corpus cardiacum whereas the Rhopr-AKHR transcript is highly expressed in the fat body, prothoracic glands/fat body, and reproductive tissues. Adult males that had been injected with double-stranded RNA (dsRNA) for Rhopr-AKHR exhibited increased lipid content in the fat body and decreased lipid levels in the haemolymph. Injection of Rhopr-AKH into Rhopr-AKHR dsRNA-treated males failed to elevate haemolymph lipid levels. Clearly Rhopr-AKH is used to elevate lipid content of the haemolymph, although we do not know its functions associated with reproductive tissue. We have also identified the first insulin like peptide (ILP) in *R. prolixus* (Rhopr-ILP). The transcript is expressed in NSCs in the brain, and reduced levels of Rhopr-ILP transcript result in increased carbohydrate and lipid levels in hemolymph and increased lipid content in fat body. Our results indicate that Rhopr-ILP is a modulator of lipid and carbohydrate metabolism, probably through signaling the presence of available energy and nutrients in the hemolymph. We have also identified an insulin-growth factor (Rhopr-IGF) and find its transcript is expressed in a variety of tissues, but mostly in the fat body, the dorsal vessel and CNS. Insects with reduced Rhopr-IGF transcript have elevated hemolymph lipid and carbohydrate levels, but no differences in fat body lipid or carbohydrate content. Insects with reduced transcript levels were followed through ecdysis, and phenotypically were shorter in body length, and had shorter and narrower wings. Our results indicate that Rhopr-IGF modulates growth in *R. prolixus* most likely through altering the usage of nutrients available in the haemolymph. Clearly, the ultimate control of nutrient balance will depend upon the interplay of a variety of signalling pathways. Acknowledgements: Supported by: NSERC Canada.

S12-4) Michel, Maximilian (USA)

MODELING OF ENERGY HOMEOSTASIS IN ZEBRAFISH

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Leptin is the primary adipostatic hormone in mammals, and leptin and its cognate receptor (LepR) are conserved across the vertebrate phylum and have been identified in a variety of fish and bird species. In mammals, it is released by adipose tissue and regulates food intake and energy expenditure in order to maintain homeostatic control of adiposity. Failure of leptin signaling in mammals results in extreme obesity but also infertility and diabetes. Interestingly, leptin expression in fish adipose tissue is low or undetectable while expression in the liver has been correlated with fasting rather than feeding in different species. We investigated the role of leptin in a zebrafish line mutant for LepR. We found no effects on larval, juvenile or adult growth parameters. Furthermore, neither body weight nor adiposity showed significant differences between genotypes. In order to aid with the discovery of adipostatic factors in teleosts, we have now developed a model of energy homeostasis in fish. Zebrafish fasted until they lost between 10-20% of their body weight in high density conditions show full compensatory growth within 5 days. In female fish, a large proportion of weight gain is due to oocyte mass, however in male fish we saw a two fold increase in whole body adipose mass. This data shows that homeostatic regulation of adipose mass is intact in zebrafish. We are currently looking into differentially regulated transcripts in adipose tissue using RNAseq and the role of the LepR mutation in this homeostatic regulation of adipose mass. Acknowledgements: This work was supported by USDA NIFA grant 2015-67015-23488 to RDC.

S12-5) Ukena, Kazuyoshi (Japan)

IDENTIFICATION AND BIOLOGICAL ACTION OF A NOVEL SMALL SECRETORY PROTEIN, NEUROSECRETORY PROTEIN GL, INTHECHICKENHYPOTHALAMUS

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To find novel neuropeptide and/or peptide hormone precursors in the avian brain, we performed a cDNA subtractive screen of the chicken hypothalamic infundibulum, which contains one of the feeding and neuroendocrine centers. After sequencing 596 clones, we identified a novel cDNA encoding a previously unknown protein. The deduced precursor protein consisted of 182 amino acid residues, including one putative small secretory protein of 80 amino acid residues. This small protein was flanked at the N-terminus by a signal peptide and at the C-terminus by a glycine amidation signal and a dibasic amino acid cleavage site. Because the predicted C-terminal amino acids of the small protein were Gly-Leu-NH_2, the small protein was named neurosecretory protein GL (NPGL). Quantitative RT-PCR analysis demonstrated specific expression of the NPGL precursor mRNA in the hypothalamic infundibulum. Furthermore, the mRNA levels in the hypothalamic infundibulum increased during post-hatching development. *In situ* hybridization and immunohistochemical analysis revealed that the cells containing the NPGL were localized in the medial mammillary nucleus and infundibular nucleus within the hypothalamic infundibulum. Subcutaneous infusion of NPGL in chicks increased body weight gain without affecting food intake. On the other hand, the chronic intracerebroventricular infusion of NPGL stimulated food and water intake, with a concomitant increase in body mass. Our findings indicate that NPGL may participate in the growth process and energy homeostasis in chicks. Acknowledgements: Supported by: MEXT/JSPS KAKENHI grants to KU and EI-U

S12-6) Habroun, Stacy (USA)

EFFECTS OF FOOD CONSUMPTION ON CELL PROLIFERATION IN THE BRAIN OF *PYTHON REGIUS*

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Neurogenesis is an important and vastly unexplored area in reptiles. While the ability to generate new brain cells in the adult mammalian brain is limited, reptiles are able to regenerate large populations of neuronal cells. What makes pythons a particularly interesting subject in this field is their characteristic specific dynamic action (SDA) response after food intake with an increase in metabolic rate in order to process the meal. Further, they exhibit impressive plasticity in their digestive and cardiovascular physiology due to the sheer magnitude of the increase in organ growth that occurs after a meal to allow digestion and to absorb and assimilate nutrients from it. While this systemic growth in response to food consumption is well documented, what is happening in the brain is currently unexplored. For this study, juvenile male ball pythons (*Python regius*) were used to test the hypothesis that postprandial neurogenesis is associated with food consumption. We used the thymidine analog 5-bromo-12'-deoxyuridine to quantify and compare cell proliferation in the brain of fasted snakes and at two and six days after a meal: during and after the SDA response, respectively. In all groups the retrobulbar and olfactory regions had the highest numbers of proliferating cells, consistent with other reptile species. Throughout the telencephalon, cell proliferation was significantly greater in the six-day group, with no difference between the two-day group and controls. Most postprandial systemic plasticity occurs within a day or two after a meal and decreases after digestion, however, the brain displays the opposite result, with a surge of cell proliferation after most of the digestion and absorption is complete. Our results support our hypothesis and indicate that food consumption does affect neurogenesis, increasing cell proliferation at specific time points after a meal.

Wednesday PM S13

ENVIRONMENTAL REGULATION OF REPRODUCTIVE PROCESSES

Chair: Vance Trudeau and Glen Van Der Kraak

S13-1) Meuti, Megan (USA)

THE CIRCADIAN CLOCK'S CONTROL OF OVERWINTERING DORMANCY AND SEASONAL DIFFERENCES IN MOSQUITO REPRODUCTIVE PHYSIOLOGY

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Females of the Northern house mosquito, *Culex pipiens*, are the major vectors of West Nile Virus and overwinter as adults in a reproductively dormant state known as diapause. Diapausing females fail to take a blood meal, and after mating in the first two weeks of their lives, store sperm and keep it viable until diapause is terminated approximately 6-9 months later. The hormonal and signaling pathways regulating diapause in females of *Cx. pipiens* are well-characterized: Juvenile Hormone (JH) is suppressed thereby preventing ovarian maturation, and the Forkhead Transcription Factor (FOXO) is upregulated, leading to fat hypertrophy and elevated stress responses. The short daylengths of late summer and early fall are the environmental cues that females of *Cx. pipiens* use to anticipate the coming winter. However, how daylength is measured and whether the circadian clock genes are involved is unknown. Additionally, no one has yet investigated whether male mosquitoes, which do not enter diapause, change the composition of their accessory gland proteins in response to short daylengths to inhibit female blood-feeding and promote sperm storage. Here we present work which demonstrates that the circadian clock is involved in the female diapause response, and that male mosquitoes of *Cx. pipiens* differentially regulate their accessory gland proteins under different photoperiods. Specifically, RNAi against negative circadian regulators (*period*, *timeless*, and *cryptochrome2*) caused short-day reared females to avert diapause while knocking down the clock-associated gene *pigment dispersing factor* caused long-day reared females to enter a diapause-like

state. Several genes that promote host-seeking, blood meal-processing and egg maturation are down-regulated in short-day vs. long-day reared males of *Cx. pipiens* while genes involved in protecting sperm and enhancing immune responses are upregulated in short-day males. Together, these results suggest that the circadian clock is involved in measuring daylength in females of *Cx. pipiens* and that short photoperiods induce changes in both male and female mosquito reproductive physiology.Acknowledgements: Supported by NSF Graduate Research Fellowship and Ohio State College of Food, Agriculture and Environmental Sciences SEEDS grant.

S13-2) Yamamoto, Yoji (Japan)

GENOTYPIC AND TEMPERATURE-DEPENDENT SEX DETERMINATION IN PEJEREY

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Sex determination in pejerrey*Odontesthesbonariensis* is characterized by strong temperature dependence (TSD). Each individual can be feminized or masculinized by exposure to low and high temperatures, respectively, during the first weeks after hatching. For this reason, genotypic determinants of sex have been considered as virtually inexistent in this species. However, recently we demonstratedthe existence in this species of anorthologue of its congener's(Patagonian pejerrey*O. hatcheri*) testis-determining gene *amhy* (Ychromosome-linked, anti-Müllerian hormone) and showed that at an intermediate temperature (25ºC), its presence (XY) or absence (XX) can favor the formation of males and females, respectively.We alsoexaminedthe transcriptional profiles of *amhy* and the autosomal *amha* at female-(FPT; 17ºC) and male-(MPT; 29ºC) promoting temperatures during early larval development to evaluate their relationship with TSD processes.Transcripts of *amhy* were highly expressed in all XY larvae at the beginning of sex determination period and then declined regardless of the temperature. The autosomal *amha*, in turn, was upregulated during the sex determination period in a few XY larvae at the FPT and in both genotypes at MPT, and was highly correlated with maleness. In addition, a luciferase reporter assay with the presumptive promoters of both *amh*paralogues was performed to investigate the regulation of these two genes by cortisol,which has been implicated in the temperature-induced masculinizationof pejerrey. Transcriptional activity analyses showed that the *amhy* promoter did not respond to any cortisol doses whereas the *amha* transcription increased with cortisol in a dose-dependent manner. Overall,the results suggest that *amhy* probably acts as a trigger of masculinization, although more or less dependent on water temperature, whereas the temperature-modulated*amha*seems to have a more direct role in testis formation in pejerrey. How *amhy* actually triggers masculinization and interacts with *amha*remain to be elucidated. This study also revealed cortisol signaling as an important transcriptional regulator of *amha* gene during the process of high temperature-induced masculinization. Acknowledgements:This work is supported by JSPS KAKENHI Grant Numbers JP15K18728 (YY) and JP26241018 (CAS).

S13-3) Greives, Timothy (USA)

SONGBIRDS AS A MODEL FOR UNCOVERING MECHANISMS REGULATING VARIATION IN SEASONAL REPRODUCTIVE TIMING IN THE WILD

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Outside of the tropics, reproduction is often limited to a short window of time that ensures favorable conditions for rearing offspring. Timing of reproduction in these seasonal environments influences individual variation in reproductive success. While annual cycles are predictable, within-species variation in seasonal timing of reproductive function is considerable both across and within populations. To address mechanisms underlying this variation in reproductive timing, we primarily study a free-living songbird, the dark-eyed juncos (*Junco hyemalis*). Across the junco range, resident and migrant individuals live in sympatry for ~6 months of the year where they experience identical photoperiod and other environmental cues but differ in timing of reproduction. Further, significant individual variation in female reproduction is observed within populations. In vertebrates, the hypothalamo-pituitary-gonadal (HPG) axis ultimately regulates seasonal reproductive physiology and timing. The specific nodes, particularly outside of the brain, that may influence variations in HPG axis activity and thus transitions to breeding condition remain poorly understood. Here we report data pointing to variation in pituitary responsiveness to continued upstream stimulation of the HPG axis (i.e. repeated injections with gonadotropin-releasing hormone) as a 'control point' modulating the activity of the HPG axis. Further, recent findings in juncos and an additional songbird species, the Great tit (*Parus major*), suggest that variation in endogenous daily rhythms may also significantly contribute to within population variation in seasonal reproductive timing (i.e. clutch initiation). Research aimed at uncovering the mechanistic basis of individual variation is necessary if we are to understand if, and how, selection shapes reproductive timing of free-living vertebrates. Acknowledgements: Supported by: US National Science Foundation grants to TJG and EDK.

S13-4) Martyniuk, Chris (USA)

METABOLIC PROFILING IN RADIAL GLIAL CELLS: A NOVEL APPROACH TO STUDY REGULATION BY ENDOCRINE DISRUPTORS AND SEX STEROIDS

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Radial glial cells (RGCs) in teleost fish produce aromatase B (AroB), the key enzyme that converts testosterone (T) into 17β-estradiol (E2). These cells are regulated by a number of neuropeptides and hormones and are potentially affected by environmental contaminants. The objectives of this study were to determine the effect of the environmental contaminant 17alpha-ethinylestradiol (EE2) on mitochondrial bioenergetics in a zebrafish primary radial glial cell culture. These cells have estrogen receptors and respond to estrogen feedback to modulate steroid production. Radial glial cells were passaged four times until cultures yielded > 95% purity. Cells were treated for 24 hours with EE2 in media (1, 10, and 100 nM). RGCs (n=5 replicates/group) were then seeded at 5.0x10^4 cells/well and assessed using an XFe24 Flux Analyzer. Mitochondrial bioenergetics were quantified by subtracting respiration rates at times before and after addition of electron transport chain inhibitors that included oligomycin, FCCP, and antimycin (i.e. mitochondrial stress test). EE2 did not significantly affect basal respiration of mitochondria, ATP production, proton leak, maximum respiratory capacity, spare capacity, or non-mitochondrial function. These preliminary data suggest that EE2 does not significantly affect the metabolic capacity of RGCs at the time point and doses examined. Additional experiments with hormones and endocrine disruptors are underway to determine if physiologically relevant treatments alter mitochondrial profiles. Here we optimize a new assay for investigating endocrine disruptors and hormones on mitochondrial bioenergetics for relevant cell types related to reproduction *in vitro*. Acknowledgements: Supported by the University of Florida (CJM), NSERC Discovery Grant (VLT), NSERC Graduate Student Award (DDF), and a NASCE Student Travel Award to X. Ling and D. Da Fonte.

S13-5) Norris, David (USA)

ARE INTERSEX FISHES IN NORTH AMERICAN RIVERS A RECENT PHENOMENON?

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We reported endocrine disruption in white sucker (*Catostomus commersoni*, WS) living downstream of municipal wastewater treatment facilities (WWTFs) in Colorado rivers and elsewhere as compared to upstream reference sites. Disruption of WS in Boulder Creek included intersex, skewed sex ratio toward females, feminization of juveniles and adult males (vitellogenin production), and lowered gonadosomatic indices in both sexes (Woodling et al., 2008; Vajda et al., 2008). Furthermore, treatment of male fathead minnows (*Pimephales promelas*, FHM) in reproductive condition with effluent from the WWTF diluted to reflect downstream levels was able to demasculinize and feminize FTMs within 7 days (Vajda et al., 2011). We histologically examined the gonads from WS collected between 1915 and 1974 from these same sites as well as FHMs collected between 1943 and 1955 that were maintained in the University of Colorado Museum of Natural History. We found no evidence of endocrine disruption in either species and conclude that the observed endocrine disruption we observed in recently is a relatively new phenomenon linked to estrogenic chemicals present in wastewater effluent. Additionally, we will report on a similar nationwide examination of endocrine disruption in museum specimens of large- and smallmouth bass (*Micropterus salmoides; M. dolomieui*).

S13-6) Maclatchy, Deborah (Canada)

RESPONSES TO ESTROGENIC ENDOCRINE DISRUPTORS AREVARIABLE IN COMMON MODELTELEOSTS

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Different reproductive responses have been observed across common model teleosts to the environmental estrogen, 17α-ethinylestradiol (EE_2), which suggest that species-specific mechanisms exist. These observations point to the need to proceed with caution when extrapolating across fish species or in the use of a single species as a surrogate in screening reproductive effects. In the freshwater zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) exposure of adults to low levels of EE_2 (less than 25ng/L) causes significant reductions in the numbers of eggs that are spawned. EE_2 exposure for three weeks at 10-fold higher concentrations, however, does not inhibit spawning in an estuarine killifish, the mummichog (*Fundulus heteroclitus*). Environmental salinity has been eliminated as a factor in uptake and effects of EE_2 in mummichog. EE_2 accumulates differentially in mummichog compared to other common model teleosts. Despite differences in spawning levels, EE_2 has been shown to consistently induce vitellogenin in males and cause abnormalities in gonadal differentiation and sex reversal in embryos of all three species. Gene expression endpoints including steroidogenic enzymes and luteinizing hormone receptor are negatively affected by EE_2 exposure in zebrafish and fathead minnow; in mummichog, analyses are pending to establish whether responsiveness at the molecular level differs, including at different stages of ovarian development. Understanding gonadal physiology and control of steroidogenesis in mummichog may be key to determining why responses at the level of egg production vary. Overall, there is a growing body of research showing that the reproductive effects of endocrine disruptors in mature fish is species-dependent and may be linked to differences in basic biology of the species. Acknowledgements: NSERC Discovery and Strategic grants to DM

Wednesday PM S14

ECO-EVO-DEVO: THE PHYSIOLOGY OF PHENOTYPIC VARIATION
Chair: Christen Mirth and Nadia Aubin-Horth

S14-1) Aubin-Horth, Nadia (Canada)

AN INTEGRATIVE APPROACH TO STUDY A BEHAVIOR-MODIFYING PARASITE AND ITS HOST
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The cestode *Schistocephalus solidus* is a model parasite with a complex life cycle that successively parasitizes a copepod, a fish – the threespine stickleback, *Gasterosteus aculeatus* – and a bird. Sticklebacks infected by this parasitic flatworm show large changes in phenotype, including a lack of the typical behavioural response to risky situations. These host changes occur specifically when the parasite is ready to move to its definitive bird host to reproduce. However, whether this drastic behaviour change is a by-product of facing a parasitic infection, a side effect of infection, or the result of a direct manipulation by the parasite is unknown. Furthermore, parasites with multiple hosts, such as *S. solidus,* undergo dramatic phenotypic transformations and endure major environmental shifts over the course of their life cycle, yet very little is known about how these are orchestrated at the molecular and physiological levels. We used this host-parasite pair as a model to study the mechanisms of behavioural modification by parasites in the host and of host transitions in the parasite. To understand the proximate mechanisms that generate these host behavioural changes, we took advantage of two approaches. We used phenotypic engineering to try to recreate the host behavioural modifications in healthy fish using pharmacological and physical manipulations. We combined this with transcriptomics to define a genomic signature of infection in the stickleback brain. In parallel, we examined the transcriptomic response of *S. solidus* over the course of its development in its stickleback and avian hosts and predicted *in silico* potential mimic proteins in the parasite genome that could disturb physiological pathways in the fish host. Our combined approach to uncover the causes and molecular consequences of behaviour modification by a parasite in a host and the molecular signature of host transitions in the same parasite will contribute to shed new light on parasite-host interactions. Acknowledgements: Supported by a FRQ-NT projet de recherche en équipe grant to NAH and CRL, NSERC Discovery grant to NAH, RAQ Travel fellowship to FOH and LG, University of Leicester funding to IB, a Vanier Canada Graduate Scholarship to FOH, a UK BBSRC MITBP fellowship to SG and a Explo' RA Sup de la région Rhône-Alpes fellowship to CSB.

S14-2) Mirth, Christen (Australia)

FROM PLASTICITY TO ROBUSTNESS:COORDINATING ORGAN SIZE AND PATTERN
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Changes in environmental conditions affect a wide range of developmental processes. This poses a particular challenge; to ensure robust development, animals need to coordinate the development of their organs to maintain correct form and function of the body's parts. However, while developmental processes like organ growth are sensitive to environmental conditions, others like patterning - the process that generates distinct cell identities - remain robust to perturbation. This is particularly surprising given that the same hormones that regulate organ growth also regulate their pattern. We are interested in the mechanisms that coordinate organ development with whole-body development across a range of environmental and physiological conditions. To do this, we use the fruit fly, *Drosophila melanogaster,* as a genetic system to uncover how environmental cues regulate larval physiology to control the growth and patterning of the developing wing. We find that the signalling pathways from at least two hormones, insulin-like peptides and the steroid moulting hormone ecdysone, jointly regulate growth. However, the contribution of each pathway to growth changes over time. Further, we find that although the final pattern of discs is robust against environmental and physiological perturbation, patterning rates are highly variable across conditions. This variability in patterning rates arises because patterning genes differ in their requirements for ecdysone signalling. This work provides a framework for understanding how hormones coordinate animal development in the face of changing environmental conditions. Acknowledgements: Supported by: FCT project grant (PTDC/BEX-BID/5340/2014) to CKM. MMO and TK were the recipients of an FCT studentship (SFRH/BD/51181/2010) and FCT fellowship (SFRH/BPD/74313/2010).

S14-3) Lavine, Laura (USA)

ENDOCRINE CONTROL OF CONDITION-DEPENDENT TRAITS.
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The most striking examples of condition-driven variation in phenotype can be found in the iconic weapons and ornaments typical of sexual selection. Examples include the prominent antlers of buck deer, the claws of fiddler crabs, the mandibles of stag beetles, and the horns of rhinoceros beetles. Sexually selected weapons take condition dependence to an extreme – these traits are more sensitive to stress, parasites, and access to nutrients than are other body parts. The heightened condition-sensitive growth characteristic of these structures is thought to make them unusually reliable, or "honest", if they are used by either rival males or choosy females as signals of individual quality. While the weapons of high condition males attain grossly exaggerated shapes and sizes, those of low condition males are either smaller or are not produced at all. As a result, weapon size is indicative of fighting ability, and these structures can serve as effective deterrents as well as implements of battle. Consequently, the exquisite condition-sensitivity of exaggerated animal weapon growth is integral to their function in the context of sexual selection, and this has generated intense interest into the developmental mechanisms of conditional expression. Endocrine signals are well known to provide mechanistic links between the environment and the expression of whole-organism traits. In insects, the most important of these signals include juvenile hormone (JH), ecdysteroid signaling, and insulin-like peptides and growth factors of the insulin signaling pathway (INS). In this study, we show that endocrine control of condition-dependent traits is lineage specific and that interactions between pathways are likely more important that previously recognized. Acknowledgements: Supported by: NSF IOS grants to LCL and DJE and USDA NIFA funds to LCL. HG was the recipient of a JSPS International Postdoctoral Fellowship.

S14-4) Shingleton, Alexander (USA)

BREATH CONTROL: THE SYSTEMIC REGULATION OF GROWTH IN RESPONSE TO HYPOXIA IN *DROSOPHILA*
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In almost all animals, low levels of oxygen (hypoxia) during development slows growth rate and reduces final body size. Despite the near ubiquity of this phenomenon, we have a very poor understanding of how the growth response to hypoxia is developmentally regulated. In particular, it is unclear whether the response is due to the cell-autonomous effects of low oxygen on the rate of cell metabolism and proliferation, or whether it is regulated through systemic physiological mechanisms. Here we provide evidence that the developmental response to hypoxia is regulated systemically in *Drosophila melanogaster*, via insulin- and ecdysone-signaling. We show that the effect of hypoxia on body and trait size phenocopies the effect of reduced insulin-signaling, with different traits showing different sensitivities to changes in oxygen level. We demonstrate that hypoxia suppresses systemic insulin-signaling, and at the same time elevates basal levels of circulating ecdysone and increases ecdysone-signaling. Previous studies have established ecdysone as a repressor of systemic insulin-signaling, suggesting that the negative effect of oxygen on growth may be mediated by elevated ecdysone. This hypothesis is supported by data that show that genetic-ablation of the prothoracic gland, which synthesizes ecdysone, rescues larval growth-rate in hypoxic conditions. Additional data suggest that oxygen affects ecdysone synthesis via HIF-signaling in the prothoracic gland. Collectively, these data indicate that hypoxic *Drosophila* larvae grow at rate slower than can be supported metabolically, through systemic mechanisms. More generally, we expect that, for any reliable environmental factor that affects growth rate, animals should evolve physiological mechanisms that allow growth to respond to environmental change in a controlled and coordinated manner. Further, we predict that selection acts upon genetic variation in these mechanisms to allow the adaptive evolution of the plastic response

S14-5) Renn, Suzy (USA)

MECHANISMS OF BEHAVIORAL PLASTICITY ON MULTIPLE TIMESCALES.
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Behavioral plasticity allows animals to adjust to environmental pressures on different timescales. Mechanistically, regulation of a behavioral plasticity is facilitated by changes in hormone levels or patterns of gene expression. These changes may happen on rapid timescales within the lifetime of the organism or on longer evolutionary timescales due to changes in the genetic code that lead to behavioral evolution. Here, I investigate behavioral plasticity on these different timescales by examining changes in gene expression associated with parental roles in African cichlid fishes. In the cichlid species *Julidochromis transcriptus*, males are generally larger, more aggressive, and more territorial than females which are submissive and provide the majority of the nest care. These conventional sex-biased parental roles can be reversed on a

fairly rapid timescale by changing intra-pair sexual size dimorphism to be female-biased. In a closely related species, *J. marlieri*, the sex-biased behavior has been reversed on an evolutionary timescale such that pairs form between larger more aggressive females and smaller more submissive males. Overall, we find that evolution has co-opted a portion of the gene expression profile such that there is high correlation between the plastic and the evolved switch from submissive to aggressive in females (and vice versa for males). Interestingly, while we do find species-specific aspects of the gene expression profile regardless of behavioral phenotype, we see little signature of sex. These data suggest that behavioral phenotype, rather than sex or species predominates brain gene expression. Interestingly, on the rapid timescale within species, there is a strong signature of plasticity such that regardless of sex or phenotype, males and females in reversed parental roles turn on or off similar sets of genes. This pattern reveals the cost of plasticity and likely highlights those genes that are necessary to allow an individual to exhibit behavioral plasticity. Acknowledgements: Supported by: NIH-NIGMS Award # R15GM080727 to SCPR

S14-6) Laslo, Mara (USA)

EXPRESSION OF THYROID HORMONE RECEPTORS AND DEIODINASES IN THE DIRECT-DEVELOPING FROG *ELEUTHERODACTYLUS COQUI*

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Direct development is a novel reproductive mode that has evolved independently in at least a dozen anuran lineages and other amphibians. Direct-developing frogs, including the Puerto Rican coquí, *Eleutherodactylus coqui*, hatch from terrestrial eggs as miniature adults. While their embryonic development resembles metamorphosis in several respects, characters develop in a different sequence compared to metamorphosing frogs. In metamorphosing frogs, for example, limb growth and tail resorption both occur following thyroid gland formation and well after hatching. In contrast, limbs in direct-developing frogs begin to form early in embryogenesis and well before the thyroid, suggesting their development is thyroid hormone (TH)-independent. Thus, changes in thyroid hormone provisioning, metabolism, or action may underlie the evolution of direct development. Specifically, maternally derived TH and changes in temporal or spatial expression of the nuclear thyroid receptor (TR) α, TRβ, deiodinase type II, or deiodinase type III in target tissues could facilitate tail resorption and early development of limbs. Embryonic expression of TRs in *E. coqui* suggest that tail resorption is mediated by TH. Similarly, TR expression dynamics in the limb approximate those in the developing limb of the metamorphosing frog *Xenopus laevis*, which is dependent on TH. Liquid-chromatography mass-spectrometry indicates that maternally derived TH is present in *E. coqui* at the onset of limb development. Moreover, TRs in the limb regulate transcription of T3-response genes. The embryonic limb of *E. coqui* is at least TH competent, and TH-mediated embryonic development may begin prior to thyroid gland formation in direct-developing frogs.

Wednesday PM S15

NEUROENDOCRINOLOGY OF INVERTEBRATE DEUTEROSTOMES - A CRUCIAL LINK BETWEEN PROTOSTOMES AND VERTEBRATES

Chair: Dan Larhammar and Maurice Elphick

S15-1) Lee, Leo (Macau)

EVOLUTION OF AMPHIOXUS PTH AND PACAP/GLUCAGON RECEPTOR FAMILY AND THEIR POTENTIAL ROLE IN REGULATING GH-LIKE GENE EXPRESSION

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Pituitary adenylyl cyclase activating polypeptide (PACAP), glucagon (GCG) and parathyroid hormone (PTH) are pleiotropic peptides and their receptors are belonging to the class B1 (secretin-like) G protein-coupled receptor (GPCR) superfamily. Receptors for these peptides carry out diverse physiological functions while their sequences and functions in invertebrates were unclear. Recently, our research group reported data confirming the presence of the invertebrate class B1 ligand-receptor pairs in amphioxus (*B.floridae*) and demonstrating that functional homologs of vertebrate PACAP/GCG and PTH receptors arose before the cephalochordate divergence from the ancestor of tunicates and vertebrates. Those receptors are clustered with PTH receptor (PTHR) and PACAP/glucagon receptors in phylogenetic analysis. cAMP assays showed that the putative amphioxus peptide ligands,bfPTH1 and bfPTH2, activated one of the amphioxus receptors (bf98C) whereas the other putative PACAP/GLUC homolog, bfPACAP/GLUCs, strongly interacted with bf95. By the gene expression pattern and functional studies in*B. belcheri*, we found that the amphioxus PACAP/GCG peptides may also act as the GH regulator in the homolog/precursor of the hypothalamus/pituitary (Hatschek's pit) in amphioxus. This function is similar to the class B1 GPCR's ligands in vertebrate, including PACAP. Hence, our data provided new evidence about the functional co-evolution of ligand and receptor of class B1 GPCR family from invertebrate to vertebrate.This project was supported by FDCT101/2015/A3, MYRG2016-00075-FHS to LTOL and HKU/CRF/11G to BKCC.

S15-2) Elphick, Maurice (UK)

THE EVOLUTION AND COMPARATIVE PHYSIOLOGY OF NEUROPEPTIDE SIGNALLING: INSIGHTS FROM ECHINODERMS

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Advances in genome/transcriptome sequencing have provided opportunities for investigation of neuropeptide signaling in a range of phyla. As deuterostomian invertebrates, echinoderms (e.g. sea urchins, starfish) occupy an "intermediate" phylogenetic position with respect to vertebrates and protostomes and therefore they can provide "missing links" for reconstruction of neuropeptide evolution. Furthermore, the radial symmetry of echinoderms provides a unique context for exploration of neuropeptide function. Sequencing of the genome of the sea urchin *Strongylocentrotus purpuratus* identified several novel neuropeptides. Perhaps most remarkable is the neuropeptide NGFFFamide, which is derived from a precursor that contains a neurophysin domain, which hitherto was thought to be unique to vasopressin/oxytocin(VP/OT)-type precursors. Identification of the NGFFFamide receptor as an ortholog of vertebrate neuropeptide-S (NPS)-type receptors and protostome crustacean cardioactive peptide (CCAP)-type receptors facilitated reconstruction of the evolution of the paralogous VP/OT-type and NPS/CCAP-type signaling systems. Sequencing of the neural transcriptome of the starfish *Asterias rubens* enabled identification of 40 neuropeptide precursors, including 2 precursors of gonadotropin-releasing hormone (GnRH)-like peptides. Identification of the receptors for these peptides revealed that one peptide is the ligand for a GnRH-type receptor, whereas the other is the ligand for a corazonin-type receptor. Hitherto corazonin signaling was thought to be unique to arthropods/protostomes. Discovery of corazonin signaling in an echinoderm has revealed that the evolutionary origin of the paralogous GnRH- and CRZ-type signaling systems dates back to the common ancestor of protostomes and deuterostomes. On-going studies are investigating the physiological roles of multiple neuropeptide systems in echinoderms, using *A. rubens* and *S. purpuratus* as model systems. Acknowledgements: BBSRC (BB/M001644/1); Leverhulme Trust (RGP-2013-351).

S15-3) Larhammar, Dan (Sweden)

NEUROPEPTIDES AND RECEPTORS IN THE DEUTEROSTOME PREDECESSOR OF VERTEBRATES

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The vertebrate lineage had a flying start in evolution thanks to two rounds of genome doubling, often referred to as 1R and 2R. These events are known to have duplicated many genes that encode endocrine peptides and receptors in gnathostomes. However, reconstructing the endocrine gene repertoire of the deuterostome predecessor of vertebrates has been challenging: genome assembly difficulties forcyclostome species (lampreys and hagfishes), as well as difficulties assigning orthologyrelationships between cyclostome and gnathostome genes, has led to uncertainty regarding the gene repertoire of their early vertebrate ancestor. Furthermore, independent gene gains and losses in extant tunicates and lancelets obscures the gene repertoire of the chordate ancestor. Nevertheless, we have been able to identify severalneuroendocrine gene families that must have undergone duplications in the vertebrate predecessor even before 1R. This deuterostome had three neuropeptide Y receptorsubtypes that became seven in the gnathostome ancestor;it had two somatostatin receptors that became six in gnathostomes; two oxytocin/vasopressin receptors that became six;two QRFP receptors that became four;two CRH/urocortin peptides that became five; and two IGFBP genes that became six. Genes that were duplicated as a result of 1R and 2R in vertebrates, and thus must have been present in the vertebrate predecessor, but are apparently absent in extant invertebrate deuterostomes, are especially intriguing. These genes may constitute vertebrate novelties, although it remains possible that they arose earlier but have been lost in other deuterostomes. We are presently investigating endocrine genes that may have arisen in the deuterostome that gave rise to the vertebrates, setting the foundation for the diversity that later arose through 1R and 2R

S15-4) D'Aquila, AL (Canada)

THE ROLE OF THE TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP) FAMILY IN ENERGY PRODUCTION IN PROTOCHORDATES AND CHORDATES.

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Stress can overwhelm the survival of an organism if it cannot cope properly. Stress demands a higher energy budget in the cell in order to overcome the stress, thus mechanisms of enhancing energy production would be a potential target to aid in the stress response. The teneurin C-terminal associated peptide (TCAP) family is a novel energy regulator of glucose metabolism. As enhanced skeletal muscle function is a key component of the stress response, our work aims to elucidate the role of TCAP in stress and anxiety in these tissues. Moreover, as evolutionary analyses indicate that TCAP is an ancestral peptide, we took a comparative approach to study the conservation of its roles. First, in the vase tunicate, *Ciona intestinalis*, we designed a novel behavioural assay to determine the role of TCAP on stress-related behaviour through muscle contractions. TCAP treatment in *C. intestinalis* increased its contractile behaviour and decreased the stress response to the stimulant, suggesting that TCAP increased energy and decreased stress in this model. In zebrafish, we designed a novel stress-metabolism assay used to characterize the stress response in a confined space, by measuring their metabolic output. This assay revealed TCAP-treated animals demonstrated lower stress-related responses than vehicle-treated animals. Moreover, TCAP demonstrated a significant dose-dependent increase in energy production, by using an *in vivo* resazurin assay. Lastly, the role of TCAP as an energy regulator is conserved in rodents. After induced metabolic fatigue, TCAP-treated rats showed improved muscle contractile function under these stress conditions compared to vehicle-treated rats. In addition, TCAP-treatment significantly increases glucose uptake in muscle *in vivo*, as visualized by radioactive deoxyglucose uptake in functional positron emission tomography (fPET) scans. Behavioural tests, such

as open field test, indicate these animals are also less anxious. Our results demonstrate that TCAP's role in energy modulation is conserved in both protochordate and chordate models and that the enhanced energy budget allows for a decreased stress response, resulting in anxiolytic effects on behaviour.

S15-5) Osugi, Tomohiro (Japan)

IMAGING MASS SPECTROMETRY ANALYSIS OF MULTIPLE NEUROPEPTIDES IN THE BRAIN OF CIONA INTESTINALIS

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The localization of neuropeptides in the brain is one of the most important information to elucidate neuropeptidergic systems. However, comprehensive visualization of the localization and co-localization of numerous neuropeptides is hampered due to technical limitations of multistaining procedures. Mass spectrometry (MS) is a valid technique for detection of multiple biological molecules by a single experimental operation. Indeed, our previous MS-based peptidomic analysis detected more than thirty peptides, including both homologs and prototypes of vertebrate peptides and novel peptides in the brain extracts of tunicate *Ciona intestinalis*, a basal chordate that is the closest living relatives of vertebrates. The recent advances in MS technology enabled direct visualization of biological molecules on tissue sections. Imaging MS can peptidomically locate neuropeptides in the *Ciona* brain, and combined with the phylogenetic position, leading to elucidate the biological roles of numerous *Ciona* neuropeptides and provide novel insights into the evolutionary processes of neuropeptidergic systems in chordates. In the present study, we analyzed neural tissue sections of *Ciona* using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) imaging MS. Major *Ciona* neuropeptides were detected on a single brain tissue section, and the images of distribution and intensities of each peptide were acquired with a spatial resolution of 20 μm. We further developed a high resolution mapping method by a combination of fluorescent microscope and imaging MS in the neural tissues of transgenic animals. The high resolution imaging MS showed the distribution of peptides with a single cell resolution, which has a potential to serve as a new platform for histology. In addition, we analyzed the morphology of nervous system of adult *Ciona* using transgenic animals that express fluorescent proteins in neural tissues. Integration of detailed morphology of nervous system and high resolution imaging MS will pave the way to elucidate the net neuropeptidergic regulatory system. Acknowledgements: Supported by: Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (#26840108) to TO

S15-6) Taylor, Elias (Canada)

THE EFFECT OF THYROID HORMONES ON LARVAL SKELETOGENESIS IN THE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS*

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Biomineralization provides the rigid material for many adaptations across diverse phyla. Skeletogenesis is the process by which biomineralized structures are formed during embryogenesis. In the sea urchin *S. purpuratus*, it is a well understood process of development and the gene regulatory network (GRN) controlling skeletogenesis in the larva has been described. Previous research shows that TH accelerates the development of the juvenile rudiment in *Dendraster excentricus* and other irregular urchins while slowing the growth of larval skeleton. Here we tested the hypothesis that thyroid hormones (specifically T4, T3, and Triac) are regulators of skeletogenesis in *S. purpuratus*, upstream of the skeletogenic GRN. Treatment with T4 and T3 accelerated initiation of larval skeletogenesis in gastrulae, plutei, and the juvenile rudiments of late stage larvae, while Triac inhibited skeletogenesis. The use of fluorescently labeled THs suggested that THs bind to skeletogenic primary and secondary mesenchyme cells. Quantitative real-time PCR after TH exposure revealed upregulation of a key transcriptional initiator of skeletogenesis, Ets, as well as various skeletogenic proteins. Some evidence suggests there is a conserved mechanism of developmental regulation of mesenchyme cells between vertebrates and echinoderms, mediated in part by TH binding to a membrane receptor. Acknowledgements: Supported by: NSERC Discovery grant to AH

Thursday AM S16

GROWTH HORMONE AND PROLACTIN: NEUROPROTECTIVE AND DEVELOPMENTAL ACTIONS

Chair: Stephen Harvey and Carlos Arámburo de la Hoz

S16-1) Aramburo, Carlos (Mexico)

NEUROPROTECTIVE ACTIONS OF GH IN THE EMBRYONIC AND POSTNATAL CHICKEN RETINA

Martínez-Moreno CG, Carranza M, Ávila-Mendoza J, Luna M, Harvey S, Arámburo C
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There is increasing evidence to indicate a beneficial neuroprotective effect of growth hormone (GH) in the nervous system. During embryonic neuroretinal growth, GH regulates the physiological equilibrium between cell proliferation and developmental apoptosis in retinal explants and in a quail neuroretinal derived cell line (QNR/D). Neurotrophic actions of GH in the retina include axonal growth in immuno-panned retinal ganglion cells (RGCs) and neuroprotective actions against glutamate-induced cell death in QNR/Ds. While our previous studies have largely focused on RGCs, we have also found conclusive evidence of a pro-survival effect of GH in cells of the inner nuclear layer (INL) and a protective effect on the dendritic trees of the inner plexiform layer (IPL). Our results, both *in vitro* (embryo) and *in vivo* (postnatal), corroborate previous findings of the neuroprotective actions of GH against kainic acid (KA)-induced excitotoxicity in the chicken neuroretina. In addition, we have demonstrated that GH over-expression and exogenous administration increases brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NTF3) gene expression. Our results strongly suggest that these classical neurotrophins are mediators of GHs neuroprotective actions. Thus, a complex cascade of neurotrophins and growth factors, which have been classically related to damage prevention and neuroretinal tissue repair, likely mediates GH neuroprotective actions in neural tissues. Acknowledgments: We thank Gerardo Curtois (lab assistant), Nydia Hernandez Rios (confocal microscopy) and Maarten Werdler (histology) for technical assistance. Supported by PAPIIT-DGAPA UNAM (IN20613, IN206115, IA200717) and Pilgrim Pride.

S16-2) Stephen Harvey (Canada)

GROWTH HORMONE PROTECTS AGAINST KAINATE EXCITOTOXICITY (IN VITRO AND IN VIVO) AND INDUCES BDNF AND NT3 EXPRESSION IN NEURORETINAL CELLS

Martinez-Moreno CG, Fleming T, Carranza M, Avila-Mendoza J, Luna M, Harvey S, Arámburo C
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There is increasing evidence to suggest a beneficial neuroprotective effect of growth hormone (GH) in the nervous system. While our previous studies have largely focused on RGCs, we have also found conclusive evidence of a pro-survival effect of GH in cells of the inner nuclear layer (INL) as well as a protective effect on the dendritic trees of the inner plexiform layer (IPL). The administration of GH in primary neuroretinal cell cultures protected and induced neural outgrowths. Our results both, *in vitro* (embryo) and *in vivo* (postnatal), show neuroprotective actions of GH against kainic acid (KA)-induced excitotoxicity in the chicken neuroretina. Intravitreal injections of GH restored brain derived neurotrophic factor (BDNF) expression in retinas treated with KA. In addition, we have demonstrated that GH over-expression and exogenous administration increases BDNF and neurotrophin-3 (NT3) gene expression in embryonic neuroretinal cells. Thus, GHs neuroprotective actions in neural tissues may be mediated by a complex cascade of neurotrophins and growth factors which have been classically related to damage prevention and neuroretinal tissue repair

S16-3) Morales, Teresa (México)

NEUROPROTECTIVE ACTIONS OF PROLACTIN HORMONE AGAINST NEUROTOXIN LESIONS IN THE CNS OF RODENTS

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Prolactin (PRL) is a pituitary hormone with a variety of physiological actions including the CNS, in which its effects relate to reproduction, metabolism, emotional response, neurogenesis, and neuroprotection. In neuronal tissue, PRL can function as a protective or repair agent after a lesion. Lactation is considered a hyperprolactinemic state and we have documented PRL neuroprotective effects in the hippocampus of both, females and males. By taking advantage of the experimental neurotoxin lesion model we have demonstrated that the hippocampus of the lactating dam is less sensitive to kainic acid (KA) lesion compared to that of diestrus-virgin rats. Such shorter sensitivity has been proven by systemic or intracerebral administration of KA, at different time-points after the lesion, it prevails even 48h after weaning, and has been detected by apoptosis, glial, and neurodegeneration markers. In female rats, the pre- or post-lesion treatment with PRL diminishes damaging effects of the excitotoxic lesion. Pre-treatment with ovine-PRL or human-PRL and its phosphorylated mimic S179D-PRL diminishes KA-damage to pyramidal neurons in the hippocampus, which correlates with less progression of the behavioral manifestations of epilepsy activity caused by the lesion. Additionaly, PRL applied after the KA-lesion diminishes the cell loss in CA1 subfield of the hippocampus as well as the glial response, and attenuates the cognitive deficit in a novel-object recognition test. Protective effects of PRL have been documented in male mice, in which pre-treatment with this hormone decreases neuronal loss and neurodegeneration. Interestingly, paternity has protective actions in this KA-lesion model, and currently we are investigating whether PRL is involved in this phenomenon. PRL receptor presence in the hippocampus has been controversial, but we have detected it in fixed brain tissue or in vitro neuronal culture. This simposium presentation will discuss candidate mechanisms of PRL actions and intracellular pathways mediating them. Acknowledgements: Supported by: UNAM-DGAPA PAPIIT 202812, 202315; and CONACYT 128090; JR-M and IA are PhD students supported by CONACYT Scholarship.

S16-4) Luna, Maricela (México)

NEUROPROTECTIVE ACTIONS OF GH AND IGF-I AGAINST HYPOXIA-ISCHEMIA INDUCED BRAIN DAMAGE

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It is known that growth hormone (GH) and IGF-I are locally expressed in the central nervous system (CNS), indicating that they may be involved in functional roles mediated by autocrine/paracrine mechanisms. In the brain, GH and IGF-I induce neuro-protection against severe insults, such as hypoxia-ischemia. We found that GH and IGF-I concentrations increased in cerebellar cell cultures exposed to hypoxic conditions (0.5-5% O_2) when incubated with low glucose levels (1 g/L) (HLG). Both GH and IGF-I administered before injury may act as neuroprotective factors and have been implicated in cell survival. Administration of rcGH (1nM) or IGF-I (40nM) prior to the insult resulted in a significant increase of cell viability (1.7 and 2 X, respectively) in comparison with the HLG condition, while caspase-3 activity was concomitantly reduced (1.5 and 1.2 X respectively). When GH or IGF-1, were administrated after an acute injury in cultured cerebellar neurons a significant increase in cell viability was found (78.6±5.1% and 76.2±6.8%, respectively) in comparison with HLG untreated cells (59.2±3.1%). The combination of both hormones did not show a synergistic effect (65.1±4.0%). After 12 h of incubation under HLG, GH and IGF-I mRNA expression increased (by 1.79 and 1.54-fold, respectively). Treatment with exogenous rcGH significantly decreased GH and IGF-I mRNA expression (1 fold) under HLG conditions. The use of a specific GH siRNA decreased both GH (0.79-fold) and IGF-1 (0.54-fold) mRNA expression. Thus, administration of GH and IGF-1, either before or after HLG, increases cell survival. In conclusion, locally expressed GH may act as an autocrine/paracrine neuroprotective factor that preserves cellular viability and inhibits apoptotic cell death.Acknowledgements: Supported by PAPIIT-DGAPA, UNAM, IN206115, IN201817, IA200717; Pilgrim's

S16-5) Causey, Dwight (UK)

DIFFERENT ROUTES TO RAPID GROWTH: PROTEOME-WIDE OUTCOMES OF SELECTIVE BREEDING VS. GROWTH HORMONE TRANSGENESIS

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Selective breeding is commonly used to increase the growth rate of domesticated animals, though similar gains can be achieved by growth hormone (GH) transgenesis of wild strains. These two routes to 'unnaturally' fast growth are hypothetically underpinned by distinct mechanisms, considering that selective breeding may target a broader set of genes and pathways than those governed by GH. We are using a teleost fish study system and an 'omics approach to characterize the divergent molecular level regulation of fast growth; contrasting selective breeding with GH-transgenesis, focusing on skeletal muscle as the main target for growth and protein deposition. We sampled wild-type Coho salmon (Oncorhynchus kisutch) and compared them with fast-growing strains attained by GHtransgenesis (in a wild background) and selective breeding. Muscle protein extracts were analyzed on a Q-Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer using standard protocols. Mass spectra profiles were bioinformatically analyzed using MaxQuant, with protein identification completed against a rainbow trout (O. mykiss) database. Over 1,000 proteins were identified, of which 90 showed a significant group effect (GLM; FDR-adjusted P

S16-6) Biga, Peggy (USA)

VARIABLE ORGANISMAL GROWTH POTENTIAL CORRESPONDS TO DIFFERENTIAL GROWTH HORMONE SIGNALING MECHANISMS

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Myogenic precursor cells (MPCs) isolated from species with varied growth potential exhibit different proliferation capacities *in vitro* and biomarkers that represent different phenotypic stages in myogenic cell lineage commitment. Giant danio (*Devario aequipinnatus*; indeterminate growth) MPCs exhibit greater proliferation *in vitro* compared to zebrafish (*Danio rerio*; more determinate-like growth) MPCs. Consistent with proliferative capacity data, zebrafish MPCs express higher levels of the myogenic lineage marker myf5, while giant danio MPCs express low levels of myf5 but high levels of the early myogenic stem cell marker Pax-3. In addition, growth hormone (GH) induces *in vitro* proliferation to a greater extent in MPCs from giant danio compared to zebrafish. These data are consistent with increased overall giant danio body mass observed *in vivo* following GH treatment, while zebrafish fail to exhibit a maintained body mass increase in response to GH *in vivo*. In this study, we investigated the involvement of GH in local muscle proliferation regulation. Corresponding to observed growth effects (or lack thereof), we observed changes in *myostatin (MSTN)* and *Pax-3b* expression. Growth hormone reduced *MSTN* in giant danio, but increased *MSTN* expression in zebrafish muscle *in vivo*. Additionally, GH increased *Pax-3b* expression in giant danio but did not affect *Pax-3b* in zebrafish muscle. To further analyze local GH action in MPCs, we investigated the involvement of intracellular signaling mechanisms in response to GH in relation to growth-related gene expression activation. In general, GH appears to reduce *MSTN* via MEK and Jak2/Stat5 in indeterminately growing myoblasts, *in vitro*. These data suggest that muscle tissue receptivity to GH may be important in

overall growth potential and understanding variation in hormone sensitivity across growth potentials could lead to novel understanding of local tissue regulatory mechanisms leading to growth variability. Acknowledgements: Supported by: UAB Faculty Development Grant

Thursday AM S17

DEVELOPMENT OF THE NEUROENDOCRINE SYSTEM
(Sponsored by the Cumming School of Medicine and Alberta Children's Hospital Research Institute)

Chair: Deborah Kurrasch and Per-Erik Olsson

S17-1) Kurrasch, Deborah (Canada)

THE INFLUENCE OF HORMONES ON HYPOTHALAMIC NEURAL PROGENITORS DURING EMBRYONIC DEVELOPMENT
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The neuroendocrine hypothalamus is important for controlling various physiologies, such as hunger, thirst, thermoregulation, and reproduction. Despite considerable knowledge about the hormones that regulate these physiologies and the circuits responsible for transmitting their cues, very little known about the developmental programs that govern hypothalamic formation the first place. Indeed, questions still remain about how individual hypothalamic neurons acquire a particular cell fate and then migrate to an exact location to enable proper circuit formation. For the past several years, our lab has been using mice and zebrafish as complementary model systems to understand neuroendocrine developmental programs. Here, I will outline some of our recent work exploring the influence of hormones on progenitor behaviour in the developing hypothalamus. Our previous work showed that the estrogen-like contaminant bisphenol A caused precocious neurogenesis in the embryonic hypothalamus, suggesting that hormones might play an unappreciated role in controlling neural precursor cell behaviour long before they are produced by the gonads. Currently we are following up on these findings and focused on examining whether estrogen and/or testosterone directly govern cell cycle exit of these neural progenitors. Acknowledgements: Supported by: NSERC Canada operating grants to DMK. TH was the recipient of a Cumming School of Medicine Fellowship and DN was awarded an Eyes High and AI-HS Fellowships.

S17-2) Heyland, Andreas (Canada)

SEA URCHIN HISTAMINE RECEPTOR 1 REGULATES PROGRAMMED CELL DEATH IN LARVAL STRONGYLOCENTROTUS PURPURATUS
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Apoptosis is essential for the resorption of larval tissue and remodelling of structures during the metamorphic transition. In sea urchins the end of the larval period is characterized by settlement (i.e. leaving the water column to settle on the benthos) and a rapid morphological transition of the larva into the juvenile. These processes are regulated by external settlement cues (i.e. habitat-specific or other environmental cues), and endogenous signals, such as histamine, thyroid hormone and nitric oxide. In the purple sea urchin, *Strongylocentrotus purpuratus*, histamine maintains the state of metamorphic competence. Previous studies suggest that histamine receptor antagonists elicit caspase-mediated apoptosis in the sea urchin. We therefore hypothesize that suH1R regulates apoptosis in *S. purpuratus* as well. To this end, we mapped the distribution of suH1R positive cells throughout larval development using immunohistochemistry, established gene expression profiles for suH1R and assessed the result of suH1R knock-down on apoptosis and necrosis in early and late stage larvae. Our results provide functional evidence that suH1R is an inhibitor of apoptosis throughout larval development and regulates apoptosis during the metamorphic transition. These results therefore provide critical new insight into the mechanisms of metamorphosis in sea urchins and have important implications improve our mechanistic understanding of sea urchin development and metamorphosis

S17-3) Dewey, Deborah (Canada)

BRAINS AND BEHAVIOUR IN YOUNG CHILDREN EXPOSED PERINATALLY AND IN EARLY CHILDHOOD TO ENDOCRINE DISRUPTING CHEMICALS
Dewey D, Ejaredar M, Liu J,Grohs M,Ten Eckye K, Giesbrecht GF, LetourneauN, Lebel C, Martin JW
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Bisphenol A (BPA) and phthalates are considered endocrine disrupting chemicals. They are known to cross the blood-brain barrier and pass through the placenta. Prenatal and early childhood exposure to their endocrine disrupting properties could alter brain development and ultimately neurobehaviour. Biomonitoring studies suggest that more than 90% of Canadians have detectable levels of BPA and phthalates in their urine with children between the ages of 3 to 11 years displaying the highest levels of exposure. Results of our recently completed systematic reviews, support associations between prenatal and early childhood exposure to these chemicals and adverse neurobehavioral outcomes, including lower IQ, problems in attention and hyperactivity, and higher levels of anxiety, depression, and aggression in children. Findings from our ongoing longitudinal study investigating brain and neurodevelopment in preschool aged children exposed perinatally to BPA and phthalates will be presented. This study is following ~500 children for whom we have maternal biofluid concentrations of BPA (phthalate analyses ongoing) at <26 weeks gestation. Children's neurobehavioral development is assessed at 3 three times points between 2 and 7 years of age and a subset of the children participated in magnetic resonance brain imaging (MRI). Preliminary analyses suggest that level of exposure to BPA is associated with behavioral and cognitive outcomes at two years of age. Cross-sectional regression analysis of the first 58 children to undergo MRI revealed relationships between perinatal BPA and brain surface area. Specifically, significant sex-specific relationships between prenatal exposure and cortical surface area in the frontal pole, and middle and inferior temporal areas in the right hemisphere were found. Finally, prenatal exposure to BPA was associated with sex-specific alterations of the infant hypothalamic–pituitary–adrenal (HPA) axis. Together, these findings suggest that level of prenatal exposure to BPA is associated with sex-specific alterations in children's brains and the HPA axis, which are possible mechanisms underlying behavioral and neurodevelopmental problems in children.
Acknowledgements: Supported by: CIHR, NIEHS and AIHS operating grants to DD, GFG, NL CL and JWM. JL was the recipient of an AIHS studentship; KT was the recipient of U of C Eyes High and ACHRI Neurodevelopment Disorders Fellowships; ME and MG were recipients of QEII scholarships; ME was a recipient of an ACHRI studentship.

S17-4) Nishimura, Hiroko (Japan)

STRESS AND REDUCED NUTRITION DURING DEVELOPMENT PROGRAM ABNORMAL GROWTH OF THE EMBRYO
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Stress and insufficient nutrition in developmental life impose a risk of structural and functional alterations in humans and experimental animals in their later lives. This developmental programming (plasticity) causes serious health problems in human society and animal kingdoms, but studies on nonmammalian vertebrates are limited. We found that Japanese quail, *Coturnix japonica*, in which partial (8-10%) egg-white was withdrawn (EwW) before incubation had low birth weight, low hatching rate, and enhanced apoptosis of renal glomeruli; after maturation, they had fewer glomeruli than controls (CT) did and had glomerular mesangium lesions partly resembling nephrosclerosis. We further determined whether the stress imposed on the *Coturnix* embryo during the early developmental period induces structural abnormalities, whereas insufficient nutrition causes impaired growth and maturation. Also, we examined whether ✔-smooth muscle actin (✔-SMA) may serve as an injury/inflammation marker in glomerular mesangium. We found: 1) At embryonic day 8-9 (E8-9), 45.1% (n = 31) of embryos derived from eggs in which 10% of EwW before the start of incubation showed abnormal growth, malformation, and/or early death. At E15-16 (hatch, E17), the rate of abnormal growth/structure was lower (26.4%, n = 34). 2) The weight of the EwW embryos that showed good growth (0.74 ± 0.03 g, n = 8) was not significantly different from that of controls (CT, 0.79 ± 0.02 g, n = 14) at E8-E9, but it was lower ($P < 0.01$) at E16 (CT, 5.9 ± 0.2 g, n = 13; EwW, 5.2 ± 0.1 g, n = 14). 3) ✔-SMA signals were present in renal arteries and arterioles, glomerular mesangium, and peritubular capillaries of embryonic kidneys. ✔-SMA signals were stronger in EwW groups both in E16 embryos and in mature quail. These results suggest that partial withdrawal of egg-white (92% is protein) before incubation induces structural defects, possibly due to mechanical stress of EwW (such as internal pressure change by gentle suction), whereas the effect of low nutrition was more obvious in the later embryonic period. ✔-SMA in glomerular mesangium may serve as an injury/inflammatory marker in quail kidneys. These studies suggest that "developmental programming" has evolved phylogenetically early and that it needs more global attention from researchers and environmental care providers.

S17-5) Nesan, Dinushan (Canada)

INVESTIGATING THE ACTIONS OF STEROID HORMONES AND XENOESTROGENS ON HYPOTHALAMIC NEUROGENESIS DURING DEVELOPMENT.
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The neuroendocrine hypothalamus is a key regulatory brain region involved in maintenance of various physiological processes including hunger and thermoregulation, as well as the initial source of trophic hormones that regulate endocrine axes including the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis. Given the importance of this brain region, there is a striking lack of knowledge regarding hypothalamic development. Increasingly, there has been evidence that neural stem cells may be a specific target for steroid hormones during embryogenesis. Here I will present our recent findings on the action of steroid hormones on neural stem cells, using the primary culture neurosphere assay as well as further evidence that neural stem cells are steroid-responsive. Additionally, our lab recently showed that bisphenol A exposure causes precocious neurogenesis in the developing zebrafish hypothalamus. I will also present our work extending this finding to mouse models and examining how altered hypothalamic development results in behavioural changes in the mature animal. Together this data provides an interesting linkage between endocrine disruption, hypothalamic neurogenesis, and altered behaviour, further reinforcing the role of steroid hormone signaling in hypothalamic development and providing a putative connection to

neurodevelopmental disorders. Acknowledgements: Supported by: NSERC Canada operating grants to DMK. TH was the recipient of a Cumming School of Medicine Fellowship and DN was awarded an Eyes High and AI-HS Fellowships.

S17-6) Miller, Annie (USA)

SEX AS AN ENRICHMENT TO RESCUE REPRODUCTIVE DEFICITS IN MALE TRANSGENIC MICE

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Gonadotropin-releasing hormone (GnRH) neurons activate and maintain reproduction in all vertebrates and ensure the propagation of species. Emerging evidence suggests fibroblast growth factor (FGF) signaling is important for the maintenance of the postnatal GnRH system. FGF-deficient mice were shown to undergo accelerated deterioration of the postnatal GnRH system, leading to compromised reproduction at an earlier age. Unexpectedly, we found that the declining GnRH system in FGF-deficient mice could be rescued by a simple environmental intervention: opposite-sex (OS) housing, suggesting a very novel plasticity in the reproductive brain. However, the full rescue of the compromised GnRH system required a very lengthy period of OS housing (up to 300 days), making it somewhat inefficient. We hypothesize that the previous paradigm of prolonged OS housing with a single cagemate desensitized the response of the reproductive brain to sexual cues, but the rescue may be accelerated by the intermittent introduction of a novel OS cagemate in a repeated OS pairing paradigm. In this study, control and FGF-deficient (named dnFGFR) male mice were paired repeatedly with a novel same-sex (SS) or OS cagemate every 50 days for 150 days beginning at weaning. This repeated OS pairing paradigm significantly rescued the dnFGFR animals' declining GnRH system and downstream reproductive functions within an accelerated period of 150 days. Specifically, GnRH mRNA transcript in the preoptic area, pituitary luteinizing hormone content, and paired testis mass returned to normal in repeated OS paired dnFGFR animals. Further, the number of litters produced during the repeated OS pairings increased significantly for dnFGFR animals from the first to second pairings. In summary, our data suggest that enriching the environment of reproductively compromised animals with novel sexual partners can accelerate the rescue of a declining GnRH system and downstream reproductive function. These results suggest that the reproductive brain of diseased individuals remain highly plastic and responsive to positive environmental cues. Importantly, our results suggest that the reproductive health of diseased animals can be rescued by manipulating the highly plastic postnatal GnRH system via environmental interventions.

Thursday AM S18

STEROID RECEPTOR ACTIONS AND THEIR SIGNALING: NONGENOMIC VS. GENOMIC

Chair: Yong Zhu and Peter Thomas

S18-1) Shi, Yunbo (USA)

NUCLEAR ACTION OF TRA CONTROLS METAMORPHIC TIMING AND RATE DURING *XENOPUS* DEVELOPMENT

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Thyroid hormone (T3) receptor (TR) mediates the effects of T3 on organ metabolism and animal development. There are two TR genes, TRα and TRβ, in all vertebrates. During animal development, TRα expression is activated earlier than zygotic T3 synthesis and secretion into the plasma, implicating a developmental role of TRα both in the presence and absence of T3. Using T3-dependend amphibian metamorphosis as a model, we have previously proposed a dual functional model for TRs, in particular TRα, during development. That is, unliganded TR represses the expression of T3-inducible genes during premetamorphosis to ensure proper animal growth and prevent premature metamorphosis while during metamorphosis, liganded TR activate target gene transcription to promote the transformation of the tadpole into a frog. To determine if TRα has such a dual function, we have generated homozygous TRα knockout animals. We show that indeed, TRα knockout affects both premetamorphic animal development as well as metamorphosis. Surprisingly, we observed that TRα is not essential for amphibian metamorphosis as homozygous knockout animals completes metamorphosis within a similar period of time after fertilization as wild type siblings. On the other hand, the timing of metamorphosis for different organs is altered by the knockout, with limb metamorphosis occurring earlier while intestine completing metamorphosis later than the wild type siblings. Thus, our studies have demonstrated a critical role of endogenous TRα not only in regulating both the timing and rate of metamorphosis but also in coordinating temporal metamorphosis of different organs

S18-2) Vijayan, Matt (Canada)

NONGENOMIC CORTISOL SIGNALING IN RAINBOW TROUT HEPATOCYTES

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Cortisol rapidly activates cell signalling in trout hepatocytes, but the mechanisms are far from clear. We tested the hypothesis that rapid action of cortisol involves modulation of intracellular Ca^{2+} levels in trout hepatocytes. Trout hepatocytes in primary culture were exposed to cortisol at concentrations ranging from unstressed to stressed levels reported in trout plasma. There was a dose-related increase in intracellular Ca^{2+} level, as determined by Fura-2AM ratiometric imaging, which was rapid and maintained over several minutes. This response was not affected by mifepristone, a glucocorticoid receptor antagonist. The increase in intracellular Ca^{2+} level in trout hepatocytes was also seen with the membrane impermeable form of the steroid (cortisol-BSA). We determined the extent of extracellular and intracellular stores of calcium in affecting cortisol-induced elevation in Ca^{2+} levels by using EGTA (a chelator) and BAPTA-AM (inhibitor), respectively. Furthermore, we determined if IP3R pathway was involved in the activation of intracellular stores by using various inhibitors, including PLC inhibitor (U73122), thapsigargin (SERCA blocker) and ryanodine (RyR blocker). Also, L-type calcium channel blocker nifedipine and Ca^{2+}release-activated Ca^{2+} (CRAC) channel blocker Cpd5J-4 were utilized to determine the mode of entry of extracellular calcium in response to cortisol stimulation. Overall, our results suggest that cortisol rapidly increases intracellular calcium levels and this may be mediated by the activation of CRAC channel in trout hepatocytes. Acknowledgements: This study was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV

S18-3) Zhao, Xiao Fan (PRC)

G-PROTEIN-COUPLED RECEPTOR TRANSMITS STEROID HORMONE SIGNAL ON CELL MEMBRANE

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G-protein-coupled receptors (GPCRs) are involved in animal steroid hormone signaling, but their mechanism is unclear. In this research, we report that a GPCRs control steroid hormone 20-hydroxyecdysone (20E) signaling on the cell membrane of the lepidopteran insect *Helicoverpa armigera*. We used RNA interference, calcium ions assay, protein phosphorylation detection, overexpression of GPCRs, chromatin immunoprecipitation assay and [^{3}H]Pon A-binding assays for the studies. The ecdysone-responsive GPCR (ErGPCR-2) was highly expressed during molting and metamorphosis. 20E via ErGPCR-2 regulated rapid intracellular calcium increase, protein phosphorylation, gene transcription, and insect metamorphosis. ErGPCR-2 was located in the cell surface and was internalized by 20E induction. GPCR kinase 2 participated in 20E-induced ErGPCR-2 phosphorylation and internalization. The internalized ErGPCR-2 was degraded by proteases to desensitize 20E signaling. *ErGPCR-2* knockdown suppressed the entrance of 20E analog [^{3}H] ponasterone A ([^{3}H]Pon A) into the cells. ErGPCR-2 overexpression or blocking of ErGPCR-2 internalization increased the entrance of [^{3}H]Pon A into the cells. ErGPCR-2 is a key control factor for the entrance of 20E into cells. 20E via ErGPCR-2 regulates rapid intracellular Ca^{2+} increase and phosphorylation of USP1 and CDK10, which induce gene transcription in the 20E pathway, thereby regulating metamorphosis. ErGPCR-2 is phosphorylated and internalized via GRK2 for degradation to desensitize 20E signaling. 20E via GPCRs-, G-protein-, phospholipase C-, Ca^{2+}- and PKC-axis transmits signal to regulate gene expression. 20E via GPCRs-, cAMP-, and PKA-axis enhances 20E pathway gene expression.
Acknowledgment**:** This work was supported by grants from the National Natural Science Foundation of China (31230067), the National Basic Research Program of China (973 Program, Grant no. 2012CB114101). ∗Corresponding author: xfzhao@sdu.edu.cn

S18-4) Pang, Yefei (USA)

COORDINATE CONTROL OF MEIOTIC ARREST OF ZEBRAFISH OOCYTES BY GPER- AND NATRIURETIC PEPTIDE RECEPTOR 2- MEDIATED SIGNALING

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Natriuretic peptide type C (NPPC) and its receptor, natriuretic peptide receptor 2 (NPR2), have essential roles in maintaining meiotic arrest of oocytes in several mammalian species. However, it is not known if a similar mechanism exists in non-mammalian vertebrates. Using zebrafish as a model, we show that both NPPC and NPR2 are expressed in ovarian follicles. RT-PCR and immunohistochemistry results show that the NPPC is mainly expressed in the ovarian follicle cells, whereas NPR2 is detected in both follicle cells and oocytes. Treatment of intact and defolliculated oocytes with 100 nM NPPC for 6 hrs caused a significant decrease of germinal vesicle breakdown (GVBD), a visible sign of oocyte maturation. The mRNA level of NPR2, but not NPPC, in intact oocytes was significantly upregulated by 6 hrs treatments with 20 nM E2 and G-1, the specific GPER agonist. Treatment with NPPC caused a marked increase in oocyte cGMP concentrations, whereas E2, G-1, and ICI182780 caused much smaller increases in cGMP levels. Both cilostamide, a phosphodiesterase 3 (PDE3) inhibitor and rolipram, a PDE4 inhibitor, significantly decreased the GVBD of intact and defolliculated oocytes, indicating that both PDEs are involved in oocyte maturation. E2-and G1-induced decreases of oocyte maturation were enhanced by the co-treatment of oocytes with cilostamide and rolipram, suggesting that both cGMP and cAMP are critical in maintaining meiotic arrest of zebrafish oocytes. These results demonstrate that cGMP signaling caused by NPPC through its receptor NPR2 cooperates with E2 through GPER in maintaining meiotic arrest of zebrafish oocytes.

S18-5) Zhu, Yong (USA)

GENERATION AND CHARACTERIZATION OF ZEBRAFISH KNOCKOUT MODELS FOR STUDYING FUNCATIONS OF GENOMIC AND NONGENOMIC PROGESTIN RECEPTORS

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Involvement of three distinct classes of progestin receptors, i.e., multiple membrane progestin receptors (mPRα, β, γ, δ, ε,), two progestin receptor membrane components (Pgrmc1 and Pgrm2), and one nuclear progestin receptor (nPR or Pgr), in the progestin signaling and various functions in different cell lines and animal models have been demonstrated. However, *in vivo* functions and molecular mechanisms of these receptors, especially their roles in the genomic and nongenomic signaling of progestins in animals have not been fully resolved. We have generated zebrafish knockout lines for all these receptors using gene editing technologies, and examined *in vivo* functions of these receptors. We found completely anovulation in nPR knockouts, subfertility and various defects in the oocyte maturation and the embryonic development and growth in the knockouts of Pgrmcs and mPRs. Our results clearly suggest that progestin signaling and these receptors play important roles in fertility, development, growth, oocyte maturation, and ovulation in zebrafish. We will report *in vivo* functions and molecular mechanisms underlying these defects. Acknowledgements: Supported by: NIH and NCBC grants to YZ.

S18-6) Mohapatra, Sipra (USA)

ESTROGEN RECEPTORS: MAJOR PLAYERS IN SEX-BIASED REGULATION OF AUTOPHAGY IN FISH

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Autophagy is a cellular process that delivers cytoplasmic material to the lysosome for recycling. It is stimulated above the basal or resting rate when nutrients are scarce, cells are under stress, or damaged organelles need to be degraded. Using several fish species, we found that, despite evolutionary distances and variations, basic autophagy mechanism involving mTOR/HK2-AMPK-ULK-BECLIN1-LC3 pathway is strikingly conserved between these fish and human. High throughput transcriptional profiling suggested that, autophagic involvement in early disease resistance and stress management differed between male and female fish. Considering the fact that, sex steroid and their responsive receptors, especially estrogen and estrogen receptors (ERs), abundances are sexually dimorphic, we deduced that sex-biased autophagy is somehow regulated by ERs. To prove that, we used ERα and ERβ2 knockout (KO) medaka and analyzed the alterations in the autophagic genes and protein expression, in both fed and starved conditions. We found significantly increased *mTOR* expression in ERα-KO, but not in ERβ2-KO fish. However, *HK2* transcriptions were strongly altered in ERβ2-KO fish. This suggested differential involvement of ERs in autophagic regulation, which was further confirmed by *ULK* and *Beclin1* transcription, and positive cell and mitochondrial population. Interestingly, the LC3 (the last major autophagy factor) contents/cell and LC3 positive cells were increased significantly in ER-KO fish. In depth analysis showed that, LC3 nuclear-cytoplasmic transports were partially (ERβ2-KO) or completely (ERα-KO) compromised due to SIRT/DOR protein regulation in the nucleus. CHIP analysis confirmed the steroid receptor specificity and sex biasness in *mTOR*, *NRF* (upstream mTOR regulator) and *LC3* binding, but failed to find any direct impact on *HK2*. We also found that, ERβ2-KO induces a non-canonical pathway, which is probably regulated by calcium signaling. Cumulatively, our data highlights the multipoint sex-biased ER association in autophagy and suggests that, ERα is instrumental for constitutive HK2/mTOR/AMPK mediated autophagy, while ERβ2 is essential for non-canonical, probably AMPK independent-Ca^{+2}/regulated, autophagy. Acknowledgements: Supported by: JSPS, MOFF, Japan

Friday AM S19

ENDOCRINE DISRUPTION IN AQUATIC VERTEBRATES - LESSONS LEARNED AND FUTURE PROSPECTS A TRIBUTE TO PROFESSOR LOUIS J. GUILLETTE, JR.

Chair: Charles R. Tyler and Thea Edwards

S19-1) Edwards, Thea (USA)

GRANULATED MAST CELLS, AN UNEXPECTED OCCUPANT IN TILAPIA HEPATO-PANCREAS

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In the Okavango Delta, Botswana, Africa, there is a gradient of pesticide and heavy metal pollution, with low levels in the northern panhandle and higher levels in the southern reaches. To determine if pollutants are affecting fish health, three species of tilapia were collected along the gradient, with the hypothesis that changes in health status would be dose-dependent. Tilapia health was also assessed in the context of basic water quality and normal environmental changes in the Delta. Despite previous descriptions of the pollution gradient, we found evidence of endocrine disruption and histologic pathologies in fish throughout the Delta. In particular, the hepato-pancreas tissues showed evidence of acinar cell degradation and loss, immune cell infiltration, fibrosis, and mild hemorrhage. Notably, acinar clusters were surrounded by granulated, basophilic mast cells punctuated with bundles ofceroid-filled melano-macrophages. Both mast cells and ceroid bundles are evidence of contaminant exposure in tilapia throughout the Delta, despite otherwise excellent water quality measure

S19-2) Hamlin, Heather (USA)

NITRATE AS AN ENDOCRINE DISRUPTING CONTAMINANT IN AQUATIC VERTEBRATES

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Humans have altered the nitrogen cycle more than any other natural cycle, and nitrogen in aquatic ecosystems across the world has increased 10 to 15 times in the last 10 years alone. Nitrate, a principal form of environmental nitrogen, has not traditionally been considered a material water quality hazard in many aquatic ecosystems, despite a growing number of studies describing nitrate's ability to cause a variety of physiological dysfunctions. Animals raised in aquarium or aquaculture environments could be especially vulnerable as nitrate in recirculating systems is often considerably above natural concentrations. A series of studies will be discussed investigating the ability of nitrate to disrupt endocrine function in several aquatic vertebrates including zebrafish, sturgeon, alligators, and sharks. Understanding the effects of ubiquitous environmental pollutants, such as nitrate, is critical to understanding both ecosystem and human health concerns.

S19-3) Kohno, Satomi (USA)

EVERYTHING BEGAN WITH THE QUESTION, "CAN YOU CLONE ALLIGATOR ESTROGEN RECEPTOR?"
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The American alligator has been investigated as a sentinel of endocrine disruption in the wild. A variety of reproductive and endocrine alterations have been identified in the American alligator at Lake Apopka (FL, USA) which is highly contaminated with *p,p*-DDE. To understand the mechanisms of action of endocrine disruptors on reproduction, investigation of estrogen signaling is critical. Thus, in the early 2000s Dr. Guillette asked Dr. Iguchi "Can you clone alligator estrogen receptor?" That was the beginning of this investigation, and we contributed to the Alligator genome project as well as transcriptome analyses. The American alligator exhibits temperature-dependent sex determination, which is sensitive to estrogen signaling during the thermosensitive period. Estrogen and estrogenic endocrine disruptors can induce ovarian development at male-producing temperatures. Utilizing these characteristics and well after cloning and characterizing the American alligator estrogen receptors, we evaluated the endocrine disruption of dispersant and chemically-dispersed oil used in the 2010 Deepwater Horizon oil spill. Both dispersant Corexit 9500 and Corexit-enhanced water accommodated fraction of crude oil (CWAF) activated the alligator estrogen receptors as measured in transactivation assays *in vitro*. To further pursue this result, isolated gonadal tissues were exposed to CWAF and estrogen *in vitro* during the thermosensitive period. Although CWAF exposures increased the female ratios, gonad-related mRNA expression patterns induced by CWAF were different from the estrogen-induced, indicating that the observed endocrine-disrupting effects of dispersant and CWAF are not solely mediated via estrogen receptors. These results highly warrant further comprehensive studies of endocrine disruptors and their impacts on sex ratios in aquatic reptiles at the population level, including the testing of multiple estrogenic and non-estrogenic pathways with species-specific *in vitro* assays.

S19-4) Parrott, Benjamin (USA)

A MEANS TO ADAPT, A MEANS TO DISRUPT: EPIGENOME-BY-ENVIRONMENT DYNAMICS UNDERLYING SEX DETERMINATION AND REPRODUCTIVE PERTURBATIONS IN THE AMERICAN ALLIGATOR
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Unlike traditional models for reproductive health, the American alligator is a long-lived apex predator that undergoes temperature-dependent sex determination (TSD). Further, these animals are oviparous and field collected eggs allow for the investigation of environmental effects on developmental processes. Here, we use the alligator as a model to (1) examine the role of DNA methylation patterning during TSD and (2) investigate how exposures to endocrine-disrupting contaminants (EDCs) during this period may alter the epigenetic landscape in a manner that influences subsequent reproductive function. When compared to their counterparts living in relatively pristine environments, alligators undergoing natural exposures to EDCs display a severely abated ovarian transcriptional response to gonadotropin stimulation. Here, we employ reduced-representation bisulfite sequencing to explore the sexually dimorphic DNA methylome in gonads from embryos exposed to either male- or female-promoting temperatures. We identify numerous temperature-dependent methylated regions within the alligator genome. In addition, we use targeted bisulfite sequencing on the Illumina platform to examine how DNA methylation status of the *CYP19A1* promoter, a gene displaying sexually dimorphic expression and DNA methylation patterns, varies in field-collected embryos originating from contaminated environments. We find that the robustness of sexually dimorphic *CYP19A1* promoter methylation is reduced in embryos originating from a site contaminated with EDCs. We next probe the consequences of developmentally inappropriate Estrogen Receptor activity on subsequent *CYP19A1* expression and find that treatment with a selective ESR1 agonist prior to gonadal differentiation results in down regulation of *CYP19A1* in stage 27 ovaries. Results presented here suggest that DNA methylation patterning may play an integral role in mediating the effects of incubation temperature on sex determination and that EDCs may exert their effects by compromising sexually dimorphic epigenetic patterns acquired during development.

S19-5) Trudeau,Vance (Canada)

PROZAC DISRUPTS THE STRESS RESPONSE ACROSS FOUR GENERATIONS

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Targeting serotonin using selective serotonin reuptake inhibitors (SSRI) is a major theme in antidepressant pharmacology. The SSRI Prozac (fluoxetine) has been in prescribed since the late 1980s. This represents nearly 2 human generation intervals of therapeutic use. Moreover, newer SSRIs and other neuroactive pharmaceuticals are also considered environmental contaminants. They are prescribed and consumed at high rates and are released with human sewage to aquatic systems and may affect wildlife. We set out to model the transgenerational impact of Prozac in zebrafish. Larvae of a clean population were exposed for the first 6 days of life during the critical formation of serotoninergic systems and then raised in clean water. Under constant rearing conditions, descendants of these fish were followed up to F4. Stress responses were assessed by whole body cortisol levels under basal conditions and following standardized handling stress or adrenocorticotropic hormone (ACTH) injections. We observed decreased basal, stress- and ACTH-induced induced cortisol responses in males only, and this effect persisted over the 4 generations ($p<0.001$). Stress can be associated with behavioural alterations, so we also quantified exploratory behaviours in the novel take paradigm. Males displayed reduced exploratory behaviour across 4 generations ($p<0.001$). This reduction is associated with decreased cortisol because experimental inhibition steroidogenesis with the 11β-hydroxylase inhibitor metyrapone caused the same behavioural modification. RNA sequencing of interrenals of F3 fish descendant from control and Prozac-exposed ancestors indicates an array genes with altered (FDR<0.0001) expression that reflect the multifunctionality of the teleost interrenal (adrenal steroidogenic plus renal functions). There are 2 main points of significance for these data. (1) Persistent low cortisol levels have recently been linked to behavioural problems in children and chronic fatigue and burnout in adults. (2) As we enter a period of increasing usage of antidepressants, these data are a cause for concern for the potential long term negative impacts on humans and aquatic organisms exposed to these chemicals. Acknowledgements: FRQNT scholarship (MVC) and grants from NSERC (TWM, VLT), University Research Chair Program (VLT) and Health Canada (CLY).

S19-6) Helbing, Caren (Canada)

LINKING BEHAVIOURAL EFFECTS OF THYROID ACTIVE CHEMICALS WITH MOLECULAR BIOMARKERS IN THE BULLFROG TADPOLE SENTINEL.

Helbing C, Birol I, Brinkman F, Hall ER, Lesperance ML, Parker W, Pyle G, van Aggelen G, Bogart S, Brown LY, Griffiths E, Hammond SA, Heerema J, Jackman KW, Kuçuk E, Miliano RC, Partovi SH, Roberts B, Van Rossum T, Veldhoen N
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Municipal wastewater effluents introduce a variety of pharmaceuticals and personal care products into the aquatic environment, many of which are endocrine disrupting compounds (EDCs). Methods for effectively detecting EDC activity at environmentally and ecologically relevant levels are lacking, particularly for thyroid hormone (TH). Essential for normal growth, behaviour, development, and metabolism in all vertebrates, one of the most striking examples of TH action is their absolute requirement for triggering frog tadpole metamorphosis into a froglet. In Ranids - the largest frog family - the skin and olfactory systems change dramatically. In this study, we defined their gene expression programs by RNA-seq and quantitative real time polymerase chain reaction (qPCR) in response to thyroxine (T4) and 3,3',5-triiodothyronine (T3) and found that the tissue-specific profiles do not necessarily follow the classical deiodinase conversion of prohormone thyroxine to bioactive T3 schema. We placed these programs in the context of alterations to the bacterial microbiome composition on the skin and behavioral response to external olfactory cues. Our recent progress in sequencing and assembling the first "true frog" genome (*Rana* (*Lithobates*) *catesbeiana*) will enable further large scale analyses in identifying important pathways involved in the transformation of skin and olfactory systems and the impact of environmental factors on this process. Acknowledgements: Supported by: NSERC Canada, Compute Canada, and Genome British Columbia

Friday AM S20

BIOLOGICAL RHYTHMS, CIRCADIAN CLOCK

Chair: Akiyoshi Takahashi, Takashi Yoshimura, Horst-Werner Korf

S20-1) Yoshimura, Takashi (Japan)

UNDERSTANDING THE MOLECULAR BASIS OF VERTEBRATE SEASONAL ADAPTATION.

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Organisms living in temperate zones use changes in photoperiod to adapt to seasonal changes in the environment. It is well established that the circadian clock is involved in photoperiodic time measurement. However, the mechanism of how the circadian clock measures day length remains unknown. It has been reported that Medaka populations inhabiting higher latitudes require longer day lengths for reproduction than those inhabiting lower latitudes. We obtained Medaka populations, including inbred strains, closed colonies and natural populations that were derived from different latitudes. When we examined the critical day length required for reproduction, Northern populations required 14 hours of light for gonadal development, while Southern populations required 13 hours of light. To identify genes that define this critical day length, we crossed different populations and obtained F1 and F2 generations. Subsequently, we performed quantitative trait loci (QTL) analysis using restriction-site associated DNA (RAD) markers and identified a significant QTL. In addition to the above-mentioned forward genetic approach, we are currently performing a genome-wide transcriptome analysis. Functional analysis of identified gene revealed Medaka's strategy for adapting to various seasonal fluctuations in the environment. Acknowledgements: Supported by: JSPS KAKENHI "Grant-in-Aid for Specially Promoted Research" (26000013), and by the Human Frontier Science Program (RGP0030/2015).

S20-2) Suzuki, Tohru (Japan)

RHYTHMIC *PER2* EXPRESSION AT THE SUPRACHIASMATIC NUCLEUS OF THE JAPANESE FLOUNDER, *PARALICHYTHYS OLIVACEUS*, AND ITS IMPLICATIONS FOR CIRCADIAN CLOCK MECHANISM

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This session aims to describe three aspects of circadian rhythm in flounder. These are: (1) the pattern of clock gene, *period2* (*per2*), expression in the suprachiasmatic nucleus (SCN), (2) the embryonic development of rhythmic expression of *per2*, and (3) the expression of clock genes in peripheral tissues.The function of the SCN as the central pacemaker in circadian rhythms is well known in mammals. In initial investigations aimed at examining if the SCN functions as the central pacemaker in the teleost fishes, examination of the *per2* expression in the SCN by RNA *in situ* showed that *per2* had a daytime-on, nighttime-off rhythm in the flounder and amberjack *Seriola quinqueradiata* larvae. This expression pattern was maintained even when larvae were kept in DD, suggesting that the expression pattern of this clock gene is endogenous. In medaka *Oryzias latipes,* the SCN showed no *per2*expression. So, we suppose that the SCN's function as the "master clock" in regulating circadian rhythm is an ancestral trait of all vertebrates, including teleosts, with species such as medaka being examples in which this trait has degenerated. The rate-limiting enzyme in melatonin synthesis, *arylalkylamine N-acetyltransferase 2* (*aanat2*) is expressed in the pineal gland at nighttime to control daily rhythm in melatonin levels. Examination of *per2* and *aanat2* expression during embryogenesis showed that cyclic *per2* expression began in the SCN 96 hours post fertilization (hpf), coinciding with the time of eye pigmentation. This event precedes rhythmic expression of *aanat2* in the pineal gland at 114 hpf. The expression tests of *per1* and *per2* in the caudal fin under various light conditions showed that under LL although the rhythm of *per1* expression was maintained, the rhythm of *per2* expression was lost. When tested *in vitro*, the rhythmic expression in both genes was found to have disappeared even under LD. Dexamethasone, an agonist of cortisol, upregulated *per1*both *in vivo and in vitro,* suggesting that while the *per1* expression rhythms is regulated by the SCN-adrenal interaction, *per2* expression rhythm may be regulated directly by light zeitgebers through an unknown pathway. Thus, we suppose that in flounder, the SCN influences circadian rhythm in peripheral tissues, but some decentralized elements may also play a role in regulation of these rhythms.

S20-3) Takemura, Akihiro (Japan)

LUNAR CYCLE IN THE EXPRESSION PATTERN OF CLOCK GENES IN THE BRAIN OF TROPICAL GROUPERS.

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Many fishes inhabiting shallow tropical and subtropical waters exhibit lunar cycle in their activities. It remains unclear how they perceive cues from the moon and what kind of endogenous players are involved in displaying lunar related rhythmicity. Tropical groupers repeat lunar-synchronous spawning around the species-selective moon phase during the spawning season; the Malabar grouper *Epinephelus malabaricus* and the honeycomb groper *E. merra* migrate and spawn around the new moon and the full moon phase, respectively. It is hypothesized that they utilize changes in moonlight illumination during nighttime to entrain gametogenesis. One possibility is that clock genes are involved in entrainment in relation to profile and/or amplitude of "brightness at night". The present study aimed to examine the expression of *Cryptochrome* (*Cry*) gene in the brain of the Malabar grouper (immature) and honeycomb grouper (mature); the former was obtained from the Okinawa Prefecture Hatchery, while the latter was collected weekly from the coral reef of Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. When abundance of *Cry* (*mgCry1, mgCry2*, and *mgCry3*) mRNAs in the brain of the Malabar grouper was measured every 4 hours using real-time quantitative polymerase chain reaction (qPCR), *mgCry2* mRNA in the diencephalon and telencephalon fluctuated daily with increases during photophase. The expression pattern of *mgCry2* mRNA was higher around the new moon period than other moon periods. When moonlight illumination was interrupted during full moon night, abundance of *mgCry2* mRNA increased in the pituitary. *In situ* hybridization analyses revealed that *mgCry2* mRNA was highly expressed in the lower part of the pituitary. When weekly change in abundance of *Cry* (*hgCry1*, *hgCry2*, and *hgCry3*) mRNAs in the brain of the honeycomb grouper was measured using qPCR, *hgCry2* mRNA increased in the diencephalon at midnight around the last quarter and new moons. These results suggest that *Cry2* mRNA shows the similar expression pattern in the brain of two groupers, although they used different moon phase for synchronous spawning and migration. It is concluded that some clock genes act as entrainers or drivers of lunar periodicity. Acknowledgements: Supported by: JSPS KAKENHI to AT.

S20-4) Gothilf, Yoav (Israel)

NEW FINDINGS AND DEBATES ON THE ROLE OF THE PINEAL GLAND IN ZEBRAFISH

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The fish pineal gland exhibits all the characteristics of a complete circadian system, comprising photoreceptive inputs, molecular clockworks and rhythmic hormonal output - melatonin biosynthesis, and has been considered as the site of the master circadian clock in fish. In zebrafish, pharmacological and genetic manipulations suggest that melatonin is required for the circadian regulation of the sleep/wake cycle in this species. Nevertheless, the presence of peripheral oscillators that are photoreceptive and directly entrainable by exposure to light led to a decentralized view of the zebrafish circadian system, thereby questioning the central role of the pineal gland in circadian regulation. To further examine the role of the pineal gland oscillator in the zebrafish circadian system, we generated a transgenic line in which the molecular clock is selectively blocked in the melatonin-producing cells of the pineal gland by the expression of a truncated CLOCK protein that functions a dominant-negative transcription factor. As a result, clock-controlled rhythms of melatonin production are disrupted in these fish. Moreover, transcriptome analysis revealed that the circadian expression pattern of the majority of clock-controlled genes in the adult pineal gland is abolished. Importantly, circadian rhythms of locomotor activity under constant dark and dim light conditions were markedly attenuated. These results support the hypothesis that the zebrafish pineal gland is important, probably as part of a multicomponent clock system, for regulating circadian rhythms of behavior, likely through the rhythmic production of melatonin. Nevertheless, melatonin is not the only pineal-derived signal. The fish pineal gland contains projecting neurons that innervate several brain regions and could conceivably transmit photic and/or circadian information. This broad connectivity of the pineal gland and the functions its plays have been mostly overlooked. Utilizing BAC transgenesis, we identified previously uncharacterized neurons in the zebrafish pineal gland and their projections, expanding the known connectome of the pineal gland. This new finding may point to additional roles of the pineal gland and a more complex regulation of circadian biology than is currently thought

S20-5) Hur, Sungpyo (South Korea)

PHYSIOLOGICAL PROCESS DURING SILVERING OF THE NOCTURNAL EEL, ANGUILLA JAPONICA; CIRCADIAN AND PHOTOPERIOD-RELATED EXPRESSION OF CLOCK GENES IN THE JAPANESE EEL RETINA AND BRAIN

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In mammals, the suprachiasmatic nucleus (SCN) plays an important role in the maintenance and coordination of most behavioral and physiological rhythms of the body. In contrast, the existence of a master circadian pacemaker has not been proven in the organs of fish. Moreover, the details of physiological rhythms in Japanese eel is still unknown which is an economically important but endangered species. The aim of study was to clarify involvement of clock genes oscillation in nocturnal eel, Anguilla japonica. The circadian expression patterns of clock genes (Per1, 2, 3, Cry2, 3 mRNA) and melatonin synthesis enzymes (AANAT1, 2 mRNA) were measured in eel retina and brain using real-time quantitative PCR (qPCR). Statistically significant rhythmically expression in retina were found for: aanat1, Per1, Per2, Per3, Cry2, and Cry3 mRNA. No change of rhythmically expression in brain were found for all clock genes. Furthermore, in eyectomy experiment, significant rhythmically expression in brain were found for all clock genes. It may suggest that eel retina is principal circadian clock oscillator. After 1 week under short photoperiod (15L: 10D), we observed photoperiod-related changes found for: Per1, Per2, Per3, Cry2, and Cry3 mRNA. These findings suggest that the expression patterns of several clock genes exhibit seasonal variation according to seasonal changes in day length through retina and that such alteration of clock gene expression may contribute to seasonal recognition by the Japanese eel. Acknowledgements: Supported by: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A6A3A04041089).

S20-6) Mogi, Makoto (Japan)

EXPRESSION OF THREE CLOCK GENES, *PER1*, *PER2* AND *CRY1* IN THE CAUDAL FIN OF JAPANESE FLOUNDER

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Circadian rhythm is an important system that gives organisms the flexibility to adapt to environmental changes. In mammals, the circadian clock rhythm is adjusted to 24-h cycles in the suprachiasmatic nucleus (SCN) by the photic signals from retina, and the SCN functions as a sole master circadian pacemaker that regulates the rhythmicity of peripheral organs. In sharp contrast, in zebrafish, the function of the SCN

as pacemaker is not ascertained, and instead, the retina and pineal gland (PG) are reported to function as pacemakers. Additionally, peripheral tissue cells can adjust their biological clock to 24-h cycle using light *per se*. The circadian axis is not described in detail in teleost fish, except for zebrafish. We previously reported that in flounder, the SCN exhibits daily rhythm in *per2* expression. To examine whether a hierarchy exists in the system to maintain the rhythm of peripheral clocks, the present study analyzes expression of three clock genes, *per1*, *per2* and *cry1,* in the caudal fin *in vivo* and *in vitro,* and the effects of cortisol administration on their expression. *In vivo*, fin maintained daily expression rhythm of all three clock genes, even in 24-h darkness (DD), but fin explants lost expression rhythm after a short period of culture even under LD conditions. Cortisol significantly upregulated expression of three clock genes in fin both *in vitro* and *in vivo*. When the larvae were kept in DD, the expression of *per2* and *cry1* expression was upregulated by exposures to light in short period. Therefore, we suppose that the SCN-pituitary-adrenal pathway plays a role in the oscillation of the peripheral clock in flounder, but unknown light-responsive oscillation system also functions in the photo-entrainment of the peripheral clocks.

Friday AM S21

KISSPEPTINS: MANDATORY OR OPTIONAL FOR REPRODUCTION

Chair: Berta Sivan Sivan and James Nagler

S21-1) Parhar, Ishwar (Malaysia)

CELLULAR IDENTITY AND REPRODUCTIVE FUNCTION OF DEEP BRAIN PHOTORECEPTORS, SEROTONIN AND KISSPEPTIN SYSTEM

Parhar I
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Light is essentially captured by image-forming photoreceptors in the retina of the eye, converted into electrical signals and sent to specialized brain regions to form images. However, there also exists non-image forming deep brain photoreceptors whose functions are unknown.We have recently cloned a group of deep brain photoreceptors, vertebrate ancient long (VAL)-opsin that have two isoforms (*valopa*and *valopb*), which are daytime-nighttime sensitive. Double–label immunochemistry revealed valopa and valopb are co-expressed in neurons secreting serotonin, GABA or thyrotropin-releasing hormone. Further, using CRISPR/Cas9 genome editing,we generated *valop*-mutant zebrafish and examined the phenotypes of loss-of-function mutants. Interestingly, most F1 eggs or embryos from F0 female *valopa/b* mutants showed either no or only partial chorion elevation. Conversely, most F1 embryos from F0 male *valopa* mutants developed normallybut hatched significantly lateor never hatched whencompared to wild-type embryos. These resultssuggestthat VAL-opsins play an important role in normal fertility via multiple neurochemical systems in the central nervous system. This talk will review recent findings on the cellular identity of deep brain photoreceptors and discuss their implications in reproduction along with neurotransmitter and the kisspeptin systems.

S21-2) Elizur, Abigail (Australia)

REPRODUCTION RELATED NEUROPEPTIDES AND THE KISSPEPTIN SYSTEM IN INVERTEBRATES

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Neuropeptides and their receptors play a key role in mediating reproductive processes. In fish molecular and physiological studies have shown the kisspeptin system is involved in both puberty and advanced reproductive stages, however in invertebrates, while components of the kisspeptin system have been identified, their role in reproduction or other processes is less well defined. We have used a bioinformatic approach to identify neuropeptides in molluscs, echinoderms and crustaceans, using both in-house generated transcriptomic data from neural tissues as well as publically available databases, and a comparison of components of the kisspeptin system identified will be described. For a couple of oyster species, we have also extended our study to peptidomic and *in-vivo* analysis to confirm the presence and function of the predicted peptides. A suite of peptides were synthesized and tested *in vivo* for their capacity to both initiate reproductive development as well induce spawning. APGWamide, buccalin, CCAP, LFRFamide and GnRH triggered spawning in oysters and both APGWamide and buccalin also advanced conditioning and gonadal maturation

S21-3) Levavi-Sivan, Berta (Israel)

CHARACTERIZATION OF NOVEL NEUROPEPTIDES MODULATINGFISH REPRODUCTION

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Reproduction in all vertebrates is controlled by the highly conserved hypothalamic-pituitary-gonadal axis. The hypothalamic regulation of GTH secretion in fish is different from that of mammals, from both endocrinal and anatomical aspects. The hypothalamic neuropeptides

GnRHs, increase the release of LH and FSH. However, new actors have recently entered the field of reproductive physiology: kisspeptins, neurokinins, LPXRFa, spexin and dynorphin, have all been implicated in controlling reproduction. Neurokinin B (NKB) was recently identified as a key regulator of reproduction in mammals and fish, when fish were found to possess a specific novel neurokinin termed NKF. NKB system was characterized in tilapia and zebrafish, in terms of receptor transactivation, and in in vivo experiments. Using ISH and fluorescent immunohistochemistry, we have shown that LH cells possess NKB and its receptor mRNAs, whereas FSH cells possess mainly NKB-Rs. LPXRFa peptides have been characterized for their ability to inhibit GTH release in birds and stimulate GH release in frogs. Administration of tilapia LPXRFa-2 peptide to primary cell culture of pituitaries, or to reproductive female tilapia by ip injection, positively regulated both LH and FSH release in vivo and in vitro. Using double-labeled fluorescent, ISH, LH cells were found to co-express both tilapia lpxrf and tilapia lpxrf-r mRNA, whereas some of the FSH cells coexpressed only lpxrf-r mRNA. Spexin is a neuropeptide identified recently by bioinformatics approach. Currently comparative studies of SPX in fish are controversial. To examine the structure and function of SPX in fish model, SPX was cloned in tilapia and found to be highly comparable with its mammalian counterparts. SPX significantly decreased plasma LH and FSH in female tilapia and FSH in male tilapia. Dynorphin (DYN) is a neuropeptide that is involved in reproduction by decreasing the level of GTHs in several fish species. Accordingly, Naltrexone - an opioid receptor antagonist - increased plasma levels of both LH and FSH in tilapia. Mapping of hypothalamic circuitry, classification of receptor expression profiles within these circuits, and a detailed analysis of the action of reproductive hormones on these pathways is required for our understanding of the reproduction process.

S21-4) Zmora, Nilli (USA)

KISSPEPTIN AND ITS PARTNERS IN THE REGULATION OF REPRODUCTION IN FISH

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Kisspeptin has been established as an important regulator of reproduction in lower vertebrates, such as teleosts and amphibians. The stimulatory effect of the kisspeptins (Kiss1&2 in most teleosts) on reproduction via Gnrh and, in turn, Lh and Fsh, was demonstrated early on in teleosts. A direct action of kisspeptin on the pituitary was then demonstrated via pituitary *in vitro* studies and direct hypophyseal innervations in the striped bass (stb). These direct innervations originated from the major Kiss2 neuronal population in the hypothalamus, but also from temporal populations in the medio-basal hypothalamus at the time of spawning. Using specific Kiss2 antagonists, we have shown that these neurons are critical for the execution of spawning in male stb. Additional reproductive pathways have since emerged: 1) kisspeptin was shown to dramatically modulate arginine-vasotocin (AVT) and isotocin, which are implicated in regulating spawning behavior and gametogenesis. AVT and isotocin neurons in the preoptic area express kisspeptin receptors in the stb and medaka; 2) in zebrafish, Gnrh-inhibiting hormone (Gnih) receptors were neuroanatomically undetectable in the brain and pituitary, despite a clear inhibitory effect of Gnih on Gnrh3. Consequently, we have demonstrated that Gnih peptides antagonize Kiss2 activation of Kissr2 and that Gnih neurons innervate Kissr2 expressing neurons in the preoptic area and the hypothalamus along the path of Gnrh3 projections toward the pituitary; 3) unlike in mammals, kisspeptin and neurokinin B (Nkb) are not co-localized in the same neurons (known as KNDy neurons) in the brain of stb and goldfish. *In vivo* and *in vitro* studies have demonstrated that Nkb exerts its effect on Gnrh via the inhibition of Kiss2 in the stb. This was supported by the finding that Nkb neurons directly innervate the neighboring Kiss2 neurons in the hypothalamus. Altogether, our studies present kisspeptin as a centralized 'hub' neuropeptide: it acts via multiple pathways, modulating a wide range of major reproductive regulators in the brain and pituitary. Kisspeptin neurons also respond to multiple reproductive regulators, among which is Nkb, and from the well-known feedback of gonadal steroids

S21-5) Dean, Semmens (UK)

THE EVOLUTION OF KISSPEPTIN SIGNALLING: INSIGHTS FROM INVERTEBRATES

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Kisspeptins are key regulators of reproductive maturation, triggering hypothalamic secretion of gonadotropin-releasing hormone (GnRH) to stimulate the release of gonadotropins from the pituitary. Accordingly, mutations in the kisspeptin receptor cause delayed puberty in humans. Kisspeptin signalling systems have been identified throughout the vertebrates and experimental studies on non-mammalian vertebrates have provided evidence of a conserved role in the regulation of reproductive maturation. However, until recently, nothing was known about the occurrence of kisspeptin signalling systems in invertebrates. In 2013, a comprehensive analysis of neuropeptide signalling systems across the Bilateria revealed the presence of kisspeptin-type receptors in non-chordate deuterostomes (echinoderms, hemichordates) and in lophotrochozoans (molluscs, annelids). Thus, the evolutionary origin of the kisspeptin signalling system can be traced back to the common ancestor of the Bilateria but with subsequent loss in some lineages (urochordates, ecdysozoans). Importantly, the analysis also revealed the presence of four genes encoding putative precursors of kisspeptin-type peptides in the cephalochordate *Branchiostoma floridae*. Recently, we reported the discovery of 40 neuropeptide precursors in the common European starfish *Asterias rubens* (Phylum: Echinodermata). Interestingly, we identified a precursor encoding two kisspeptin-type peptides (ArKP1-2). These are the first kisspeptin-type peptides to be identified outside of the chordate branch of the animal kingdom. Moreover, we have also identified ten candidate kisspeptin-type receptors indicating that the kisspeptin signalling system is more "complex" than in the vertebrates. Importantly, we have confirmed the sequences of ArKP1-2 via mass spectrometry (LC-ESI-MS/MS) and have shown that ArKP2 is the ligand for at least one of the ten kisspeptin-type

receptors. The discovery of a kisspeptin signalling system in the starfish *A. rubens* has provided a framework to investigate the function of kisspeptin signalling in an invertebrate for the very first time and provide important new insights into the role of kisspeptins as evolutionarily ancient regulators of reproduction.

S21-6) Hatef, Azadeh (Canada)

INTERACTION OF GnRH, KISSPEPTIN AND SEX STEROIDS WITH NUCB2/NESFATIN-1 IN MICE HYPOTHALAMIC NEURONS AND GONADOTROPES

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Neuroendocrine regulation of metabolism and reproduction are tightly interlinked. Nesfatin-1 is an 82 amino acid metabolic peptide derived from nucleobindin-2 (NUCB2). NUCB2 mRNA and protein significantly increase in the hypothalamus of rats during puberty-to-adult transition. Administration of nesfatin-1 modulates circulating LH and testosterone levels in male rats. However, whether nesfatin-1 acts directly on neurons and gonadotropes to elicit this action remain unknown. In addition, whether reproductive hormones of the hypothalamo-pituitary gonadal axis modulate NUCB2/nesfatin-1 is unclear. To address this, we employed murine hypothalamic (GT1-7) and pituitary (LBT2) cells *in vitro*. NUCB2 mRNA expression, and NUCB2/nesfatin-1 immunoreactivity were observed in both GT1-7 and LBT2 cells, and in the hypothalamus of mice. Nesfatin-1 co-localized GnRH in GT1-7 cells, and in the hypothalamic perikarya of mice. Cells were treated with kisspeptin, GnRH, and estradiol and testosterone, as well as nesfatin-1 for 2, 6 or 24 hours. Synthetic nesfatin-1 increased Kiss1R and GnRH mRNAs in GT1-7 cells and LHB in LBT2. Nesfatin-1 increased GnRH and LHB protein expression in GT1-7 and LBT2 at 6-hour post incubation respectively. Both NUCB2 mRNA and protein were increased in GT1-7 cells treated with kisspeptin. Testosterone increased NUCB2 mRNA and protein expression in GT1-7 and LBT2. 17b-estradiol increased NUCB2 mRNA and protein expression in LBT2. Nesfatin-1 acts directly on hypothalamic neurons and gonadotropes to elicit a generally positive influence on the endocrine milieu regulating reproduction in mice. Reproductive hormones, in turn, modulate brain and pituitary NUCB2/nesfatin-1. In conclusion, we provide new information to designate nesfatin-1 as a novel, additional factor that helps reproductive success. Acknowledgements: Supported by: Canadian Institutes of Health Research (CIHR); Saskatchewan Health Research Foundation (SHRF); Leader's Opportunities Fund from the Canada Foundation for Innovation (CFI).

Posters Abstracts

P1 (and S15-4)
THE ROLE OF THE TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP) FAMILY IN ENERGY PRODUCTION IN PROTOCHORDATES AND CHORDATES.

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Stress can overwhelm the survival of an organism if it cannot cope properly. Stress demands a higher energy budget in the cell in order to overcome the stress, thus mechanisms of enhancing energy production would be a potential target to aid in the stress response. The teneurin C-terminal associated peptide (TCAP) family is a novel energy regulator of glucose metabolism. As enhanced skeletal muscle function is a key component of the stress response, our work aims to elucidate the role of TCAP in stress and anxiety in these tissues. Moreover, as evolutionary analyses indicate that TCAP is an ancestral peptide, we took a comparative approach to study the conservation of its roles. First, in the vase tunicate, *Ciona intestinalis*, we designed a novel behavioural assay to determine the role of TCAP on stress-related behaviour through muscle contractions. TCAP treatment in *C. intestinalis* increased its contractile behaviour and decreased the stress response to the stimulant, suggesting that TCAP increased energy and decreased stress in this model. In zebrafish, we designed a novel stress-metabolism assay used to characterize the stress response in a confined space, by measuring their metabolic output. This assay revealed TCAP-treated animals demonstrated lower stress-related responses than vehicle-treated animals. Moreover, TCAP demonstrated a significant dose-dependent increase in energy production, by using an *in vivo* resazurin assay. Lastly, the role of TCAP as an energy regulator is conserved in rodents. After induced metabolic fatigue, TCAP-treated rats showed improved muscle contractile function under these stress conditions compared to vehicle-treated rats. In addition, TCAP-treatment significantly increases glucose uptake in muscle *in vivo*, as visualized by radioactive deoxyglucose uptake in functional positron emission tomography (fPET) scans. Behavioural tests, such as open field test, indicate these animals are also less anxious. Our results demonstrate that TCAP's role in energy modulation is conserved in both protochordate and chordate models and that the enhanced energy budget allows for a decreased stress response, resulting in anxiolytic effects on behaviour.

P2
RESTRAINT STRESS AND INDUCTION OF NEURODEGENERATION DEMAND DIFFERENTIAL GENE EXPRESSION AND PROTEIN ABUNDENCE OF ISOFORMS OF N_A^+/K^+ AND C_A^{2++} TRANSPORTERS IN MICE BRAIN

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Thyroid hormones (TH) are critical for the differentiation and maintenance of brain functions. It is likely that TH could interact with cortisol, a prominent stress hormone that could contribute to neurodegenerative disorders. We thus tested the action of TH on ion transporter functions and examined whether THs are involved in the progression of neurodegeneration in stressed mice brain. Gene expression and protein abundance of neuronal-specific Atp1a1, Atp1a3 and Atp1b1 isoforms of Na^+/K^+-ATPase (NKA) and Atp2b2 and Atp2b3 isoforms of plasma membrane Ca^{2+} ATPase (PMCA) were quantified in the brain of restraint-stressed and MTPTP-treated mice. Molecular analyses of the isoforms expression in cortex, hippocampus and cerebellum of eight weeks old mice brain showed differential regulation, indicating a critical role of TH in Na^+ and Ca^{2+}signaling. Immunocytochemical localization of these molecular markers further revealed spatial and temporal distributions of isoforms, confirming the decisive actions of TH in both stressed and neurodegeneration-induced mice models. Taken together, these data provided evidence for a critical role of TH in the ion transporter functions during neurodegeneration in stressed mice brain (supported by grants from H.Edn Dept of Govt of Kerala and UoK).

P3
TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-1 ANTAGONIZES CORTICOTROPIN-RELEASING FACTOR (CRF)-INDUCED INCREASES IN INTRACELLULAR CALCIUM.

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Teneurin C-terminal associated peptide (TCAP)-1 is a member of an evolutionarily ancient family of neuropeptides that regulates cellular energy production and protects against organismal stress. Encoded in the terminal exon of teneurin-1, this peptide can be expressed by the full-length teneurin-1 translation, or by a separate mRNA. TCAP-1 is antagonistic to corticotrophin releasing factor (CRF) in mammals; however, the intracellular signaling pathways involved in this interaction have remained unresolved. Calcium signalling is integral to neuronal communication, and modulation of calcium cascades has important downstream effects of neurotransmission and energy metabolism. This suggests that the TCAP-mediated suppression of CRF actions in neurons may result from modulation of CRF-induced calcium signaling. Therefore, the aim of this study was to further investigate the physiological role of TCAP-1 during CRF-associated stress in neurons, and determine if the anxiolytic effects of TCAP-1 are the result of the suppression of CRF-activated calcium signalling. Using an in vitro approach with immortalized hypothalamic neurons, in combination with live-cell fluorescent imaging, we show that CRF increases cytosolic calcium, and that pre-treatment with TCAP-1 prevents the CRF-induced calcium increase. Application of TCAP-1 alone decreases cytosolic calcium, and at the same time depolarizes mitochondrial membrane potential, indicating that cytosolic calcium decreases result from mitochondrial uptake of cytosolic calcium. Conversely, CRF alone hyperpolarizes mitochondrial membrane potential indicating

that TCAP-1 activates a signalling cascade that shunts CRF-mediated cytosolic calcium into the mitochondria. Together, these data indicate that the anxiolytic effects of TCAP-1 result from inhibition of CRF-induced calcium increases, and may also result from stimulation of mitochondrial energy metabolism.

P4

ANALYSIS OF PI3K-PDK1-AKT AND RAF-MEK-ERK PATHWAY ACTIVATION IN GOLDFISH PITUITARY GONADOTROPES AND SOMATOTROPES.

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Mitogen-activate protein kinase-dependent signaling is highly conserved in eukaryotes and is organized as linear three-kinase cascades; most common of which is the canonical Raf-MEK-ERK transduction cassette. Phosphoinositide 3-kinase (PI3K)-controlled signaling networks, including the downstream effectors phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B (Akt), commonly communicate with the Raf-MEK-ERK cascade through intricate cross-talk in order to coordinately control aspects of cellular metabolism. In the present study, we explored the architecture of the PI3K-PDK1-Akt and Raf-MEK-ERK signaling network within primary cultures of dispersed goldfish pituitary cells using validated primary antibodies, as well as selective inhibitors in imaging flow cytometry and immunoblotting studies. Results demonstrate that although the PI3K-PDK1-Akt and Raf-MEK-ERK signaling cascades are constitutively active in goldfish gonadotropes and somatotropes, PI3Ks are not a major regulator of basal Raf-MEK-ERK activity in unstimulated goldfish pituitary cells. Just as importantly, we demonstrate a clear dissociation between the influences of PI3K-PDK1-Akt and Raf-MEK-ERK signaling on total hormone protein availability in relationship to previous results on gonadotropin subunit and growth hormone mRNA expression. (Supported by NSERC, AIHS, and the Killam Trusts).

P5

DIFFERENTIAL INVOLVEMENT OF THE PI3K-PDK1-AKT-TOR SIGNALLING CASCADE DURING THE LONG-TERM CONTROL OF BASAL LH AND GH RELEASE AS WELL AS CELLULAR HORMONE CONTENT.

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Across animal models, phosphoinositide 3-kinases (PI3Ks) are central to the cellular control of protein synthesis. However, whether PI3K-dependent signal transduction contributes to the integrated control of basal pituitary hormone synthesis and release has not been examined in detail. Using goldfish pituitary cells, a classical model used for studying the neuroendocrine regulation of reproduction and growth in cyprinids, we performed pharmacological mapping of PI3K-dependent signaling using broad-spectrum inhibitors of PI3K isoforms (LY294002 and GDC0941) in addition to selective inhibitors of canonical PI3K transduction targets protein kinase B (Akt; Akt_i VIII) and phosphoinositide-dependent kinase 1 (PDK1; GSK2334470), as well as target of rapamycin (TOR) complex 1 (TORC1; rapamycin) and complex 2 (TORC2; INK128, which targets TOR kinase activity within both TORC1and TORC2) in static incubation studies across 2, 12, and 24 hrs. At each time point, LH and GH levels in the cell culture supernatants (released) and cellular protein extracts (cellular content) were quantified by radioimmunoassays. The sum of cellular hormone contents and released hormone for each individual treatment (total) were used as an index of hormonal protein availability and production. Results identify PI3K-dependent signaling, and more specifically the Akt-TOR signaling node, as an important regulator of long-term basal pituitary hormone release and availability. In addition, these data strongly support the idea that long-term basal hormone release and synthesis can be regulated by dissimilar intracellular mechanisms that are clearly time- and cell type-dependent. (Supported by NSERC, AIHS, and the Killam Trusts).

P6

CLONING, LOCALIZATION, AND PHYSIOLOGICAL EFFECTS OF SULFAKININ IN THE KISSING BUG, *RHODNIUS PROLIXUS*

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Sulfakinins (SKs) are a family of multifunctional neuropeptides that have been primarily shown to have myotropic activity on muscles of the digestive system and to function as feeding satiety factors. Our initial experiments have confirmed the presence of two sulfakinins (Rhopr-SK-1 and Rhopr-SK-2) in *Rhodnius prolixus*. Reverse transcriptase quantitative PCR (RT-qPCR) demonstrated that the *Rhopr-SK* transcript is mainly expressed in the central nervous system (CNS) in unfed fifth-instar *R. prolixus*. Fluorescent *in situ* hybridization showed transcript expression only in neurons in the brain. Immunohistochemical staining of SK-like peptides was observed in the same neurons in the brain and in processes extending throughout the CNS, as well as over the posterior midgut and anterior hindgut. Rhopr-SK-1 induced contractions of the hindgut in a dose-dependent manner, but had no significant effect on heartbeat frequency. Injection of Rhopr-SK-1 significantly decreased the overall weight of the subsequent blood meal consumed, suggesting SK's role as a satiety factor in *R. prolixus*. A seven-transmembrane Rhopr-SK G-protein coupled receptor (GPCR) was cloned and characterized. RT-qPCR of the receptor transcript

revealed that the target tissues for Rhopr-SK-1 and/or –SK-2 are primarily located in the CNS, with lower expression in the heart, gut, salivary glands, Malpighian tubules, as well as male and female reproductive tissues. Rhopr-SK-1 inhibits contractions of the oviduct in adult *R. prolixus* in a dose-dependent manner. These findings suggest that Rhopr-SKs are involved in the control of feeding and reproduction in *R. prolixus.* Acknowledgements: We thank Professor Jan Veenstra for provision of the antiserum. Supported by: NSERC Discovery grants to ABL and IO.

P7

CLONING AND CHARACTERIZATION OF SEA LAMPREY THYROSTIMULIN, A NOVEL PITUITARY GLYCOPROTEIN HORMONE

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Unlike all jawed vertebrates that have three canonical pituitary glycoprotein hormones (GpHs; luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH)) and their respective receptors, jawless vertebrates (lamprey and hagfish) represent the earliest divergence in vertebrate phylogeny and have two GpHs (lGpH, thyrostimulin) and two receptors (lGpH-R I, II). Thyrostimulin, comprised of homologous alpha (GpA2) and beta (GpB5) subunits, was discovered 15 years ago and received its name for its ability to activate the TSH receptor in mammals. GpA2 and GpB5 are found in representative species of bilaterians and are considered ancestral alpha and beta subunits to the vertebrate pituitary glycoprotein hormone family. Lamprey GpH (lGpH) has the GpA2 subunit of thyrostimulin and a typical beta subunit. We report here the cloning of sea lamprey GpB5, which forms a heterodimer with GpA2 to constitute a novel lamprey pituitary thyrostimulin. The full-length cDNA of lGpB5 encodes a protein of 174 amino acids with ten conserved cysteine residues and one glycosylation site conserved with other vertebrate GpB5 sequences. Phylogenomic and synteny analyses support the basal positioning of lGpB5 to other vertebrates. Fluorescent in situ hybridization and immunohistochemistry showed that both lGpA2 and lGpB5 are co-expressed throughout the pituitary during each life stage, i.e., larval, parasitic, and adult phases. Intraperitoneal injection with lGnRH-III (100µg/kg) increased pituitary GpA2 and GpB5 mRNA expression in sexually mature adult female lamprey. Recombinant heterodimerization was achieved by producing a single expression plasmid with two methanol promoters for independent subunits with His or FLAG tags in Pichia pastoris, followed by histidine pull down by nickel-agarose batch purification, and visualized by reducing PAGE and duel-fluorescent Western blot. These data provide evidence that lamprey thyrostimulin is a novel, functional pituitary glycoprotein hormone. Support: NSF IOS-1257476 and AES NH00624 to SAS.

P8

NEUROKININ B RECEPTOR SEQUENCE FROM THE BRAIN OF CHIROSTOMA HUMBOLDTIANUM (ATHERINIFORMES: ATHERINOPSIDAE).

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The silverside Chirostoma humboldtianum is an endemic fish from central highland and western Mexico and traditionally, it has been used as food. Neurokinin B (tac3) and its receptor (tac3r) has been reported to play an important role in reproduction. In fish, the tac3r has been reported just in a few species. In this work mature fish from Zacapu lagoon, Michoacan, Mexico were collected and the brain were removed and kept in dry ice. Posteriorly, the total RNA was extracted and the amplification was made with one step RT-PCR system SuperScript III using specific designed primers.Two products were obtained. The first product is 452bp and correspond to fragment transmembrane domain 3 to transmembrane domain 6 of tac3Ra. The second fragment is 586bp, from transmembrane domain 3 to transmembrane domain 7 of tac3Rb. The identity for fragment tac3Ra is 83%, 80% and 79% with Gasterosteus aculeatus, Tetraodon nigroviridis and Oreochromis niloticus respectively. The fragment of tac3Rb identity is 81%, 80% and 77% with Morone saxatili, Oryzias latipes and O. niloticus. In conclusión, this is the first report of two tac3 receptors subtypes in the brain of Atherinopsodae family.

P9

A GONADOTROPIN-RELEASING HORMONE 2 (GNRH2) KNOCKOUT ZEBRAFISH LINE REVEALS THE DUAL ROLE OF GNRH2 IN FEEDING AND REPRODUCTION.

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The evolutionarily conserved isoform of the gonadotropin-releasing hormone (GnRH) neuropeptide, GnRH2, is found ubiquitously in the midbrain of all jawed vertebrates except rodents. Despite the absolute conservation of the peptide sequence of GnRH2 between vertebrate groups, suggesting important conserved roles for this peptide, the functional roles of GnRH2 are not well known and have been much less studied than the hypophysiotropic form of GnRH, GnRH1 or GnRH3. Only a few behavioral studies in goldfish, zebrafish, musk shrew, and mice suggest a role of GnRH2 in decreasing feeding and stimulating reproductive behaviors. Nonetheless, no studies have examined this peptide thoroughly or deduced its mechanisms of action. Zebrafish has two forms of GnRH, including the hypophysiotropic GnRH3

and GnRH2, and has emerged as an ideal model organism to study GnRH2 due to its ease of genetic manipulation and fast maturation time. We aimed to study the functional roles of GnRH2 by knocking out the *gnrh2* gene in zebrafish and conducting loss-of-function studies to identify any differences in feeding, growth, and reproduction. Using our *gnrh2*$^{-/-}$ line, we saw increased feeding behaviors and growth associated with the loss of GnRH2. Additionally, this corresponded with differences in molecular levels of several different feeding associated peptides, such as *agrp1* and *pomca*. In terms of reproduction, *gnrh2*$^{-/-}$ fish showed decreased expression levels of *lh*, along with a reduction in oocyte size and quality. Interestingly, under a starving regime, *gnrh2*$^{-/-}$ fish had significantly reduced spawning success compared to wild-type fish. In support of these findings, ICC on wild-type fish pituitaries shows long-term starvation induces a marked decrease in GnRH3 neuronal fibers and increase in GnRH2 fibers, suggesting GnRH2 takes over the hypophysiotropic role of GnRH3. These findings suggest GnRH2 has multiple roles in controlling feeding under high metabolic states, promoting gamete maintenance under normal metabolic conditions, and enabling successful reproduction in low metabolic conditions.

P10

SEXUAL MATURATION AND DIMORPHISM IN THE BRAIN OF THE INDIAN CARP, *LABEO ROHITA* AT THE LEVELS OF NEUROSTEROIDOGENESIS

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Neurosteroidogenesis is playing a vital role in governing the physiology of reproduction along with neuropeptides and neurotransmitters. Gonadal development influences steroid synthesis in the central nervous system (CNS), and the CNS also regulates the gonadal steroid production. It is well known that the receptors of estrogen modulate the production of GnRH, and serotonin, dopamine and GABAergic neurons modulate the steroidogenic enzymes. However, the influence of neurosteroids and variations of synthetic pathway towards reproductive cycle is not studied in detail in Indian carps. Here we examined the presence of various steroids in specific areas of the brain, including the quantitative difference in estradiol (E2), testosterone (T), 11-ketotestosterone (11-KT), androstenedione (A), DHEA, and 21-hydroxyprogesterone (21-P), and the conversion of 5α-DHP to 5α,3α-THProg and 5α-DHT to 5α,3α-THT by 3α-HSD. The brains of reproductively active female fish showed high testosterone levels when compared with the male brain. It has been derived from the expression of Cyp19 and Cyp17 are higher than the Cyp21. The steroidal production in the incubated tissues of brain highlights the augmented presence of 5α- or 3α-reductase evidence the elimination pathway. The quantitative expression of mRNA analysis of 3α-HSD, 3β-HSD, Cyp17, Cyp19 and Cyp21 substantiate the variation in sex and maturation of gonadal stages. Aromatase indicate the shift in the sex dependent pathway. The sulphated steroids of pregnenalone and DHEA indicate the presence of hydroxysteroid sulfotransferase (HST) in its exclusion pathway. The study suggests that the sexual modulation can be carried out at the CNS by manipulating the steroids and their receptors, more particularly in the thalamus region of the brain. Planned studies on transcriptomics using NGS with region-specific analysis of the brain will unravel the pathways involved in the integration of steroidal signals in gonadal development.

P11

TRANSGENIC MODELS AND IN VIVO IMAGING OFFER NEW INSIGHTS INTO THE DEVELOPMENT AND FUNCTION OF THE TELEOST GNRH-GONADOTROPE AXIS.

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Reproduction in vertebrates is dependent upon the release of the gonadotropins LH and FSH from the pituitary, mainly under the control of GnRH. The longstanding perception is that in fish the neuroendocrine control of pituitary cells is achieved via direct communication between neuroendocrine axons and their endocrine cell targets and not through the portal system as in tetrapods. However, we recently provided evidence underscoring the importance of neurovascular delivery mode of GnRH to gonadotropes. Furthermore, we found distinct differences in the proximity of the two gonadotrope types to the vessels as well as to the GnRH terminals. By studying functional pituitary cell networks we were able to show that LH and FSH cells also differ in their level of cell-cell coupling. An additional pathway for gonadotropin regulation was revealed by studying the largely neglected stellate cells of the fish pituitary. We describe a long-range stellate cell network that may regulate FSH release through follistatin.
Next, to enhance our understanding of the development of the reproductive axis we generated transgenic zebrafish expressing calcium-sensitive proteins in GnRH cells. Using these fish we are able to use in vivo calcium imaging to monitor GnRH neuron activity throughout their migration from the nasal placode to the brain. We found that at early stages, GnRH neurons exhibit highly synchronized activity between the two sides of the brain. By following the projections of single neurons we are able to identify contralateral connections that may mediate the synchronization within the GnRH circuit. Genetically-induced lectin tracing and electrophysiological recordings further validate the notion of both ipsilateral and contralateral GnRH cell coupling that is mediated within the circuit. Finally, by silencing the activity of individual GnRH neurons, we show a strong correlation between calcium activity and the ability of the neurons to migrate into the brain. Taken together, our findings shed new light on the development of the GnRH-gonadotrope axis in fish and have important implications regarding the differential regulation of LH and FSH that underlie their distinct release patterns. Acknowledgements: MG is supported by a Marie Skłodowska Curie individual fellowship.

P12
MOLECULAR MECHANISM OF GONADOTROPIN-INHIBITORY HORMONE ACTION ON REPRODICTION

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Since GnIH was discovered in 2000 as the first hypothalamic neuropeptide actively inhibiting gonadotropin release, it has been demonstrated that GnIH acts as a pronounced negative regulator of reproduction. Inhibitory effect of GnIH on reproduction is mainly accomplished at hypothalamic-pituitary levels; GnRH neurons and gonadotrpoes are major targets of GnIH action based on the morphological interaction with GnIH neuronal fibers and the distribution of GnIH receptor (GnIH-R). We have demonstrated the molecular mechanism of GnIH action by investigating the signaling pathways of GnIH-R occurring in these target cells. For the mechanistic study of target cell-specific action, we used *in vitro* models, GnRH neural GT1-7 and gonadotrope LβT2 cells. We examined GnIH-mediated second messenger pathways by GPCR reporter assay, and then analyzed the change in downstream MAPK phosphorylation. GnIH specifically inhibited the AC/cAMP/PKA-mediated ERK and/or p38 pathways in GnRH neurons as well as gonadotropes. The physiological relevance of the inhibitory effect of GnIH on each target cell was indicated by the reduction of GnRH or gonadotropin levels by GnIH treatment. GnIH effectively suppressed the stimulatory effect of VIP and kisspeptin on GnRH release, and GnRH-induced LH release was also decreased by GnIH. Our results indicate that GnIH may govern the hypothalamic neuronal activities of GnRH by inhibiting the action of VIP and kisspeptin, and eventually reduce pituitary gonadotropin secretion. We have also demonstrated how imbalance between endocrine systems affects reproduction *via* GnIH regulation. Activation of adrenal system by stress stimuli increased GnIH expression through the direct binding of GR to its promoter, and abnormal thyroid status altered GnIH expression with chromatin modification changes. Together, our findings indicate the significance of GnIH as a key hypothalamic inhibitor to regulate reproduction.

P13
SIGNAL TRANSDUCTION MECHANISM MEDIATING PROLACTIN CONTROL OF GILL NA, CL COTRANSPORTER (NCC2B) IN MEDAKA

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Prolactin is a potent stimulator of Na,Cl cotransporter (Ncc2b) expression in various teleosts including Japanese medaka, *Oryzias latipes*. The mechanism by which prolactin regulates Ncc2b expression, and hence extra-renal ion uptake, in fresh water is poorly understood. In this study we used in vitro gill explants from medaka to examine whether prolactin might stimulate Ncc2b expression through activation of Stat5, Akt (downstream of PI3 kinase) or Erk1/2. Using overnight treatment, we found that ovine prolactin induced a concentration dependent stimulation of *ncc2b* with significant effects of 10, 100 and 1000 ng of hormone per ml media (2-6 fold). The effect was abolished by co-incubation with the STAT5 inhibitor N-((4-oxo-4H-chromen-3-yl)methylene)nicotinohydrazide. We also tested involvement o f the PI3K/Akt and Erk1/2 pathways using an inhibitor of Akt (Carb) and Mek (U0126). Neither of these latter pathway inhibitors interacted with the effect of prolactin on Ncc2b. To understand the molecular mechanisms, we analyzed the early effects of prolactin on signaling kinase activation. Prolactin induced a concentration dependent stimulation of Stat5 phosphorylation (5-15 fold), while no significant activation of Akt of Erk1/2 was observed. These data suggest that prolactin elicit its Ncc2b dependent ion-regulatory effect by downstream activation of Stat5. The cellular underpinning of the signalling pathway involved in the above changes is currently being investigated by localization studies within the gill.

P14
MELANOCORTIN RECEPTOR-4 REGULATES CELL FUNCTION AND THE COUNTER-REGULATORY RESPONSE TO INSULIN-INDUCED HYPOGLYCEMIA IN MICE.

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Previous studies have demonstrated POMC-null mice lack a counter-regulatory response to insulin-induced-hypoglycemia and lack accompanying glucagon secretion (*Endocrinology* (2003) 144:5194-202). In the current study we used melanocortin receptor antagonists to test the role of peripheral melanocortin receptors in the counter regulator response. The MC3R/MC4R antagonist SHU9119 inhibited the counter- regulatory response to insulin-induced hypoglycemia in wild type mice. Then the hypothesis that alpha cells themselves can respond to a melanocortin peptide from the POMC gene was tested. RT-PCR analysis demonstrated that mouse islets express MC4R but not MC3R and immunofluorescence of islet cells indicated that pancreatic alpha cells but not beta cells express MC4R. The POMC-derived peptide MSH t riggered an increase in intacellular Ca^{2+} in isolated mouse alpha cells. Finally, the counter regulatory response to insulin-induced hypoglycemia was greatly impaired in MC4R-null mice as compare to wild type littermates. Together these findings point toward an important role for alpha-cell MC4R in control of glucagon secretion. Acknowledgements: Supported by NIH grant R15 DK89442.

P15
ADVANCED FOLLICLE ACTIVATION IN THE ZEBRAFISH OVARY BY MODIFICATION OF THE TRANSCRIPTION FACTOR YBX1/*ybx1* WITH GENOME EDITING TECHNOLOGY

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In mammals, folliculogenesis contains gonadotropin-independent preantal stage and gonadotropin-dependent antral stage. This is similar in non-mammal vertebrates. For instance, zebrafish ovarian follicle development is divided into gonadotropin-independent primary growth (PG) and gonadotropin-dependent secondary growth (SG). Recruitment of follicles from dormant PG stage to fast growing SG stage (follicle activation) is a key event in ovarian development, which is crucial for female fertility. Using comparative proteomics approach, we have previously identified Y-box binding protein-1 (YB-1; Ybx1/ybx1), a germ cell abundant transcriptional factor and mRNA binding protein to be a potential factor that controls early follicle development. Our previous data suggested that the function of Ybx1 in the oocyte depended on its cellular localization. Its localization in the cytoplasm as determined by the cytoplasmic retention signal (CRS) at the C-terminus would favor its function as mRNA binding protein, which blocks follicle development; whereas the loss of CRS promotes its nuclear translocation and function as a transcription factor, favoring follicle development or activation. To test this hypothesis, we generated a Ybx1 mutant zebrafish with the C-terminus truncated by using the recently developed genome editing approach - Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9. The mutated Ybx1 was preferably located in the nucleus as a truncated form. Phenotype analysis demonstrated that the activation of PG follicles was significantly advanced in the mutant ovary than that in the wild type control. Moreover, the mutant female fish started to spawn ten days earlier than the wild type siblings, but with a lower fecundity. Further studies are now being undertaken to understand the mechanisms by which Ybx1 regulates early follicle development. Acknowledgement: This work was funded by grants from University of Macau and The Macau Fund for Development of Science and Technology.

P16
ROLE OF ARTHROPOD INSULIN SIGNALING IN PARASITE DEVELOPMENT WITHIN THE ARTHROPOD VECTORS
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In vertebrates, members of insulin-like peptide (ILP) super family are important growth factors and multifunctional hormones. As many as ten different ILPs, including insulin, insulin-like growth factors (IGF), and relaxin, occur in humans and other mammals. Multiple ILPs are also present in invertebrate species, including mosquitoes. Previously, we identified eight different ILPs and components of an insulin-signaling pathway in female *Aedes aegypti* mosquitoes. In bioassays, one ILP, ILP3, stimulated egg maturation and ovarian steroid production. This peptide showed high specific binding to ovary membranes, and expression of the insulin receptor was required for ILP3 action, as determined by RNA interference. Insulin receptor (IR) knockdown in *Culex quinquefasciatus* mosquitoes completely prevented development of filarial nematode, *Wuchereria bancrofti*, to the infective L3 stage, and reduced, but did not prevent, development of another filarial nematode, *Brugia malayi*, in *A. aegypti* mosquitoes. Additionally, IR knockdown in *Anopheles gambiae* mosquitoes resulted in reduced number of *Plasmodium falciparum* oocysts on mosquito midguts. However, at present essentially nothing is known regarding the mechanisms by which mosquito insulin signaling affects the development of multi-cellular filarial parasites and the protozoan malaria parasites in the same mosquito species. Additionally, most insecticides currently in use for mosquito control work by binding to the Na+ channels in the nervous system of the mosquitoes, thus altering its gating properties. IR, a receptor tyrosine kinase, provides a unique target that has not previously been selected for insecticide resistance.

P17
ORIGIN AND DIVERSIFICATION OF THE INSULIN SUPERFAMILY AND ITS RECEPTORS IN VERTEBRATES: REVISITED

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The vertebrate insulin superfamily is a diverse group of peptides that share a similar 3-dimensional structure, and are involved in broad range of endocrine and paracrine functions regulating cell growth, metabolism and reproduction. Gaps remain in the understanding of the origins of the vertebrate insulin superfamily ligand-receptor systems. Here we take advantage of recent genomic and transcriptomic data and state of the art computational techniques to revisit the origins of the superfamily and the processes by which it diversified in vertebrates. In particular, we collect genomic sequence and transcriptomic data for the insulin-like peptides (ILP) in protochordates and echinoderms, and then use a combination of ancestral genome reconstruction mapping, small-scale synteny, gene structure, tissue-specific gene expression, motif analyses and phylogenetic reconstruction to probe the relationships and mechanisms of diversification of the two major subfamilies of ILPs in vertebrates: 1) insulin (Ins) and insulin-like growth factors (Igf) and 2) relaxin (Rln) and insulin-like peptides (Insl), and their diverse receptors. We characterize multiple ILPs from early deuterostomes and show that a single Rln and two linked IGF-INS genes were most likely present in the ancestral vertebrate genome. These three ancestral genes (anc-RLN, anc-IGF, anc-INS) predominantly (but not exclusively) duplicated during the 2R and 3R whole genome duplication events that occurred during vertebrate evolution as did three of the ancestral receptors (anc-RXFP1/2, anc-RXFP3/4, anc-IR). These findings help understand the relationship and identity of members of the insulin superfamily in different taxa and shed light on the processes that shaped the repertoire in vertebrates.

P18
NEUROSECRETORY PROTEIN GL, A NOVEL HYPOTHALAMIC SMALL PROTEIN, PROMOTES ADIPOSITY IN RATS.

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The mechanisms underlying the central regulation of adiposity in peripheral tissues are not fully understood. We found a novel cDNA in chicken hypothalamus and deduced a precursor protein including a secretory protein of 80 amino acids. This small protein has Gly-Leu-NH_2 at its C-terminal and was named neurosecretory protein GL (NPGL). NPGL was robustly produced in the mediobasal hypothalamus of rats. Histological analyses showed that both *Npgl* mRNA and its mature protein were localized in the lateroposterior part of the Arc (ArcLP) and the ventral tuberomammillary nucleus (VTM). ArcLP and VTM are known as one of centers regulating energy homeostasis. We speculated that NPGL regulates feeding behavior and energy metabolism. Using protein administration and gene overexpression in rats, we established that NPGL increased lipid accumulation in white adipose tissue (WAT) without remarkable change in food intake. This adiposity was associated with an induction of *de novo* lipogenesis in WAT but not in liver. NPGL did not change the food intake under the normal chow, but increased under the high calorie diets. NPGL selectively induced carbohydrate intake during the day time, when the frequency of food intake is low, and increased blood insulin level. *Npgl* mRNA expression was upregulated by fasting and low insulin levels and NPGL-producing cells were responsible to insulin. These results point to NPGL as a novel neuronal regulator that drives fat deposition through *de novo* lipogenesis in WAT and acts to maintain steady fatty level in harmony with insulin. Acknowledgements: Supported by: Grant-in-Aid for JSPS Fellows to KS and MEXT/JSPS KAKENHI grants to KU and EI-U.

P19
CORTISOL AND LIPOPOLYSACCHARIDE MODULATE THE IMMUNE RESPONSE OF RAINBOW TROUT LIVER.

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Many studies have shown that stress-induced cortisol levels negatively influence growth and immunity in finfish. Despite this knowledge, few studies have assessed the direct effects of cortisol on liver immune function in finfish. Our goal was to determine how cortisol and the pathogen mimetic, lipopolysaccharide (LPS), affect immune function in rainbow trout liver. Precision cut liver slices were incubated in media containing the following treatments: control (0.01% ethanol), cortisol (100 nano-grams/ml), LPS (30 micro-grams/ml) and cortisol + LPS. Tissues were sampled at 1, 4, 6, and 8 h post-treatment. Using real-time PCR, the expression of two cortisol-responsive genes (GR: glucocorticoid receptor -1 and SOCS-1: suppressor of cytokine signaling-I), genes involved with innate and adaptive immunity (Lyz: lysozyme and IgM: immunoglobin-M), and liver-specific antimicrobial peptides (hepcidin and LEAP-2: liver-expressed antimicrobial peptide-2A) were studied. Abundance of GR mRNA was significantly elevated by LPS treatment (above the cortisol and cortisol + LPS-treated groups) and, within treatment, levels increased over time. SOCS-1 mRNA abundance was also elevated by LPS treatment. Abundance of IgM mRNA was similarly higher in the LPS-treated group compared with the other groups, but there was no effect of sampling time. Abundance of hepcidin and LEAP-2A mRNA levels was highest in the LPS-treated group and suppressed in the cortisol-treated groups. Unlike hepcidin, levels of LEAP-2A mRNA were transiently elevated over time. These results showed that cortisol directly suppresses abundance of GR, SOCS-1, IgM, hepcidin and LEAP-2A mRNA transcripts in the rainbow trout liver. We believe this is the first report of a suppressive effect of cortisol (within 8 h of treatment) on AMP mRNA expression in rainbow trout liver, which further demonstrates that acute stress negatively influences liver immune function in rainbow trout

P20
NEUROKININ B STIMULATES ESTRADIOL PRODUCTION BY ACTING DIRECTLY ON THE OVARY

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Neurokinin B (NKB) and its receptor, NK3R, play critical roles in reproduction by regulating the secretion of the hypothalamic gonadotropin-releasing hormone. NKB and NK3R genes are also expressed in the ovary; however, little is known about their physiological roles within the ovary. The aim of this study was to determine if NKB acts directly on the ovary to regulate reproduction. Injection of NKB into zebrafish accelerated follicle development, increased the mRNA levels of *cyp11a1* and *cyp19a1*, and enhanced estradiol production. Similarly, NKB induced *cyp11a1* and *cyp19a1* expression in primary cultures of zebrafish follicular cells and stimulated estradiol production from cultured follicles. Furthermore, NKB activates CREB and ERK and ERK inhibitors abolished the effect of NKB on *cyp11a1* whereas PKA and CAMKII inhibitors that blocked the activation of CREB, attenuated the effect of NKB on *cyp19a1* expression. In a human granulosa cell line, COV434, a NKB agonist, senktide, also increased CYP11A1 and CYP19A1 mRNA levels and enhanced aromatase protein levels and activities. SiRNA-mediated knockdown of NK3R reduced senktide-induced CYP11A1 and CYP19A1 mRNA levels. Finally, we found that NK3R mRNA was strongly down-regulated in granulosa cells obtained from polycystic ovary syndrome (PCOS) patients when compared with non-PCOS subjects. Taken together, our findings establish a direct action of NKB to induce ovarian estrogen

production and raise the possibility that defective signaling of this pathway may contribute to the development of PCOS. Acknowledgements: Supported by: NSERC discovery grant to CP and FWO-Flanders (G.0343.13) to JS.

P21
INVOLVEMENT OF VASOPRESSIN AND OXYTOCIN SYSTEMS IN BEHAVIORAL BOLDNESS

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Across vertebrate species, vasopressin and oxytocin systems have been implicated in the modulation of neural circuits controlling social behaviors, including effects on social anxiety and attachment. However, the effects of these neuropeptides are not always consistent, and have been shown to vary across social contexts. This variability is at least partly due to different populations of vasopressin and oxytocin neurons becoming active under different contexts. In this study, rather than examining activation of these neurons during social interactions, we investigated how baseline differences in vasopressin and oxytocin neuron number and activity, at different brain nuclei, relate to a general behavioral boldness in sexual and agonistic social contexts. Furthermore, we examined hormone levels and measures of testes and body size as additional variables, since these may influence the neural circuits and behavioral interactions. These studies were conducted in male green anoles (*Anolis carolinensis*), using immunohistochemical characterization of neurons that produce the lizard Ile^3-vasopressin (vasotocin) and Ile^8-oxytocin (mesotocin), as well as the immediate early gene product Fos, a marker of recent neural activation. We characterized populations of neurons within the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus as positive for vasopressin, oxytocin, or colocalizing both neuropeptides. Repeated testing identified a range of behavioral boldness scores (based on intensity, frequency, and latency) that were maintained across repeated testing sessions. Our results demonstrate a negative correlation between the number of vasopressin neurons in the SON and sexual boldness. Although steroid hormone levels were not found to vary with behavioral boldness, testes mass (perhaps an indicator of longer-term androgen levels) was also found to correlate negatively with vasopressin neurons in the SON, as well as positively with sexual boldness, and with oxytocin neurons within the PVN. Surprisingly, baseline levels of Fos were almost nonexistent in either vasopressin or oxytocin neurons. Overall, these results suggest potential long-term effects of androgen levels on numbers of neurons expressing vasopressin and oxytocin, and associated changes in behavioral boldness.

P22
TRANSCRIPT LEVELS OF CORTISOL SIGNALING-RELATED GENES IN THE GILLS OF ATLANTIC SALMON (*SALMO SALAR*) FOLLOWING SEAWATER AND FRESHWATER ACCLIMATIONS.

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Cortisol is a major mineralocorticoid of teleosts and known to have important roles in regulation of numerous osmoregulatory processes during seawater (SW) and freshwater (FW) acclimations. However, it is unclear how the cortisol signaling systems are regulated under these contrasting osmotic conditions. To answer this question, we conducted a series of SW and FW challenge experiments to examine the physiological responses and the transcript levels of cortisol signaling-related genes, glucocorticoid receptors (GR1, GR2), mineralocorticoid receptor (MR) and 11β-hydroxysteroid dehydrogenases (11β-HSD2, 11β-HSD3) in gills of Atlantic salmon (*Salmo salar*). Transcript levels of growth hormone receptors (GHR1, GHR2), prolactin receptor (PRLR) cystic fibrosis transmembrane conductance regulator (CFTR) 1, CFTR2, Na^+/K^+ ATPase (NKA) α1a and NKAαlb were also measured. As expected, gill NKAα1b and CFTR1 transcripts increased and NKAα1a and PRLR transcripts decreased after transfer from FW to SW, whereas NKAα1a and PRLR transcripts increased and CFTR1 transcripts decreased after transfer from SW to FW. Transcript levels of GHR1 and GHR2 showed no change after SW transfer and a slight increase after FW transfer. 11β-HSD3 transcript levels showed no change after transfer into SW and were slightly lower in FW acclimated fish than those in SW acclimated fish. Interestingly, levels of transcripts encoding GR1, GR2 and MR in SW acclimated fish were consistently lower than those in FW acclimated fish, whereas 11β-HSD2 transcript levels in SW acclimated fish were consistently higher than those in FW acclimated fish. Our results indicate that there is a complex regulation of corticosteroid receptors and that 11β-HSD2 may be important for controlling intracellular cortisol levels during salinity change. Acknowledgements: Supported by: JSPS Grants-in-Aid for Scientific Research.

P23
A NOVEL OSMOREGULATORY ROLE OF AN ADIPOKINETIC HORMONE IN THE GASTROPOD MOLLUSK, *APLYSIA CALIFORNICA*

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Adipokinetic hormone (AKH) is a small neuropeptide related to gonadotropin-releasing hormone. In insects, AKH elevates hemolymph trehalose and mobilizes lipid stores during times of high energetic demands. We recently characterized a novel AKH in an intertidal gastropod mollusk, *Aplysia californica.* Immunoreactivity for *A. californica* AKH (ap-AKH) is present in cell bodies of the pleural, cerebral,

and abdominal ganglia, as well as in fibers throughout the central nervous system. Our previous data showed that ap-AKH induced an acute weight reduction in *A. californica* that was likely due to water loss. This suggests that ap-AKH may have an osmoregulatory role, but where and how ap-AKH exerts this effect has not been examined. The goal of the present study was (1) to infer the target tissues of ap-AKH by examining the distribution of ap-AKH and a putative ap-AKH receptor (ap-AKHR) in key osmoregulatory organs, and (2) to examine changes in ion balance induced by ap-AKH. RT-PCR revealed that the putative ap-AKHR is expressed in the heart, gut, kidney, and gills, suggesting a wide range of osmoregulatory organs that can be targeted by ap-AKH. Immunohistochemistry revealed that ap-AKH immunoreactivity is absent in the esophagus, the crop, the intestine, and the osphradium (a chemosensory organ), suggesting the site of ap-AKH production is primarily central. Lastly, ap-AKH injection significantly increased hemolymph conductivity; we are currently examining changes in ion concentrations resulting from ap-AKH treatment. In sum, these data suggest centrally produced ap-AKH may stimulate water loss by acting on multiple osmoregulatory organs, which, in part, lead to increased blood conductivity. Importantly, these results reveal a function of an AKH that has not been observed previously in ecdysozoans, suggesting a lineage-specific functional divergence of this versatile peptide. Acknowledgements: Supported by NSF Grant IOS 1352944 to PST. The authors would like to thank Nate Anderson for his assistance with system assembly and maintenance.

P24
CHARACTERIZATION AND POSSIBLE FUNCTIONS OF GONADOTROPIN-RELEASING HORMONE-LIKE PEPTIDES IN BIVALVE.

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A clear neuroendocrinological regulation of vertebrate gonadotropin releasing hormone (GnRH) controlling gonadal maturation infers possible presence of ancestral molecule in invertebrates. Recent next-generation sequencing (NGS) techniques have identified a number of GnRH-like molecules from a wide variety of invertebrate species, even though their functions are not fully elucidated. In this study, we investigated the GnRH-like molecule in the scallop (py-GnRH), *Patinopecten yessoensis* (Mollusca: Bivalvia) associated with its reproductive parameters. First, we identified full-length cDNA and peptide sequence of the scallop GnRH by PCR cloning and mass spectrometry, respectively. Second, we performed functional study with synthetic py-GnRH peptides for understanding possible functions. Our previous study found that the addition of py-GnRH peptides promoted spermatogonial proliferation in the *in vitro*-cultured testis. Therefore, we conducted *in vivo* administration of py-GnRH peptides into scallop gonad. In the study, we developed a slow-release peptide delivery system at scallop gonad and were able to observe long-term effects of peptide administration (i.e., ~6 weeks). We found that py-GnRH peptide administration influenced gonad development in the scallop during a reproductive phase. Specifically, this py-GnRH administration could promote gonad development for testis with an increase in the number of spermatogonia and gonad index (GI). On the other hand, an inhibitory effect of this administration was seen on oocyte growth. These findings proposed that py-GnRH functions on gonad development through its receptor (py-GnRHR) as a side of invertebrate GnRH function. Acknowledgements: This work was supported by JSPS Grant-in-Aid KAKENHI (23380109/16H04978) and Tohoku Ecosystem-Associated Marine Sciences (TEAMS) grants from the Ministry of Education, Culture, Sports, Science, and Technology-Japan to support the recovery of aquacultural production of marine bivalves from the Great East Japan Earthquake to MO.

P25
TRANSCRIPTOMES OF INFANTS AND ADULTS OF THE OVARY OF AN ASCIDIAN, *CIONA INTESTINALIS*.

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In vertebrates, oogenesis and folliclogenesis are induced by the hypothalamus – pituitary – gonad (HPG) axis and the HPG axis-independent process. The former is activated after puberty, and induces oocyte maturation and ovulation, whereas the latter is responsible for the growth of early-stage follicles that are not regulated by gonadotropins. The HPG axis-independent system mainly functions in the premature ovary. However, the molecular mechanisms underlying the HPG axis-independent oogenesis and folliclogenesis remain unknown. Invertebrates are not endowed with the HPG axis (no hypothalamus, pituitary, or circulation system), suggesting that the HPG axis might have emerged along with acquirement of the hypothalamus, pituitary, and circulation system during the evolution of vertebrates. In other words, it is presumed that HPG axis-independent system might have acquired in common ancestors of vertebrates and invertebrates or ancestral invertebrate chordates, and is conserved between vertebrates and invertebrate chordates such as ascidians. Ascidians belong to the phylum Chordata. Their phylogenetic position as a protochordate has provided attractive and useful targets for wide-ranging biological research. *Ciona intestinalis* is a cosmopolitan ascidian species, and has outstanding advantages as a model organism. In particular, the whole genome sequence, various ESTs, and microarray analysis data enable a variety of gene model prediction, homology search, and comprehensive comparison of genomes and transcriptomes of other species. Recently, approximately 17,000 ESTs of the adult *Ciona* ovary have been available, and the microarray analysis between the *Ciona* ovary and central nervous system detected several ovary-selective gene expression. By contrast, gene expression profile for the *Ciona* premature ovary has yet to be verified, which hampers investigation of the developmental process of the *Ciona* ovary. In this presentation, we show the gene expression profile of the premature ovary of *C. intestinalis*, which is expected to contribute a great deal not only to the investigation of the maturation process of the *Ciona* ovary but also evolutionary aspect of the oogenesis and folliculogenesis within chordates. Acknowledgements: This study is financially supported by Suntory Holdings and JSPS.

P26

BIOLOGICAL ACTIVITY OF GIANT GROUPER (*Epinephelus lanceolatus*) RECOMBINANT FOLLICLE STIMULATING HORMONE

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Reproductive development in vertebrates is tightly regulated by the two pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). It is firmly established in mammals that during ovarian development FSH assumes key role in steroidogenesis while LH stimulates oocyte maturation and ovulation. In contrast, the definitive roles of gonadotropins in gonadal development in protogynous hermaphroditic fish, which matures as female and later on reverse into male is less well understood. In this study, we have produced recombinant giant grouper (gg) (*E. lanceolatus*) FSH and currently investigating its effects on grouper reproductive development. The gg-rFSH was produced in single-chain form using yeast (*Pichia pastoris*) expression system. The *in vitro* biological activity of gg-rFSH was demonstrated by activation of its cognate receptor in a luciferase reporter assay. This will further be assessed in an *in vitro* ovarian assay using as indicator of potency the secretion of 17β-estradiol.
Weekly *in vivo* treatment of gg-rFSH on sexually immature tiger grouper (*E. fuscoguttatus*) is currently being undertaken. Samples collected two months after treatment showed higher gonadosomatic index in the treated fish compared with the control. Plasma vitellogenin was detectable in some of the treated fish while totally undetectable in any of the control fish. Treatment is continuing for another two months. Gonadal histology and gene expression analysis of key reproductive hormones will be conducted.
Acknowledgements: Funded by the Australian Centre for International Agricultural Research through Research Project No. FIS/2012/101 granted to AE. PP is supported by Australia Awards – John Allwright Fellowship.

P27

DEVELOPMENT OF GONADAL AND BEHAVIORAL SEX DIFFERENCES IN ZEBRAFISH

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Sex determination and differentiation is a process that relies on multiple genetic, endocrine and environmental signals that occur during different stages of development. While the mechanisms show high similarities between species, there are also unique features for different organisms. Development of sexual dimorphisms require a coordination between the gonadal sex and the brain. The mechanisms responsible for mammalian sex determination and differentiation are well studied and it has been shown that male gonad development is regulated by the presence of the Sry gene on the Y-chromosome. So far functional sex chromosomes have not been identified in zebrafish. Rather a complex pattern of gene expression appears to govern gonadal development in this species. Our research has focused on the involvement of inflammatory signals such as NFκB, Cox-2 and prostaglandins in gonadal differentiation in zebrafish. We have shown that induction of inflammatory signals results in feminization of zebrafish while inhibition of these signals results in masculinization. PGE_2 and PGD_2 are key regulators involved in the decision between male and female gonadal development where activation of PGE_2 results in inhibition of Sox9 and activation of the wnt/β-catenin pathway. Up-regulation of wnt/ β-catenin appears to block PTGDS and thereby reduce the PGD_2 and Sox9 signaling, resulting in ovarian development. Conversely activation of PGD_2 is needed to override the female pathway, activate Sox9 and induce juvenile-ovary to testis transition. In contrast zebrafish mating behavior appears to be controlled by steroid hormones rather than prostaglandins. Thus, while estrogen induced PGE_2 up-regulation is involved in masculinization of the mammalian brain this does not appear to be the case for zebrafish. We are now focusing on the cross talk between gonadal signaling and neuronal sex differentiation in order to determine the contribution of genes, steroid hormones and environment to the development of sex specific behavior.

P28

CHARACTERIZATION OF A FEMINIZING POWER BY GERM CELLS DURING SEX DIFFERENTIATION IN MEDAKA

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In genetically sex-determined fish, sex of germ cells obeys the direction by surrounding somatic cells under natural conditions – if the somatic cells become male by the expression of sex determination gene, the somatic cells send signals to make germ cells fated to spermatogenesis. Medaka is one of the genetically sex-determined fish. However, we have been revealing a core mechanism in medaka that can determine the sex independent of the direction of sex determination gene. Artificial modification of the core mechanism could develop a tool to manipulate the sex according to our desire.
In the core mechanism, germ cells have a critical function for feminization of the gonad (ovarian formation). Our studies indicate that the enough number of germ cells is necessary and sufficient for forming ovary. However, we did not know if the feminizing power of germ cells is germ cell-developmental stage or not. Here I show, using several medaka mutants, that it is not developmental stage-dependent, but can be attributed to an original and inherent nature of germ cells. Interestingly our results also suggest that a fate decision of germ cells towards eggs or sperm by *foxl3* (sex determination gene of germ cell) can be distinct from the inherent nature of germ cells.

P29
FUNCTIONAL ANALYSIS OF Figla/*figla* IN ZEBRAFISH GONADAL DIFFERENTIAITON.

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Sex determination and differentiation are complex processes in vertebrates. So far only a few teleost species have sex-determining genes identified including medaka and tilapia. In most species, such as the zebrafish, sex determination is believed to be governed by multiple genes (polygenic determination). By comparison, the process of sex differentiation and its controlling factors are better documented in teleosts. A large number of endocrine hormones, paracrine factors and transcriptional factors have been implicated in the process. Despite this, there had been a lack of definitive genetic evidence for functional importance of these factors in fish until recently. In mammals, Figla (Factor in the Germline Alpha) is one of the critical transcriptional factors involved in regulating early follicle development. In this study, we analyzed spatio-temporal expression of Figla*/figla* in early gonadal development of zebrafish by RT-PCR and FISH. To demonstrate the importance of Figla in controlling gonadal differentiation, we established a *figla* mutant zebrafish line by CRISPR/Cas9 gene editing method. The *figla*-deficient fish were all males in adults. Detailed analysis of early stages of development showed that the germ cells in the undifferentiated gonads could initiate meiosis, but failed to form typical perinucleolar oocyte-like cells, leading to all-male phenotype. Compared to the phenotype of *cyp19a1a* mutant we recently reported, it seemed that the deficiency of Figla led to an arrest of germ cell development and differentiation even earlier than that induced by *cyp19a1a* deficiency. Interestingly, treatment of larval fish with estrogen could rescue the phenotype of *cyp19a1a* mutant but not *figla.* Whether there is a regulatory relationship between Figla and aromatase will be an interesting issue to explore in the future. Acknowledgement: This work was funded by grants from University of Macau and The Macau Fund for Development of Science and Technology.

P30
***AMH*, A NECESSARY GENE FOR MALE DIFFERENTIATION BUT NOT SUFFICIENT FOR SEX DETERMINATION IN ALL THE NILE TILAPIA POPULATIONS?**

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Teleost fish do not have Müllerian ducts, nevertheless the anti-Müllerian hormone (amh) and its signaling pathway play a key role in their sex-determining cascade. In the Nile tilapia, *Oreochromis niloticus*, sex is determined by the interactions between genetic factors with an XX/XY system and the environment (temperature). Interestingly in this species, a precocious and dimorphic *amh* gene expression has been evidenced in the central nervous system (CNS) just before the beginning of gonadal sex differentiation, with *amh* being predominantly expressed in the male CNS. A similar dimorphic expression appears later in the gonads. In XX fry, temperature-induced masculinization (TIM) at 36°C causes a rapid and strong overexpression of both the *amh* and *dmrt1* genes in the gonads followed by the repression of the female pathway. In the brain, TIM also causes an up-regulation of the *amh* gene expression. Recently two tandem Y copies of the *amh* gene (*amhΔY*, *amhY*) have been identified on LG23 with the X chromosome carrying the *amhX* gene. *AmhΔY*, is a truncated gene lacking the TGF-β domain, whereas *amhy* has a missense SNP. *AmhY* has a male-specific expression at the beginning of the critical period of sex differentiation. Its knockdown and overexpression reciprocally induced an ovarian development in XY fry and a testis differentiation in XX gonads. Therefore *amhy* has been considered as the sex determining factor at least in the Japanese strain, and the Y chromosome assigned to LG23. Using markers from the *amhX*, *amhΔY* and *amhY*, we have analyzed the sexual genotypes in 2 wild thermosensitive populations (Hora/Ethiopia & Kou valley/Burkina Faso), as well as in a thermosensitive (Manzala) and a non-sensitive (Japanese/) domestic strains evidencing that *amhY* might not be the sex-determinant in all strains/populations of the Nile tilapia. Furthermore we have correlated these with the phenotypic sex analyzing the *amh* gene expression during sex-differentiation at the CNS and gonad levels in temperature treated and control groups. Acknowledgements: Funded by SexTil and Climsex ANR Projects

P31
SEX STEROIDS DRAMATICALLY ALTER THE PREVITELLOGENIC OVARIAN FOLLICLE OF COHO SALMON (*ONCORHYNCHUS KISUTCH*)

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Recent studies using several teleost fish models indicate that androgens and estrogens play important roles in previtellogenic ovarian follicle development. In coho salmon, the non-aromatizable androgen 11-ketotestosterone (11-KT) promotes development of primary (perinucleolar) ovarian follicles *in vitro*, while estradiol-17β (E2) has little effect. Conversely, both 11-KT and E2 stimulate growth of

previtellogenic secondary (early cortical alveolus stage) follicles but E2 has a much more substantial effect on the formation of cortical alveoli. Similar phenotypes are seen after *in vivo* steroid treatment at both stages, although E2 also promoted growth of the primary follicle, albeit with an action that was slower than with 11-KT. Notably, 11-KT treatment resulted in elevated plasma E2 levels. Our overarching hypothesis is that 11-KT drives the transition from primary to secondary growth, and prepares the early secondary follicle for increased E2 signaling. To identify the transcriptional events underlying these actions, we implanted late primary or early secondary stage coho salmon with pellets containing 11-KT (primary stage) and with E2 or 11-KT (early secondary stage) and assessed changes in the ovarian transcriptome after one and/or three days of treatment using RNA-Seq analyses. The ovarian transcriptome was dramatically and dynamically altered by the steroids, including disparate effects between ovarian stages after 11-KT treatment, and between the two steroids in the early secondary growth stage. Pathway analysis software was used to identify and predict potential global ovarian outcomes from these treatments. Included among the hundreds of ovarian transcripts altered by steroid treatments are those encoding proteins involved in steroidogenesis and steroid and growth factor signaling, lipid metabolism, cellular differentiation, and the extracellular matrix. These results give insights into the fundamental mechanisms driving previtellogenic ovarian development, and have identified androgen- and estrogen-sensitive genes for further study. Acknowledgements: Supported by: National Science Foundation grants OISE-0914009, IOS-0949765 and EPA-STAR grant R835167

P32

GONADAL GnRH AND GnIH ARE INVOLVED IN PARACRINE CONTROL OF TESTICULAR DEVELOPMENT AND SPERMATOGENESIS IN ZEBRAFISH (*DANIO RERIO*).

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Control of testicular development is multifactorial, and involves a number of hypophysial, peripheral and gonadal hormones. Here we investigate the role of local testicular peptides, GnIH and GnRH in the control of testicular development in zebrafish. The present study demonstrate direct action of GnIH and GnRH on basal and gonadotropin (GtH)-induced response in testicular development and function in adult male zebrafish. FACScan cell cycle analysis with Propidium Iodide (PI) as well as morphometric histological analysis were used as experimental approach to investigate direct actions of GnIH and GnRH on cultured testis in vitro. The result demonstrate both inhibitory and stimulatory effects of GnRH and GnIH on spermatogonial proliferation and differentiation in adult zebrafish. Treatment with both GnRH and GnIH were found to significantly alter production of postmeiotic haploid cell populations after treatment for 7 days, in vitro. Measurement of the secretion of testosterone level by ELISA also demonstrated that the effects of GnRH and GnIH may in part be due to changes in testicular steroidogenesis. This finding suggests that GnIH & GnRH local peptides are important components of complex multifactorial system that regulate testicular development and function in adult zebrafish.
Funded by NSERC.

P33

THE ROLE OF THE IMMUNE SYSTEM DURING OVULATION IN THE ZEBRAFISH (DANIO RERIO).

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Ovulation is regulated by a combination of hormones and local signaling molecules which are induced in a specific sequential manner. Previous studies on both mammals and fish have noted that the ovulatory process involves the swelling of the oocyte (inflammation) and the subsequent rupturing of the follicular layer (apoptosis). The immune system has many roles including the induction of apoptosis and phagocytosis, and recruiting pro and anti-inflammatory molecules to the sight of infection. The process of ovulation has thus been likened to an inflammatory response. The aim of this project was to determine whether immune regulating genes are present in the ovary and to characterize their role during ovulation in the zebrafish (*Danio rerio*). We hypothesized that immune regulating genes will play a role during ovulation and be dynamic over time. We predict an increase/ decrease in immune genes (tumor necrosis factors (TNFs), interferons (ifn), interleukins, and chemokines (cxc)) during the ovulatory process in conjunction with or in response to ovarian hormones and signaling molecules. We used quantitative PCR and showed that interleukin 1β, ifn gamma, cxc l14, and TNFs were expressed in ovarian follicles. In other studies, ovaries were collected at various times during the ovulatory period and we found that TNF expression was dynamic with a general increase in expression seen at 7am, corresponding to the time of ovulation. Subsequently we showed that TNF was expressed in the intact ovarian follicle and isolated follicular layers but not in the oocyte. To evaluate how TNF is regulated, ovarian follicles were incubated *in vitro* with human chorionic gonadotropin (hCG), maturation inducing steroid 17α, 20β dihydroxy-4-prenen-3 one (17, 20 βP) and the protein kinase C activators phorbol 12-myristate 13-acetate (PMA) and A23187. Treatment with PMA/ A23187 led to a 40 fold increase in TNF ($p<0.001$) whereas hCG and 17, 20 βP were ineffective. Treatment of zebrafish follicles with murine TNF had no effect on steroid production (17β estradiol, testosterone), or on prostaglandins (E_2, $F_{2\alpha}$). Collectively these results demonstrate that immune regulating genes, particularly TNF, are expressed by the zebrafish ovary but their precise role in the ovulatory process remains to be established.

P34
SEXUAL PLASTICITY AND GERM LINE STEM CELLS IN ADULT TELEOSTS

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Compounding effects of global warming and pollution increases the risks of reproductive failure and intersexuality, thus posing a threat to population existence. Hence, it is high time to understand the molecular mechanism of sexual plasticity in vertebrates. Stem cells and their potency can be crucial to understand the sexual re-orientations in adulthood. Although mammalian adult gonadal plasticity and sex reversal is a debatable matter, our recent studies with medaka have shown that the female fish can transdifferentiate into fertile males at any stages of life by exogenous steroid treatment or genetic mutation. We also found that, Oct4 (undifferentiated stem cell marker) expresses in stem cells and early oocytes in females, but not in males, suggesting that sexual plasticity might have some commonality and differences among lower and higher vertebrates. The present study was conducted to explore the mechanism of bidirectional gonadal sexual plasticity in adult medaka. We found that immature males are significantly more plastic, and prone to alterations in DNA methylation and complete sex reversal upon estradiol-17β (E2) administration, than breeding ones. Apart from more permanent changes in sex-biased markers (*Gsdf, Sox9*, etc.), we observed strong correlation between E2 and Oct4 positive early gonia proliferation in both sexes. The undifferentiated stem cell marker genes were initially reduced and then re-induced from 45 days after treatment (dat), while the differentiated stem cell marker genes showed totally opposite pattern. We isolated these germ cell clusters (hereafter named as germ line stem cells, GSCs) from both stages of each sex, and validated their stemness *in vitro* and *in vivo*. When transplanted with PKH-26 treated-GSCs, the surrogate fish produced donor-specific-fertile gametes depending on host's genetic makeup, irrespective of donor cell sex. In-depth analysis confirmed the DNMT-Oct4-retinoic acid interconnected gonadal stemness regulation, and the differential role of Oct4 in both GSCs and oocytes. Our study suggests that bi-directional sexual plasticity mostly depends on GSCs and their rejuvenating potential and methylation patterns. However, the maturation-related inhibition of sex change needs further investigation. Acknowledgements: Supported by JSPS, MOFF, Japan.

P35
RELATIONSHIP BETWEEN SEXUAL PLASTICITY AND BODY GROWTH IN THE OVARY OF KOI CARP (*Cyprinus carpio*)

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Recently, it had been shown that chronic inhibition of estrogen biosynthesis induces complete masculinization in adult female of some gonochoristic teleosts, indicating that sexual bipotentiality is maintained far beyond the embryonic labile period. Workable methods for sex control is desired in aquaculture of koi carp (colored carp), a major ornamental fish, because females have higher commodity value. However, growth rate of carp is highly variable depending on population density and feeding rate, and has been inferred to influence on gonadal sex differentiation. We have investigated the relationship between sexual plasticity and body growth. Genetically controlled female (all-XX) populations of koi carp with two different growth conditions were fed a diet containing exemestane (1mg/g diet), an aromatase inhibitor (AI), for 4, 8 and 12 months from 1.5, 2.7, 3.9, 5.8 and 10.0 months after hatching (MAH). Each group of fish was subjected to histological analysis at the end of the treatment and 12 months after. In the higher growth rate groups, 4 months treatment started before 2.7 MAH induced well developed testes, whereas ovarian tissues remained when started from 5.8 MAH. In the lower growth rate groups, complete masculinization was observed even when started from 3.9 MAH, while testicular tissues less developed suggesting the pubertal suppression under restricted growth. The longer administration gave a similar trend. Consequently, the masculinization rates showed close relationship with body size at the start of treatment rather than the timing. These findings suggest that sexual bipotentiality in female carp gonad is maintained after the initiation of ovarian differentiation, while gradually attenuated in the process of puberty in connection with body growth. Further investigations will be required to fully clarify the mechanism underlying sexual plasticity.

P36
THE ROLE OF THYROID HORMONES (T3 and T4) IN ZEBRAFISH SPERMATOGENESIS IS MEDIATED BY NUCLEAR RECEPTOR IN GERM CELLS

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The relationship between thyroid function and reproduction has been investigated for many years in different species. It has been increasingly clear that thyroid hormones (THs, T3 and T4) can influence reproduction either directly by affecting hormones of the brain–pituitary–gonadal axis or indirectly by influencing metabolic pathways and energetic allocations required to support reproduction, growth, or catabolic response. Most of our current understanding of TH action is based on studies focusing on the intracellular thyroid receptors

(TRs), which are members of the nuclear receptor superfamily. In zebrafish, it has been shown that T3 stimulated the formation of new cysts by promoting the proliferation of both Sertoli cell and type A undifferentiated spermatogonia (A_{und}). TH acts through nuclear receptor (genomic effects, T3 effects), and more recent via integrin membrane receptor mediating a rapid and nongenomic action (T4 effects). In this context, we evaluated the effects of T3 and T4 in zebrafish spermatogenesis using an *in vitro* tissue culture system. Testes were incubated in 24 well plate, containing 1 mL of media (L-15 or L-15+treatment) per well and kept at 28ºC for 07 days, changing the media every 03 days. Gene expression for the following testicular targets *3β-hsd*, *amh*, *cyp17a1*, *igf3*, *nanos2*, *piwil1*, *shippo1*, *sypc3* e *insl3* were evaluated by real time quantitative PCR (qPCR). Moreover, analysis of cell cycle by cell flow cytometry were performed after hormone treatment using FACSCalibur machine (BD Biosciences). Our results showed no effects of T4 in zebrafish spermatogenesis by qPCR and cell flow cytometry. On the other hand, T3 increased *nanos2* (stem cell marker) expression levels and also elevated the diploid population on G2 phase, suggesting that T3 promoted spermatogonial proliferation (including stem cell). Moreover, T3 stimulated meiosis as seen by increase of synaptonemical complex gene, *sypc3*. No expression changes were observed for the other genes evaluated. Our data suggested that TH effects in zebrafish spermatogenesis are mediated exclusively through nuclear receptor via T3. Moreover, T3 stimulated zebrafish spermatogonial proliferation (including stem cell population) and meiosis. These effects seemed to be directed on germ cells and not via somatic cells (Sertoli and Leydig cells). Acknowledgements: Supported by: Ciência sem Fronteiras, CNPq, Processo 234548/2014-2 and grants from NSERC.

P37

NEW TYPE OF 20B-HYDROXYSTEROID DEHYDROGENASE IS THE KEY ENZYME FOR PRODUCTION OF MATURATION-INDUCING HORMONE BY MEDAKA (*ORYZIAS LATIPES*) PREOVULATORY OVARIAN FOLLICLES

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17α,20β-dihydroxy-4-pregnen-3-one (DHP) is the maturation-inducing hormone (MIH) controlling resumption of meiosis of oocytes in many teleost species including medaka (*Oryzias latipes*). Pituitary luteinizing hormone (LH) dramatically increases 20β-hydroxysteroid dehydrogenase (20β-HSD) activity, resulting in production of DHP by preovulatory follicles, through an action that requires *de novo* mRNA and protein synthesis. Here, we show that recombinant protein of medaka *hsd17b12-like* produced in HEK293T cells displays strong 20β-HSD activity, converting 17α-hydroxyprogesterone (17α-P) to DHP. *hsd17b12-like* transcript levels increase acutely in full-grown ovarian follicles, coincident with the increase in DHP levels. Both recombinant LH and forskolin increased *hsd17b12-like* transcripts and DHP production in preovulatory follicles *in vitro*. *hsd17b12-like* transcripts were observed in the granulosa cells, varying in a stage specific manner. By contrast, levels of transcript encoding carbonyl reductase-like/20β-HSD were constant throughout ovarian follicle development. These results suggest that the novel 20β-HSD encoded by *hsd17b12-like* is the key enzyme involved in DHP production in medaka.

P38

THE ASSOCIATION BETWEEN MALE-BIASED SEX RATIO AND INDICATORS OF STRESS IN RED-SPOTTED NEWTS

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Corticosterone (CORT; a glucocorticoid in amphibians) release rates may vary as a function of the operational sex ratio (OSR) during the mating season. Specifically, a male-biased (OSR) could result in greater activation of the stress response, resulting in higher CORT release rates. A male-biased OSR results in a decrease in body condition and immune function in female red-spotted newts (*Notophthalmus viridescens*). We measured CORT release rates of red-spotted newts from a population with a male-biased OSR. We predicted that females in a male-biased OSR treatment would have higher CORT release rates than those in a female-biased treatment, because of greater male harassment. We also predicted that males will have higher CORT release rates in male-biased treatments due to higher levels of competition. We found that females, but not males, in a male-biased OSR treatment have higher CORT release rates than those in a female-biased OSR treatment. Our results support the hypothesis that a male-biased OSR leads to a higher stress response, which may underlie the observed decrease in immune function and body condition in female red-spotted newts exposed to a male-biased OSR.

P39

ENDOCRINE CHALLENEGES OF A MIDWEST UPBRINGING: INVESTIGATING THE IMPACTS OF AGRICULTURAL RUNOFF ON STEROID RECEPTOR GENE EXPRESSION, GROWTH AND DEVELOPMENT IN LARVAL FISH FOLLOWING IN SITU AND LABORATORY EXPOSURES.

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Waterways of the Midwestern United States are subject to seasonal agricultural pulses that contain pesticides, pharmaceuticals, fertilizers and suspended sediments. Such mixtures present a risk to early life stages of fish inhabiting impacted watersheds. Recent studies have found that altered gene expression of endocrine biomarkers including: androgen receptor (AR), estrogen receptor α (ERα) and insulin-like growth factor 1 (IGF1), is a consistent response of minnow larvae following exposure to agricultural runoff. These studies were conducted during spring runoff events in the Elkhorn River watershed from 2014 – 2016. In 2014 and 2015 fathead minnow larvae were exposed to seasonal runoff under natural conditions in outdoor mesocosms at the Elkhorn River Research Station. Along with changes in expression of AR and IGF1, growth depression in larval mass and condition factor were common responses when minnow (*Pimphales promelas*) larvae were exposed to this complex and seasonally-dynamic agrichemical mixture. Interestingly, our findings also demonstrate compensation in both growth, and in the expression of some, but not all, endocrine responsive genes. Further investigation during the 2016 field season revealed that androgenic and (anti-)estrogenic responses of minnow larvae were dependent on whether minnow larvae were exposed to the aqueous or suspended sediment phase found in agricultural runoff. The Elkhorn River exposed larval fish to a complex environment. The response of the larvae to it is much more nuanced than was originally anticipated, highlighting the importance of evaluating early life stressors under natural conditions. Acknowledgements: Supported by: Water for Food Global Institute Graduate Fellowship to JMA. National Science Foundation grant award CBET-0966858 to ASK.

P40

CONTINUOUS EXPOSURE TO WATERBORNE CORTISOL AS A MEANS TO STUDY CHRONIC STRESS IN ZEBRAFISH: COPING MECHANISMS AND EFFECTS ON FOREBRAIN NEUROGENESIS

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Chronic exposure to anthropogenic or environmental stressors can lead to a condition of allostatic overload that has negative consequences for animal welfare. Beyond its detrimental consequences for health, growth, and reproduction, there is evidence that chronic stress also negatively affects neurogenesis in many species, including mammals and fish. However, current methods for modeling chronic stress, such as cortisol-infused food, cortisol injections, and variable stressor schedules may be inadequate due to inconsistent dosage between individuals, invasiveness, or fluctuating whole body cortisol levels. In this study, to further characterize the effects of chronic stress on neurogenesis, we developed a flow-through system which exposed zebrafish, *Danio rerio*, to a constant level of exogenous cortisol. Surprisingly, despite continuous exposure to a broad range (0-20 mg/l) of consistently high water cortisol levels for 5 days, whole body cortisol levels did not differ between any of the treatments. Similarly, forebrain expression of *neurod*, *pcna*, *bdnf* and *c-fos*, key markers of neuronal differentiation, proliferation and activation, were not affected by the chronic cortisol treatments. In contrast, cortisol treatment was associated with a marked dose-dependent increase in whole body 20β-hydroxycortisone levels (an inactive cortisol metabolite) and a parallel dose-dependent increase in forebrain 20β-hydroxysteroid dehydrogenase type 2 (20β-HSD) gene expression (the enzyme responsible for the production of 20β-hydroxycortisone). We are now characterizing the relationships between whole body cortisol, cortisone and 20β-hydroxycortisone levels, 20β-HSD gene expression, and forebrain neurogenesis at different time points throughout the 5-day continuous cortisol exposure to better understand the dynamics of this response. Results to date suggest that zebrafish have a large capacity to inactivate cortisol during continuous exposure, thereby mitigating the detrimental consequences of chronic stress. Additionally, our results suggest that habituation alone may not be responsible for the return of whole body cortisol to baseline levels during extended exposure to a single, continuous stressor. Acknowledgements: Supported by NSERC Canada operating grants to NJB and MAB.

P41

MOLECULAR AND FUNCTIONAL EVOLUTION OF NEUROHYPOPHYSIAL HORMONE SYSTEM: WITH SPECIAL REFERENCE TO A POSSIBLE FUNCTION OF NEWLY DISCOVERED V2B RECEPTOR IN CATSHARK.

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Vasopressin/vasotocin (VT) and oxytocin (OT) family peptides are secreted from the neurohypophysis and exert various actions including salt and water homeostasis and reproduction through distinct G protein-coupled receptors. From teleosts to mammals, four neurohypophysial hormone receptors (V1aR, V1bR, V2R, and OTR) had been identified, and we recently found and characterized a fifth neurohypophysial hormone receptor (V2bR) from the holocephalan elephant fish, *Callorhinchus milii*. This receptor is similar to conventional V2 receptor (V2aR) in sequence, but induced Ca^{2+} signaling in response to VT, which is typical signaling of V1-type receptors. On the other hand, we could not find conventional V2aR from the elephant fish. The same case was evidenced in the elasmobranch catshark, *Scyliorhinus torazame*; V1aR, V2bR and OTR have been identified. Although the timing of diversification of V2aR and V2bR still remains to be clarified, the cartilaginous fish V2bR, which uses Ca^{2+} as an intracellular second messenger, most likely resembles the ancestral V2aR/V2bR. In the catshark, V1aR mRNA was intensely expressed in the rostral pars distalis of pituitary, and colocalized with the mRNA signal of proopiomelanocortin, implying that VT controls the secretion of adrenocorticotropic hormone via V1aR. Meanwhile, intense expression of V2bR was observed in the posterior part of oviduct (PPO). *In vitro* administration of VT caused contraction of the PPO in a dose-dependent manner, while VT treatment had no contractile effect on the anterior part of oviduct, where V2bR is not expressed. Considering that the chicken V2bR, published as a VT1 receptor, is also expressed in the oviduct (Tan et al., 2000), our observation strongly suggests that V2bR

contributes to egg laying process throughout vertebrates. In this talk, molecular and functional evolution of neurohypophysial hormone system will be discussed by focusing on the newly identified V2bR.

P42
BIOSYNTHESIS OF 1α-HYDROXYCORTICOSTERONE IN THE WINTER SKATE, *LEUCORAJA OCELLATA* (MITCHILL 1815): EVIDENCE TO SUGGEST A NOVEL STEROIDOGENIC ROUTE

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Description of the complete biosynthetic pathway of the elasmobranch corticosteroid 1α-hydroxycorticosteroid (1α-OH-B) has proven challenging with the presence of the final enzymatic step thus far eluding researchers. Here we explore the potential role of intracellular bacteria isolated from the renal/interrenal tissue in the Winter Skate *Leucoraja ocellata* to metabolize steroids and contribute to the synthesis of 1α-OH-B. In the original identification of 1α-OH-B the rarity of C1 hydroxylation in eukaryotes was noted, however, the literature provides evidence for steroid C1 hydroxylation by microorganisms. We have identified eight bacterial strains isolated from renal/interrenal tissue of *L. ocellata* under aseptic conditions. One isolate – UM008 of the genus *Rhodococcus* was noted to metabolize a variety of corticosteroids and produce novel products via HPLC analysis. Furthermore, the metabolic actions of this isolate was could be manipulated through the addition of cations Zn^{2+} and Fe^{3+} suggesting inhibition of *Rhodococcus* steroid catabolism. Genome sequencing of UM008 identified strong sequence and structural homology to that of *Rhodococcus erythropolis* PR4, and we were able to assign a complete enzymatic pathway for steroid ring oxidation as documented within other Actinobacteria. We provide evidence to suggest an alternate pathway for steroidogenesis in elasmobranchs that may include a form of mutualism between intracellular bacteria and their Elasmobranch host Acknowledgements: Supported by: NSERC Canada operating grants to WGA, AKB and PJW.

P43
VITELLOGENIN: A BETTER INDICATION OF REPRODUCTIVE STATUS IN LOGGERHEAD SEA TURTLES

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Vitellogenin (VTG) is an egg yolk-precursor protein that serves as a nutrient source for developing embryos in oviparous vertebrates. The hormonal control of this protein has been studied in a variety of taxa, but details about the dynamics of this protein remain to be elucidated in sea turtle species. VTG production is induced by estradiol-17ß (E_2), and testosterone (T) is also thought to have a regulatory role, though its function in sea turtles is currently unknown. To investigate the dynamics of VTG in a multi-clutch species under natural conditions, 38 adult (SCL > 80 cm) Loggerhead females entrained in the Florida Power and Light St. Lucie Nuclear Plant intake canal in Hutchinson Island, FL were sampled from May-August of 2014. Blood samples were taken to measure T, E_2, and VTG using enzyme-linked immunosorbent assays (ELISAs). Ultrasound images of the gonads were used to determine ovarian status and to measure ovarian follicle and oviductal egg size. Results showed that mean VTG concentration increased from May (8.27 mg ml^{-1}) to June (15.37 mg ml^{-1}) and declined into July and August (9.44 mg ml^{-1}); this decline corresponded with the end of the nesting season. Mean E_2 (718.02 pg ml^{-1} in May to 95.89 pg ml^{-1} in July-August) and mean T (2,008.35 pg ml^{-1} in May to 1,221.24 pg ml^{-1} in July-August) declined over the summer months, and mean concentration for both steroids was significantly higher in reproductively active females than means of reproductively inactive females, though overlapping concentrations of the steroids occurred between the active and inactive groups. However, VTG concentration was high in reproductively active turtles and undetectable in reproductively inactive turtles. We conclude that the addition of VTG measurement in conjunction with the gonadal steroids provides a more accurate and easily-interpretable way to predict reproductive status of adult Loggerhead females. Additionally, gonadal steroid and vitellogenin concentration in our study corresponded only with late nesting animals, indicating that early season females do not become entrained in the intake canal of the power plant.

P44
LEPTIN RECEPTOR EXPRESSION AND DOWNSTREAM SIGNALING IN IMMUNE ORGANS OF XENPOUS TADPOLES AND JUVENILES

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Leptin, a pro-inflamatory adipokine hormone, is best known for its role in regulating an organism's food intake and energy balance, but in mammals it has been shown to modulate both the adaptive and innate immune responses. Previous work in the lab showed that leptin treatment reduced mortality and non-lethal effects of gram-negative bacterial infections in Xenopus laevis tadpoles, suggesting that leptin also is an immunomodulator in amphibians. In this study, we confirmed using in situ hybridization that the long form of the leptin receptor

is present in the thymus, spleen and liver of the X. laevis tadpoles and juveniles. We also conducted experiments to show that phosphorylated STAT3 and ERK1/2, downstream molecules activated by leptin receptor signaling, are upregulated by homologous leptin protein injections (200ng/animal) in these tissues. This research suggests that, like mammals, leptin is an immunomodulator as early as the tadpole stage, and future research will investigate specific immune outcomes that result from leptin activation of both of these signaling pathways.

P45

PHYSIOLOGICAL ROLES, MOLECULAR REGULATION, AND DISRUPTION OF THE PROGESTERONE RECEPTOR SIGNALING PATHWAYS IN AMPHIBIANS

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Environmental gestagens are an emerging class of contaminants that have been recently measured in surface water in North America and have the ability to interfere with reproduction in aquatic vertebrates. Gestagens include endogenous progestogens, such as progesterone (P4), which bind P4-receptors and have critically important roles in vertebrate physiology and reproduction. Gestagens also include synthetic progestins, which are components of human and veterinary drugs, such as melengestrol acetate (MGA). Endogenous progestogens are essential in the regulation of reproduction in mammalian species, but the role of P4 in amphibian larval development remains unclear. This project aims to understand the roles and the regulatory mechanisms of P4 in amphibians and to assess the consequences of exposures to environmental gestagens on the P4-receptor signaling pathways in frogs. Here, we established the developmental profiles of the P4 receptors (*ipgr, mpgr, and pgrmc1*) and the steroidogenic enzyme, 3β-hydroxysteroid dehydrogenase *(3β-hsd)* in *Silurana tropicalis* embryos using real-time RT-PCR. Progesterone receptor mRNAs were detected throughout embryogenesis. Transcripts for *ipgr* and *pgrmc1* were detected in embryos at Nieuwkoop and Faber (NF) stage 2 and 7, indicative of maternal transfer of mRNA. We also assessed the effects of P4 and MGA exposure in amphibian early development and during metamorphosis through transcriptional analysis of a suite of gene targets of interest, including: *ipgr, mpgr, pgrmc1, 3β-hsd, ar, er, fshβ, prl,* and *srd5a*. Larval exposure (NF 12 - 46) to P4 induced 3- to 6-fold change increase of *ipgr, mpgr, pgrmc1*, and *ar* mRNA levels at the environmentally relevant concentration of 314 ng/L P4. Chronic exposure (NF 12 – 60) to MGA and the binary mixture of P4 and MGA caused conspicuous abnormal development, including: retarded growth, narrowing of head, and lack of forelimb emergence. Effects of chronic exposure on the suite of genes of interest in target organs will be presented. Our data suggest that exposure to P4, MGA, and their mixture induces multiple endocrine responses and adverse effects at environmentally realistic concentrations in *S. tropicalis*.

P46

EMBRYONIC ORIGINS OF ALTERED OVARIAN GONADOTROPIN RESPONSIVENESS IN AN ENVIRONMENTAL MODEL OF ENDOCRINE DISRUPTION, THE AMERICAN ALLIGATOR

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Exposures to endocrine disrupting contaminants are thought to broadly impact reproductive health in humans and wildlife. The American alligator has provided significant utility to our understanding of population-level effects of exposure to endocrine-active chemicals on vertebrate reproduction. Reports from a contaminated lake (Lake Apopka, FL) have indicated a causal role for these contaminants in the rapid decline of alligators living there in the 1980s-90s. Juvenile alligators from this site are characterized by genital abnormalities, aberrant sex-steroid hormone production, and abated ovarian responsiveness to gonadotropin signals. Studies from our lab have provided evidence that this collective reproductive disorder has embryonic origins. We seek to test the hypothesis that precocious exposure to hormone signals during critical windows of embryonic development is responsible for suppressed gonadotropin response later in life. We have exposed alligator embryos to 17β-estradiol or dihydrotestosterone prior to sex-determination and raised resulting hatchlings for five months, and then administered exogenous ovine follicle-stimulating hormone (FSH). We aim to investigate morphological and gene expression response in the ovary and modulation of this response by developmental exposure. We have thus established changes in ovarian GSI and gene expression of eleven targets in response to FSH-stimulation in control and treated animals from a reference site, Lake Woodruff, and Lake Apopka. We have detected significant impacts of FSH on gene expression of aromatase (CYP19A1), follistatin (FST), FSH-receptor (FSHR), progesterone receptor (PR), androgen receptor (AR), estrogen receptor b (ESR2), and anti-mullerian hormone (AMH), and GSI. Furthermore, we have detected the modulation of expression by developmental exposure in ESR2, arylhydrocarbon receptor 2 (AHR2), and in GSI. Incidental or experimental exposure during development to endocrine-active compounds significantly affects the expression of these genes in a manner not observed in untreated animals. Given the broadly conserved role of these genes in vertebrate reproduction, these findings implicate developmental endocrine disruption in altered gonadotropin responsiveness later in life in a potentially broad range of taxa.

P47

SKIN SECRETOME OF CAECILIANS FROM THE WESTERN GHATS, INDIA: AN INVESTIGATION ON THE SKIN BIOACTIVE MOLECULES.

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The amphibian skin is a complex organ in its morphology, biochemistry and physiology that perform the wide range of functions necessary for their survival in the harsh environment. Enormous bioactive molecules are present in the secretions of amphibians. The order Gymnophiona is a group of limbless amphibians, which are elongated worm- like and subterranean. India is home to diverse forms of caecilians. There are total 36 species of caecilians reported from India. Among the 36 species, 25 are found in the Western Ghats. The Western Ghats, is a biodiversity hotspot with high rate of endemic caecilian species. The state of Kerala covers around 600km of the Western Ghats. This bountiful landscape with its moist wet forests and rich porous soil has become a paradise for the caecilians. As the caecilians live in the wet humus soil, the environment has a significant role in their skin secretomics. Compared to frogs and salamanders, knowledge about caecilian secretome is rudimentary or rather nil to date. Their skin is moist and highly glandular in nature. The caecilian integument has granular glands, which produce bioactive molecules, but there are no comprehensive studies existing for caecilian secretome yet. Therefore, our present study explores the secretomics of *Ichthyophis tricolor* and *Uraeotyphlus cf. oxyurus* and *Gegeneophis ramaswamii* – belonging to two familes –Ichthyophiidae and Indotyphlidae from Western Ghats. We carried out the antimicrobial, antioxidant and anticancerous assays with the crude skin peptide extracts of these caecilians and found positive results. SEM And TEM analysis of their skin shows numerous granular glands and mucus glands in them. Further investigation on the skin bioactive peptides by 2D gel electrophoresis followed by MALDI TOF - TOF analysis confirmed the presence of bioactive molecules. This is the first report on the secretomics of Indian caecilians. Acknowledgements: DBT BioCARe, KSCSTE,Central University of Kerala.

P48

WHAT DRIVES SEXUAL SIZE DIMORPHISM IN SQUAMATE REPTILES? MALE GONADAL ADROGENES VERSUS OVARIAN HORMONES VERSUS FEMALE ALLOCATION TO REPRODUCTION

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Sexual size dimorphism (SSD) reflects sex-specific solutions to the allocation of energy among growth, reproduction and survival; however, the proximate mechanisms behind these trade-offs are still poorly known even in vertebrates. In squamates, the vertebrate clade encompassing more than 10.000 species, SSD used to be attributed to energetically demanding processes, especially female reproduction. In addition, SSD is assumed to be controlled by specific endogenous mechanisms regulating growth in a sex-specific manner, namely masculinization by male gonadal androgens, or feminization by ovarian hormones. We performed the complex manipulative growth experiments in the male-larger gecko *Paroedura picta* to test the reproductive cost hypothesis, the male androgen hypothesis and the ovarian hormone hypothesis by comparing growth of experimentally treated animals to control males and reproductively active females. Specifically, in females we tested the growth effects of social isolation preventing reproduction, exogenous testosterone, early and later total ovariectomy, unilateral ovariectomy and total ovariectomy followed by exogenous estradiol, dihydrotestosterone or testosterone treatment; in males, we focused on castration and castration followed by testosterone treatment. The results did not support the hypotheses that SSD reflects direct energy allocation to reproduction and the involvement of male gonadal androgens. All lines of evidence were concordant with the control of growth and hence SSD by ovarian hormones. Based on our complex experiments and indirect evidence from other squamates we suggest that feminization of growth by female gonadal hormones should be taken into consideration as a major endogenous pathway responsible for the ontogeny of SSD in this important vertebrate clade. Acknowledgements: Supported by the Czech Science Foundation (project no. GA16-24619S).

P49

ADRENOMEDULLIN 5 GENE IS EXPRESSED IN THE HEMATOPOIETIC TISSUES IN WESTERN CLAWED FROG, *XENOPUS TROPICALIS*.

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Adrenomedullin (AM) family consists of three to five distinct peptides in vertebrates. AM1 have been initially identified in human and the presence of 'family' was first determined in teleost fish by the discovery of five distinct AM genes (AM1-AM5). The following identification of AM2 and AM5 genes in mammalian lineages confirmed that multiple AMs make a family across the whole vertebrates. AM family genes have been originated from three ancestral molecules (AM1, AM2 and AM5) which are conserved in living tetrapods, whereas additional two types were generated by the whole genome duplication in teleosts.
We have been studying on the biological functions of AMs in non-mammalian species since the discovery of multiple AM genes. We focused on the function of AM5 gene in this study. Although AM5 gene is conserved in most mammalian species, its sequence is disrupted

in human and the whole gene is deleted in mice and rats. To explore the biological function of AM5 in tetrapods, we chose western clawed frog, *Xenopus tropicalis*. AM5 gene was highly expressed in the *Xenopus* spleen, which support the previous finding that AM5 gene was expressed in the porcine spleen and thymus. AM5 mRNA was also detected in the blood and the liver which is the responsible tissue for amphibian hematopoiesis. In the 1 dpf embryo (NF stage 29-31), AM5 mRNA was detected in the ventral blood island, the hematopoietic tissue at these stages. The density gradient centrifugation on adult blood sample showed that the expression of AM5 gene was higher in the leukocytes than in the erythrocytes. Therefore, we suggest that AM5 is involved in hematopoiesis, and in particular, leukopoiesis in *Xenopus tropicalis*.

P50
NUTRITIONAL REGULATION OF LIMB REGENERATION IN XENOPUS LAEVIS TADPOLE: A POSSIBLE ROLE FOR LEPTIN

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The regeneration of structures is locally controlled by injury-activated gene networks, but the rate and quality of regeneration can depend upon the nutritional state of the animal. In Xenopus laevis tadpoles, food restriction slows limb regeneration, but intraperitoneal (ip) injections of homologous leptin protein at the time of amputation partially rescues this effect. This finding suggests that leptin is a nutritional modulator of early regeneration processes, such as wound healing, apical epidermal cap formation, or blastema formation. We used in situ hybridization to confirm that the long form of the leptin receptor is expressed throughout the blastema, similar to its expression pattern in early limb buds of developing limbs. We also used fluorescent to show that phosphorylated STAT3 expression increases in blastema cells 6 hr after leptin injection (200 ng, ip) relative to saline-injected tadpoles. This finding suggests that leptin receptors are active in the blastema and JAK-STAT3 signaling pathway is mediating leptin effects during this stage of regeneration. We aim to use the same approach to determine whether the ERK1/2 pathway is associated with leptin-mediated effects on early limb regeneration, and ultimately describe the ways in which leptin modulates limb regeneration at different stages (supported by Sigma Xi Grant-in-Aid to ME, Louis Stokes Alliance for Participation and WSU School of Biological Sciences research awards to KT, WSU ADVANCE Transitions Award to EJC)

P51
WHAT CAN MAKE MALE LIZARDS GROW BIGGER: TESTOSTERONE, TEMPERATURE, OR ALTERED ENERGY ALLOCATION?

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Body size is one of the most fundamental properties of an organism. It is related to many aspects of life history and ecology of an individual and it is also often a key sexually selected trait. Over the years we performed several manipulative laboratory experiments focused on factors and mechanisms affecting final body size and growth rate in lizards, particularly in males of the male-larger Madagascar ground gecko (*Paroedura picta*). We tested the effect of castration with and without testosterone implants, as testicular androgens were suggested to have positive effect on growth in male-larger lizards. Nevertheless, we found repeatedly and independently that male gonadal androgens do not affect growth in the studied species. Structural growth of the gecko males was also unaffected by considerable energetic burden cost by tail autotomy and its subsequent regeneration in rapidly growing individuals, although tail loss represented up to 14% of body mass which needed to be at least partly regenerated. The only instance when we observed differences in growth pattern and in final snout-vent length was in the experiment where we manipulated with environmental temperature during the embryonic development and ontogenetic growth. We found that males kept at 27 °C were significantly larger than those kept at 24 °C and 30 °C. We demonstrated that in males of our gecko model species growth rate and final snout-vent length was phenotypically plastic with respect to temperature regimes, but body size and structural growth was surprisingly not related to energy allocation to tail regeneration and to circulating androgen levels. Acknowledgements: Supported by the Czech Science Foundation (project no. GA16-24619S).

P52
SODIUM IODIDE SYMPORTER EXPRESSION IN RED DRUM, (*SCIAENOPS ECELLATUS*)

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Iodine is essential for normal thyroid function. In mammals, iodide is obtained from the environment and transported into the thyroid by the sodium iodide symporter (NIS), an intrinsic membrane protein expressed in the thyroid and intestine. To determine whether a homologous NIS serves a similar function in the red drum, a euryhaline sciaenid fish, RT-PCR was used to identify NIS expression.in thyroid tissue (located by 124I PET-CT), intestine, and gill. Since environmental iodide is only absorbed via the gut in mammals, NIS expression in gills would suggest a novel pathway for iodide uptake in fish. Development of qPCR methodology will be used to further examine how subpharyngeal, gill and gut NIS expression changes in response to TSH stimulation and alterations in environmental or dietary iodide availability.

P53
T3 AND T2 DIFFERENTIALLY REGULATE THE EXPRESSION OF GENES ASSOCIATED TO THYROID FUNCTION IN CEREBELLUM.

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Thyroid hormones (TH) are key regulators of physiological process and are essential for growth and development in vertebrates. Specifically in the cerebellum, during most stages of the ontogeny of the individual, TH regulate the expression of genes that play critical roles in neuronal differentiation, neurite growth, sinaptogenesis, neuronal migration and lamination. For TH to exert their actions, at least three functional events need to occur: 1) the facilitation of TH transmembranal movement, mediated by the organic anion transporter polypeptide (OATP1C1), and more specifically by the monocarboxylate transpoter (MCT8); 2) the tissue-specific activation/inactivation of the prohormone thyroxine (T4) to either the bioactive 3,5,3-triiodothyronine (T3) , or the inactive rT3, catalysed by deiodinases D2 and D3, respectively, and 3) the binding of bioactive TH to nuclear thyroid hormone receptors type alpha and beta (THRA and THRB) which function as ligand dependent transcription factors. In concert with their thyroid-associated function, these genes are known to be tightly up-or-down regulated by T3 in most studied tissues. We have previously described that as T3, 3,5-diiodothyronine (T2) is a bioactive TH. Indeed, T2 has been shown to regulate gene expression in liver but its actions upon other tissues have never been explored. In this study, we analyzed the effects of the bioctive TH, T3 and T2 up on the regulation of OATP1C1, MCT8, D2, D3, THRA and THRB by treating cerebellum organotypic cultures from juvenile tilapia (Oreochromis niloticus) (n=12) with 0.1, 1, 10 and 100 nM of T2 or T3 administered for 24h in the culture medium. We analyzed mRNA expression by RT-qPCR. Of the 6 analyzed genes, our results showed that D3 did not respond to any treatment, while OATP1C1 only responded to T2. Furthermore, T2 was a more potent regulator of the expression of MCT8, THRB and THRA. These data supports the notion that not only T3, but also T2 is physiologically relevant in cerebellar function.
Acknowledgements: Supported by: CONACYT 219833, PAPIIT IN204517-2.

P54
EFFECT OF 3,5-DIIODOTHYRONINE ON THE MORPHOLOGY OF JUVENILE *Ambystoma Mexicanum*

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Previous work from our group and others has shown that 3,5-diiodothyronine (T2), a naturally occurring metabolite of T3 outer-ring deiodination, is an alternative ligand for the thyroid hormone receptor β1 (TRβ1), at least in some teleostean and mammalian species. To gain insights about effects of T2 in other vertebrates, we took advantage of the biology of *Ambystoma mexicanum*, a neotenic amphibian that only undergoes metamorphosis when treated with exogenous THs. Thus, we tested the hypothesis that if T2 were bioactive, it would induce metamorphic changes in this specie.The experimental approach was to administer 1 or 50 nM of T2 in rearing water for 30 days and compared external morphological (gills, dorsal fin, limbs) and internal changes (liver cytoarchitecture). As internal controls, a group of animals were treated with vehicle or 50nM of T4, a concentration reported as effective to induce a full metamorphosis. Our preliminary data shows that while 1 nM of T2 has no effect in external morphological changes, 50 nM modifies the secondary external gills, towards the structure and morphology of a metamorphic individual. Interestingly, these changes were reverted when T2 was withdrawn. These preliminary results suggest that T2 is responsible for some changes in morphology during metamorphosis induction. Acknowledges: Supported by; CONACYT 219833, PAPIIT IN204517-2 and postdoctoral fellowship from DGAPA, Instituto de Neurobiología, Campus UNAM, Juriquilla.

P55
DEVELOPMENTAL AND T3-DEPENDENT DNA DEMETHYLATION IN *XENOPUS* TADPOLE BRAIN DURING METAMORPHOSIS

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Methylation of cytosines in the genome (DNA methylation) is an epigenetic modification that affects gene transcription, and has been shown to be critical for normal brain development. We used *Xenopus* tadpole metamorphosis, a thyroid hormone (T3)-dependent postembryonic developmental process, to investigate a possible role for T3 in the regulation of DNA methylation in developing brain. Using three independent assays, we found a progressive decline in DNA methylation in tadpole brain during metamorphosis, which correlated with increases in mRNAs for genes that code for enzymes that catalyze DNA demethylation (*tet2, tet3, apobec2, gadd45β, gadd45*γ and *tdg*). Treating premetamorphic tadpoles with T3 caused time-dependent increases in
*tet3, gadd45*γ and *tdg* mRNAs. We used three independent assays to investigate possible changes in DNA methylation at T3 response elements (TREs) of known T3-regulated genes; Krüppel-like factor 9 (*klf9*) and DNA methyltransferase 3a (*dnmt3a*). Using methyl-sensitive restriction digest and bisulfite sequencing, we discovered that the regions of the TREs underwent DNA demethylation, both during

spontaneous metamorphosis and in response to exogenous T3. Using immunoprecipitation for 5 hydroxy methyl cytosine (5hmC) an active DNA demethylation intermediate, we found that exogenous T3 can induce an increase in 5hmC, at regions of the TREs. This led us to investigate T3-dependent recruitment to TRE regions of ten eleven translocase 3 (TET3), a methylated DNA-binding dioxygenase that catalyzes conversion of 5mC to 5hmC. Using chromatin immune-precipitation we found that exogenous T3 induced TET3 recruitment to the two TREs within the *dnmt3a* locus. Additionally, DNA fragments containing *klf9* and *dnmt3a* TREs sub-cloned into a CpG-less luciferase reporter plasmid exhibited T3-dependent transactivation in a transient transfection assay, which was eliminated by methylating these plasmids *in vitro* before transfection. Our findings support that T3 induces DNA demethylation at TREs of known T3 responsive genes in the tadpole brain. Furthermore, we provide evidence that DNA demethylation at the TREs is catalyzed by active recruitment of TET3. We hypothesize that these changes in DNA methylation at known TREs are important for the coordination of T3-dependent gene regulation programs that underlie tissue morphogenesis.
(Supported by NSF grant IOS 1456115 to RJD).
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P56
THE AFFINITY OF TRANSTHYRETIN FOR T3 OR T4 DOES NOT DETERMINE WHICH FORM OF THYROID HORMONE ACCUMULATES IN THE CHOROID PLEXUS.

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Normal development of the brain is dependent on the required amounts of thyroid hormones (THs) reaching specific regions of the brain during each stage of ontogeny. Many proteins are involved with regulation of TH bioavailability in the brain: transthyretin (TTR), TH transmembrane transporters (e.g. MCT8, MCT10, LAT1, OATP1C1) and deiodinases (D1, D2 and D3) which either activate or inactivate TH. Previous studies revealed that in mammals, T4, but not T3, accumulated in the choroid plexus and then entered the cerebrospinal fluid. In all species studied so far, transthyretin in mammals binds T4 with higher affinity than T3, whereas TTR in non-mammalian vertebrates binds T3 with higher affinity than T4. We investigated if the form of TH preferentially bound by TTR influenced the form of the TH accumulated in the choroid plexus and consequently other areas of the brain. We measured the mRNA levels corresponding to TTR, MCT8, MCT10, LAT1, OATP1C1, D1, D2 and D3 in the brains of chickens at 11 days post-hatching. Furthermore, we measured the uptake of intraveneously injected ^{125}I-T3 and ^{125}I-T4 into chicken brains at this age. ^{125}I-T4 but not ^{125}I-T3 accumulated in the choroid plexus and optic lobes of chicken brains. Therefore, the form of TH preferentially bound by TTR does not determine the form of TH that accumulates in the choroid plexus and other areas of the brain. T3 present in the brain is therefore mainly produced locally by conversion of T4 into T3 by D2.

P57
TEMPERATURE AND HYPERTHYROID EFFECTS ON THE ACTIVITY AND TRANSCRIPT ABUNDANCE OF METABOLIC ENZYMES IN THE COOL-WATER TELEOST, LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS*)

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Many important aspects of biological function are strongly influenced by temperature, including metabolism. While many fish can adjust metabolic processes to counteract these effects though a process known as thermal acclimation, the mechanisms and endocrine control of these shifts have not been fully elucidated. Therefore, we set out to examine the interactions between temperature and hyperthyroidism on metabolism in the cool-water teleost, lake whitefish (*Coregonus clupeaformis*). Thyroid status of fish was altered using T_4 implants and fish were exposed to 13 (control), 17 or 21 ºC for up to 24 d. Metabolic responses were assessed by quantifying mRNA transcript abundance and activity of key enzymes. Transcript abundance of cytochrome c oxidase subunits 1 and 4 were not consistently altered by elevated temperatures or hormone treatment within the 24 d period. However, exposure to 21 ºC triggered a sharp decrease in citrate synthase mRNA transcript abundance in euthyroid fish after 4 d, followed by a recovery towards control levels by 24 d. This initial decline in citrate synthase was not observed in fish treated with exogenous T_4, suggesting thyroid hormones affect transcript abundance of certain metabolic enzymes following heat stress and may be particularly influential in the early stages of temperature response. Immediate and short-term impacts of elevated temperature and hyperthyroidism on citrate synthase activity, cytochrome c oxidase activity and mRNA abundance of key enzymes in lipid metabolism will also be discussed.

P58
IOXYNIL AND DIETHYLSTILBESTROL DISRUPTION OF THYROCYTE DEVELOPMENT AND HOMEOSTASIS

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Thyroid hormones are key regulatory signaling molecules involved in vertebrate development and adult homeostasis. Widely used industrial chemicals have the potential to disrupt the endocrine function of the thyroid axis. Waterborne micromolar concentrations of IOX and DES affect the second stage of development of the thyroid and the heart. Existing evidence highlights the potential role of heart-angiogenic-thyrocyte interactions as being essential for thyroid gland and HPT-axis development and function. The aim of the present study is to establish the cross-talk that occurs between thyroid tissue development and the angiogenic system in zebrafish and how this is modified by IOX and DES. In order to understand the effect of IOX and DES, Zebrafish embryos of Tg(fli1::GFP) transgenic line were exposed to 0.1uM IOX or 0.1uM DES from 12 to 48hpf. At the end of the exposure, the GFP (endothelial)-positive cells were isolated after cell sorting and were used to isolate total RNA for RNA-seq transcriptome analysis. In both IOX and DES treated groups, the results suggested that the most significantly changed pathways were neuroactive ligand-receptor interaction, cell adhesion molecules, calcium signaling pathway, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy and hypertrophic cardiomyopathy. Chemical specific effects were observed for each treatment. The most significantly changed pathway in IOX treated embryos was glycosphingolipid biosynthesis - ganglio series, whereas in DES treated groups it was steroid biosynthesis. Taken together the evidence shows that IOX and DES affect the homeostasis and function of the vascular system, which indirectly affects thyroid gland development and most likely HPT-axis function. In conclusion, IOX and DES have thyroid endocrine disrupting action.
Acknowledgements: Funding from the Foundation for Science and Technology (FCT), Portugal (CCMAR/Multi/04326/2013). MAC is a recipient of a FCT Investigator grant (IF/01274/2014). YFL is funded by Shanghai Peak Disciplines Project of Fisheries (A1-2035-17-0001).

P59
TISSUE DISTRIBUTION AND DYNAMIC REGULATION OF CHICKEN PEPTIDE YY (PYY) EXPRESSION.

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Understanding molecular control of appetite and growth is essential in order to improve the management, animal welfare and economy of the global poultry industry. Scrutiny of avian endocrine systems can also prove useful in understanding evolution of mammalian systems. Peptide YY (PYY) is a known satiety factor contributing to control of appetite and growth in mammals however studies investigating its function in birds are lacking. Despite a broadly conserved tertiary structure, avian PYY molecules are known to differ to mammalian homologs at primary sequence level, negating proteolytic sensitivity at the N-terminus (the precise functional significance of which remains unknown). Following publication of the chicken PYY gene sequence, this work aims to characterise its tissue expression and responsiveness to nutritional state. In contrast to the mammalian dogma that PYY is primarily an intestinal peptide, the pancreas was found to be the major site of PYY expression in chickens, and intestinal PYY expression was highest around the distal jejunum, as opposed to the mammalian distal large intestine. Neither pancreatic PYY ($F_{1,22}$=0.02, p=0.898) nor PPY ($F_{1,22}$=0.15, p=0.706) expression was dependent on the region of the pancreas sampled in young chicks (2wk) but both were greater in the pancreas splenic end than duodenal end in adolescent birds (12wk) (PYY, $F_{1,7}$=13.03, p=0.009; PPY, $F_{1,7}$=6.57, p=0.037) . PYY expression was positively correlated with PPY expression in both 2wk (r_s(45)=0.506, p<0.001) and 12wk (r_s(14)=0.782, p<0.001) birds. Furthermore, it was found that pancreatic PYY expression is upregulated in ad-libitum fed birds compared to those experiencing a short-term (11-hour) fast (H=0.768,p=0.006). Taken together, these data suggest that chicken PYY is responsive to short-term nutritional state and make for an interesting comparison to mammalian PYY, offering clues as to the origin-dependence of PYY action *in vivo* and evolving function of the uniquely-structured avian PYY.
F statistics (F) are reported for variable-blocked ANOVAs (log transformation was employed to approximate normality of data where possible).
H statistics (H) are reported for Kruskal-Wallis non-parametric tests.
Spearman rank correlation coefficient (r_s) is reported for Spearman's correlation test.

P60
CHOLECYSTOKININ 1 RECEPTOR IN NILE TILAPIA: CLONING, TISSUE DISTRIBUTION, FUNCTIONAL CONFORMATION AND ITS ROLES IN REGULATING GENES RELATED TO DIGESTION AND FEEDING

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Cholecystokinin (CCK) is a peptide that mainly expressed in gut and brain. The CCK receptors can be pharmacologically subdivided into two subtypes: CCK1R and CCK2R. CCK1R is well known to be involved in regulating the secretion of digestive enzymes and inhibiting food intake by binding with CCK. However, similar information in fish is limited. In this study, the cck1r ORF was cloned and characterized in the nile tilapia (Oreochromis niloticus). The size of cck1r ORF of tilapia is 1359bp and it encodes 452 a.a. The cck1r mRNA was expressed ubiquitously in various tissues of tilapia, and particularly high levels were observed in the telencephalon, gall bladder, foregut and midgut. In vitro experiments reveal that cholecystokinin octapeptide (Cck8) combining with CCK1R will activate PKC signaling pathway and may play physiological functions by phosphorylating of PKCa/b2 isoforms. Studies carried out using i.p. injections of Cck8 in tilapia showed the following results: i) in the pyloric, Cck8 can significantly stimulate the mRNA expression of trypsin, pepsinogen and amylase after injection for 1 h; ii) in the hypothalamus, the mRNA expression levels of neuropeptide Y (NPY) and orexin were markedly increased after the i.p. injection of Cck8 for 0.5 h, 2 h and 6 h, respectively; iii) and pre-injection of Cck1r antagonist devazepide can significantly attenuate the mRNA expression of genes stimulated by Cck8 in the present study. These results collectively indicate that the Cck1r may play important roles in regulation of digestion and feeding of nile tilapia. Acknowledgements: Supported by: China Agriculture Research System (CARS-

49), the Guangdong Provincial Science and Technology Program (2015A020216006) and the Modern Agriculture Talents Support Program （2016-2020） to Dr. Li WS. *Corresponding author, email: lsslws@mail.sysu.edu.cn

P61
APPETITE REGULATORS IN CHARACIFORME FISHES: A REVIEW OF THE CURRENT KNOWLEDGE.

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In fish as in other vertebrates, food intake regulation involves intricate networks of hormones produced by both brain and peripheral tissues that affect feeding centres in the brain to either stimulate (e.g. orexin, ghrelin) or inhibit (e.g. CCK, CART) feeding. The order Characiformes includes a considerable array of freshwater fishes divided into 18 families and over 1,500 species. They include tetras, pacus, piranhas and dourados. These fishes present an incredible variety of ecological adaptations, with feeding modes including herbivory (*e.g.* pacu), omnivory (*e.g.* black skirted tetra) and carnivory (*e.g.* Mexican cave fish and voracious predators such as piranhas and dourados). Many of these species are important in the aquarium, aquaculture and sport fishing industries. Yet, very little is known about the endocrine mechanisms regulating their feeding. This review will present our current knowledge on appetite-regulators and potential interspecific differences in the endocrine mechanisms regulating feeding in these fishes. Acknowledgements: Supported by: NSERC Canada discovery grant

P62
HEPATIC MICRORNA PROFILE IN 'GLUCOSE INTOLERANT' RAINBOW TROUT AND PREDICTED CONSEQUENCES FOR THE INTEGRATION OF ENDOCRINE SIGNALS.

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Rainbow trout are a widely-used research model in the endocrine and nutritional regulation of energy metabolism. Due to their poor utilization of dietary carbohydrates characterized by a prolonged hyperglycemia following the ingestion of carbohydrate-rich diets, rainbow trout are generally considered to be 'glucose-intolerant'. In addition to the investigation of endocrine signaling, mRNA and protein abundance and function of rate-limiting enzymes in energy metabolic pathways, recent studies have pointed to metabolic roles of microRNAs. MicroRNAs are short, non-protein-coding RNA molecules with roles in the regulation of energy metabolism in both, mammals and rainbow trout. Taking advantage of a recent annotation of the rainbow trout miRNA repertoire and genome-derived UTR target sequences in this species, we used a transcriptome-level approach to identify hepatic miRNA abundance in response to dietary carbohydrate regimes inducing hyperglycemia. Using *in silico* methods, we provide evidence for the emerging hypothesis that altered hepatic miRNA profiles in response to a high dietary carbohydrate load may result in differential endocrine regulation of carbohydrate metabolism in the liver, thus contributing to a 'glucose intolerant' metabolic phenotype. Acknowledgements: Supported by: A University of Ottawa start-up grant to JAM and financial support from the INRA PHASE Department to SP and LM.

P63
THE ROLE OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN *RHODNIUS PROLIXUS.*

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Feeding and reproduction are interrelated processes in the blood-feeding insect *Rhodnius prolixus*. Nutrition is a determinant of mating motivation, egg production, and oviposition. The molecular basis of this relationship, however, is not fully understood. Corticotropin-releasing factor (CRF) is a hormone involved in the stress response in mammals. In *R. prolixus*, intake of a huge blood meal, up to ten times its body weight, puts great stress on the organism. The insect responds by initiating diuresis, orchestrated by serotonin and a CRF-like diuretic hormone (Rhopr-CRF/DH), to eliminate the excess water and salt, and the parasite transmitting Chagas disease in the process. Rhopr-CRF/DH's distribution throughout the *R. prolixus* central nervous system, however, and expression of its receptor in the female reproductive system suggests a multifaceted role for Rhopr-CRF/DH, beyond diuresis. In the grasshopper, CRF/DH is co-localized with ovary maturating parsin, and the two neurohormones are coded on the same gene, highlighting the hormone as a likely mediator of reproductive processes in insects. In the present study, feeding experiments were used to determine the possible effects of Rhopr-CRF/DH on feeding behaviour, and oviduct contraction assays and egg-laying assays were performed to determine its role in reproduction. Injection of Rhopr-CRF/DH prior to feeding resulted in intake of a significantly smaller blood meal compared to control insects injected with saline. When adult females were injected with Rhopr-CRF/DH, they produced and laid significantly fewer eggs than did controls. Finally, preliminary results from oviduct contraction assays suggest Rhopr-CRF/DH inhibits lateral oviduct contractions. To conclude, the study of the CRF pathway, its components and mechanisms of action, has implications for vector control by highlighting targets to alter diuresis, feeding, and reproduction of this disease vector. Acknowledgements: Supported by: NSERC Discovery Grants to ABL and IO.

P64
LEPTIN, A CATABOLIC STRESS HORMONE INFLUENCES THE EXPRESSION OF RATE-LIMITING ENZYMES ASSOCIATED WITH ANAEROBIC AND AEROBIC METABOLISM IN THE TILAPIA

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Leptin is known to influence energy expenditure, primarily associated with lipolysis and fatty acid oxidation in mammals. In tilapia (Oreochromis mossambicus) leptin may act to regulate carbohydrate catabolism, a function that may be common among poikilotherms. Hepatic expression of leptin increases in response to seawater challenge and other catabolic stressors (fasting), and treatment with recombinant tilapia leptin induces both hyperglycemia and hepatic glycogenolysis. Here, we employed transcriptomic (RNAseq) analysis, coupled with novel machine learning approaches, to identify leptin actions on the tilapia rostral pars distalis (RPD), which contains a nearly pure population of prolactin (PRL) cells. Our previous work shows leptin is a potent stimulator of PRL synthesis and secretion. Our analysis also revealed that leptin stimulates glyceraldehyde 3-phosphate dehydrogenase expression in a manner that covaries with gene networks involved in the hypoxic stress response. Leptin increases expression of phosphofructokinase (PFK), the rate-limiting enzyme of glycolysis, after 6 h incubation of RPD. Similarly, leptin stimulates total glycolytic activity (lactate secretion) and PFK activity within 6 h, indicating leptin is a potent stimulator of cellular glycolysis. Additional analysis using machine learning classifiers (Support Vector Machines) discovered, by contrast, that leptin down-regulates mRNA expression of pyruvate dehydrogenase (*dlat*, E2 component), isocitrate dehydrogenase (*idh*), succinate dehydrogenase (*sdhb*), and fumarate dehydrogenase (*fh1*), several of which are rate-limiting enzymes of the Krebs cycle. Interestingly, the disparate regulation of glycolysis and the Krebs cycle suggest a novel role for leptin in modifying anaerobic and aerobic pathways. Leptin may enhance anaerobic glucose catabolism while suppressing aerobic catabolism of pyruvate/citrate, activities essential to surviving acute periods of hypoxia and perhaps others stressors where leptin is known to increase and glycolytic activity is enhanced.

P65
CHARACTERIZATION OF A NESFATIN-1-LIKE PEPTIDE (NLP) IN MICE

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Nesfatin-1 (82 amino acids) is a peptide encoded in the secreted precursor nucleobindin-2 (NUCB2). Exogenous nesfatin-1 decreases food intake, and stimulates insulin secretion. When originally discovered, nucleobindin-2 was given its name due to very high sequence similarity with another secreted protein, nucleobindin-1 (NUCB1). The sequences in NUCB1, corresponding to the nesfatin-1 region of NUCB2, are highly conserved. Since nucleobindins are precursors of biologically active peptides and given the cytoplasmic presence of NUCB1; we hypothesized that NUCB1 could encode a nesfatin-1-like peptide. *In silico* analysis of NUCB1 sequence, found a signal peptide and a prohormone convertase cleavage site upstream and downstream of a 77 amino acid sequence (NLP); that could be secreted. Is NLP an endogenous peptide? NUCB1 expression was reported to be present exclusively in endocrine pancreas and is consistently associated with the golgi apparatus. Immunoprecipitation/Western blot analysis of mouse and rat pancreatic golgi-fraction and MIN6 cell protein lysates; showed a distinct band corresponding to ~8.73 kDa. In-gel digestion and subsequent nanoLC-MS/MS analysis followed by protein database search (Mascot); showed highest protein scores for nucleobindin-1 in the digest. Time of flight (TOF) analysis predicted a peptide sequence 71, 93 and 100% identical to the NLP sequence in MIN6 cells, mouse and rat pancreatic digest; respectively. Immunofluorescence analysis detected NUCB1-like immunoreactivity in murine pancreatic beta cell line (MIN6). NUCB1 also co-localized with insulin immunoreactivity in pancreatic β cells. Furthermore, transmission electron microscopy (TEM) showed NLP immunolabelling in both secretory granules and RER of mouse pancreatic islets and MIN6 cells. Static incubation of MIN6 cells with synthetic NLP upregulated both preproinsulin mRNA expression and insulin secretion into media. Conversely, treatment of cells with a scrambled peptide based on NLP sequence; did not elicit a similar response. Taken together, NLP is an endogenous NUCB1-encoded novel insulinotropic peptide that could be secreted. Acknowledgments: Supported by: Canadian Institutes of Health Research (CIHR) Open Operating Grant to SU. NR is a recipient of University of Saskatchewan Dean's Scholarship.

P66
PROFILE OF GHRELIN, GHRELIN RECEPTOR AND GHRELIN-O-ACYL TRANSFERASE IN FISH GONADS DURING METABOLIC STRESS

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Metabolism and reproduction are tightly interlinked. Various endocrine factors with key roles in energy homeostasis are involved in the regulation of reproduction. However, the hormonal inputs connecting metabolism and reproduction are not fully understood. Ghrelin is a multifunctional gut hormone involved in feeding and metabolism, circadian cycle, and reproduction. Post-translational acylation of ghrelin

by Ghrelin-O-acyltransferase (GOAT) is critical for its biological actions. It has been reported that ghrelin and its receptor (GHS-R) are expressed in gonads of several species including fishes. However, the expression of ghrelin, GHS-R and GOAT under metabolic stress remains unclear. In this research, RT-qPCR and fluorescence immunohistochemistry were used to study the expression of ghrelin, GHS-R and GOAT in goldfish gonads under metabolic stress. We found the expression of mRNAs encoding ghrelin, GHS-R and GOAT in goldfish gonads. Preproghrelin and GHS-R mRNA expression significantly decreased in the testis and ovary of sexually immature goldfish deprived of food for 3 or 7 days compared to fed goldfish. Meanwhile, preproghrelin mRNA expression significantly decreased in testis, and its expression increased in ovary of sexually mature, food deprived goldfish. GOAT mRNA expression increased in the testis, and no changes were observed in the ovary of mature goldfish under metabolic stress. GHS-R increased in both testis and ovary of unfed goldfish. Gonadal cell specific immunofluorescence signal intensity for ghrelin, GHS-R and GOAT in testis and ovary are consistent with our RT-qPCR data from mature food deprived goldfish. Further studies are required to elucidate the physiological roles of the ghrelinergic system in fish reproduction during distinct reproductive stages under various metabolic conditions.Acknowledgment: This research is generously funded by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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NESFATIN-1 LIKE PEPTIDE IS A NOVEL METABOLIC FACTOR IN RATS

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Nucleobindin-1 (NUCB1) encoded Nesfatin-1 Like Peptide (NLP) has high sequence similarity to NUCB2 encoded, anorectic and metabolic peptide, nesfatin-1. NLP is shown to suppress food intake in fish. NLP is also insulinotropic *in vitro* in mice islet cells. Our main objective was to determine whether NLP is anorectic and regulates whole-body energy homeostasis in male Wistar rats. A single intraperitoneal (IP) injection of NLP (100 µg/kg BW) decreased food intake and increased energy expenditure of rats. This was accompanied by reduced cumulative food intake and whole body fat oxidation compared to saline treated rats during the dark phase. Continuous subcutaneous infusion of NLP at the same dose using osmotic mini-pumps for 7-days showed similar effects as found after the IP injection. However, decreased physical activity was observed during the long-term treatment. Interestingly, body weight gain was not different between control and NLP treated rats. The expression of mRNAs encoding adiponectin, resistin, ghrelin, cholecystokinin and uncoupling protein 1 (UCP1) were significantly upregulated, while leptin and peptide YY mRNA expression were downregulated in NLP-treated rats. These findings indicate that administration of NLP at 100 µg/kg BW reduces food intake and modulates whole-body energy balance. In summary, NLP is a novel metabolic peptide in rats.Acknowledgments: Supported by an Open Operating Grant from the Canadian Institutes of Health Research (CIHR), an Establishment Grant from the Saskatchewan Health Research Foundation (SHRF) and a John Evans Leader's Fund (JELF) from the Canada Foundation for Innovation (CFI) to SU. SU is a recipient of the CIHR New Investigator Salary Award. KG received a postdoctoral research fellowship from the SHRF.

P68
IRISIN MODULATES FEEDING AND CARDIAC PHYSIOLOGY IN ZEBRAFISH

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Irisin is a myokine encoded in its precursor fibronectin type III domain containing 5 (FNDC5). FNDC5 forms an integral part of the muscle post-exercise, and causes an increase in energy expenditure in mammals. Irisin is abundantly expressed in cardiac and skeletal muscles and is secreted upon activation of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1 alpha). Irisin is considered to be a key promoter in the central nervous system, and it regulates cardiac contractility. More recently, irisin has gained importance as a potential biomarker for myocardial infarction due to its abundance in cardiac muscle. We aimed to study the role of irisin on feeding and cardiovascular functions in zebrafish. Irisin (0.1 nM) downregulated PGC-1 alpha, myostatin a and b; and upregulated troponin C mRNA expression in zebrafish heart and skeletal muscles. Though intraperitoneal injection of irisin did not influence feeding, its knockdown (10 ng/g B.W) caused a significant reduction in food intake. Knockdown of irisin reduced ghrelin and orexin-A mRNA expression, and increased CART mRNA expression in zebrafish brain and gut. The role of irisin on food intake is likely mediated by its actions on other metabolic peptides. Exogenous irisin (0.1 and 1 ng/g B.W) increased diastolic volume, heart rate and cardiac output in zebrafish, while irisin knockdown (10 ng/g B.W) decreased cardiac filling, heart rate and cardiac output. Irisin (1 and 100 ng/g B.W) downregulated PGC-1 alpha, myostatin a and b, while upregulated troponin C and troponin T2D mRNA expression, while knockdown of irisin resulted in opposing effects on troponin C, troponin T2D and myostatin a and b mRNAs in heart and skeletal muscle tissues of zebrafish. Irisin regulation on muscle proteins is a possible mechanism by which it modulates cardiac biology. Collectively, these results indicate that irisin is a novel modulator of cardiovascular biology and metabolic physiology in zebrafish. Acknowledgments: Supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant and Discovery Accelerator Supplement to SU.

P69
NESFATIN-1 REGULATION OF HEPATIC GLUCONEOGENESIS.

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Gluconeogenesis is a metabolic process that produces glucose from non-carbohydrate carbon substrates including pyruvate, lactate, glycerol, and gluconeogenic amino acids. However, in type 2 diabetes (T2D), dysregulation of hepatic glucose production (HGP) contributes to hyperglycemia. In recent studies, key enzymes and transcriptional factors of hepatic gluconeogenesis, and endocrine regulators of gluconeogenesis are considered important therapeutic targets for the treatment of T2D. Nesfatin-1 is an 82 amino acid metabolic peptide that plays an important role in stimulating insulin secretion, and maintaining glucose homeostasis. The main goal of this study was to analyze whether nesfatin-1 affects gluconeogenesis. NUCB2 mRNA expression and immunoreactivity was found in the liver and in hepatocarcinoma (HepG2) cells. HepG2 cells were used to determine whether nesfatin-1 regulates gluconeogenesis and key gluconeogenic enzymes. Nesfatin-1 upregulated fructose 1, 6 bisphosphatase (FBP1), phospoenolpyruvate carboxykinase (PEPCK), and downregulated glucose-6-phosphatase (G6Pase), pyruvate carboxylase (PCB) and fork head box protein O1 (FOXO1). Nesfatin-1 regulation of these enzymes could modulate HGP. Additional studies using mouse and human hepatocytes to measure glucose production and glycogen synthesis in the presence and absence of nesfatin-1 are required to determine the precise role of nesfatin-1 on hepatic gluconeogenesis. As HGP is a major contributor of hyperglycemia in T2D, nesfatin-1 regulation of HGP warrant further consideration.Acknowledgments: Canadian Institutes of Health Research, Canadian Foundation for Innovation, Saskatchewan Health Research Foundation, Department of Veterinary Biomedical Sciences

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THE NEUROPEPTIDE Y SYSTEM AND THEIR FUNCTIONS IN THE REGULATION OF FOOD INTAKE IN NILE TILAPIA *Oreochromis niloticus*

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Food intake is essential for the survival of vertebrates, which is controlled by a highly complex process involving elaborate interaction between the central and peripheral signals. Among these, neuropeptide Y (NPY) system which takes part in both the brain and peripheral regulation of food intake is crucial in this aspect. In teleost fishes, the regulation of food intake by NPY system in teleosts has not been well studied. Up to now, little is known about the effects of NPYb and PYYb on food intake and which subtypes of NPY receptor are involved in feeding regulation. In the present study, we identified the two duplicates of NPY and PYY in Nile tilapia (*Oreochromis niloticus*). Both *NPYa* and *NPYb* were primarily expressed in the central nervous system (CNS), but the mRNA levels of *NPYb* were markedly lower than those of *NPYa*. Hypothalamic mRNA expression of *NPYa*, but not *NPYb*, decreased after feeding and increased after 7-days of fasting. However, both NPYa and NPYb caused a significant increase in food intake after an intracranial injection of 50 ng/g body weight dose. *PYYb*, one of the duplicates of PYY, had an extremely high expression in the foregut and midgut, whereas another form of duplicate *PYYa* showed only moderate expression in the CNS. Both hypothalamic *PYYa* and foregut *PYYb* mRNA expression increased after feeding and decreased after 7-days of fasting. Furthermore, the intracranial injection of PYYb decreased food intake, but PYYa had no significant effect. Our results suggested that although the mature peptides of NPYa and NPYb can both stimulate food intake, NPYa is the main endogenous functional NPY for feeding regulation. A functional division has been identified in the duplicates of PYY, which deems PYYb as a gut-derived anorexigenic peptide and PYYa as a CNS-specific PYY in Nile tilapia. Acknowledgements: Supported by: the China Agriculture Research System (CARS-49) and the National Science Foundation of China (31472259) to Dr. Wensheng Li. * Corresponding author: Wensheng Li (lsslws@mail.sysu.edu.cn).

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DISRUPTION OF ZEBRAFISH GROWTH HORMONE GENE (*GH1*) CAUSES ARREST OF FOLLICULOGENESIS IN FEMALES BUT NOT SPERMATOGENESIS IN MALES

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The function of the reproductive axis is known to be influenced by the condition of the somatic growth. Growth hormone (GH) as a vital pituitary hormone plays a fundamental role in controlling body growth and metabolism, and it is also known to regulate reproduction. Studies in different mammals have revealed that mutations in GH and/or its receptor (GHR) result in severe retardation in body growth and dysfunctions of reproduction in both sexes. However, a role of GH in reproduction in lower vertebrates is still poorly defined. In the present study, we created two zebrafish *gh1* mutant lines using CRISPR/Cas 9 method. The mutant fish developed normally up to 14 dpf; however, a high rate of mortality was observed afterwards in both lines, and only a small number of mutant fish could survive to adult stage. The

body growth of the mutants was significantly retarded in both sexes in a gene dose-dependent manner compared to their wilt-type siblings. A severe dysfunction of gonadal development was observed in females with ovarian folliculogenesis being arrested completely at primary growth stage. The spermatogenesis in the testis seemed to be normal histologically, and the mutant sperm from dissected testes could fertilize eggs through artificial fertilization with normal embryogenesis. However, the mutant males could not spawn successfully with wild-type females. The expression of some relevant genes in the somatotropic axis and metabolism, including four ligands of insulin-like growth factors (*igf1, igf2a, igf2b* and *igf3*) and two leptin ligands (*lepa* and *lpeb*), were analyzed by real-time qPCR in the control and mutant fish. This study provides the first genetic evidence for dependence of female puberty onset and folliculogenesis on somatic growth but not age and will shed light on the role of GH in regulating reproduction and the potential mechanisms in a comparative perspective.
Acknowledgement: This work was funded by grants from University of Macau and The Macau Fund for Development of Science and Technology.

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TEMPORAL EXPRESSION OF HEPATIC AND GONADAL REPRODUCTIVE MARKERS IN ATLANTIC BLUEFIN TUNA CAUGHT IN MEDITERRANEAN AREA

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Atlantic bluefin tuna (*Thunnus thynnu*) is a prized pelagic fish species representing an important worldwide economic fisheries resource. Overexploitation significantly reduced tuna wild stocks and research efforts are now focusing on many aspects of their biology. Despite the increasing number of studies, many aspects of the basic biology of these species are still lacking. In this study, we investigate the variation of the expression of several reproductive signals in fish sampled at spawning (June) and post-spawning (November) season. At hepatic level, the mRNA expression of different vitellogenin isoform (*vtga, vtgb, vtgc*) and estrogen receptor α (*era)* was analyzed. In gonad, the expression of genes associated with gonadal development was considered. Focusing on the ovary, mRNA variation of zpc1, vitellin egg envelope gamma, aveolin and choriogenin L, playing a pivotal role in egg envelope formation; cathepsin S, involved in yolk proteolysis; aquaporin 1 and Tmc6-related protein 1; responsible for oocyte hydration; fatty acid binding protein (fabp) encoding for an enzyme involved in fatty acid uptake and intracellular transport, were analyzed. In the testis, the expression of T-complex associated testis-expressed protein1, a specie specific marker responsible for sperm binding to zona radiate and brain type-fabp and intestinal fabp, responsible for fatty acid uptake, were analyzed. At hepatic level, the main finding was the transcription of vitellogenin mRNA detected also in male fish, suggesting an environmental issue. Moreover, the different vtg isoform expression between spawning and post spawning season highlighted a different physiological role of these proteins, as observed in other teleosts. At gonadal level, a sex specific, slight modulation of mRNA levels was measured for some of the signals analyzed. Specifically, signals involved in oogenesis were more expressed at reproductive stage, decreasing in November. Focusing on testis, any significant mRNA changes were detected between reproductive and post reproductive seasons. This study contributes to increase the knowledge on the basic reproductive biology of tunas. Integration of these results with molecular features of growth obtained within the same project could contribute to gain knowledge on the first size of maturity of this important commercial species.Acknowledgements: Supported by Ministero delle Politiche agricole e forestali-MIPAAF

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NEW INSIGHTS INTO THE REPRODUCTIVE PROCESS OF SWORDFISH (*XIPHIAS GLADIUS*) IN THE MEDITERRANEAN SEA: THE CASE OF INTERSEX GONADS.

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Swordfish (*Xiphias gladius*) is a large pelagic migratory species of high commercial value and it is heavily exploited in the Mediterranean Sea. Studies on swordfish life-history, and particularly on reproduction, are so far limited. The objective of the present study was to better characterize the reproductive biology and seasonal maturation process of swordfish in the Mediterranean Sea. To achieve this objective, we monitored the gonadal development of individuals caught by longline fishery fleet in the Mediterranean sea over July, August, September and December. Gonadal index (GI) and Gonadosomatic index (GSI) were monitored and in the period examined, spawning was observed during July and August while in September and December spent gonads were found.
Different developmental stages of both female and male gonads, histologically described were associated to changes of gonadal macromolecular building analysed by FTIR microspectroscopy. Using this last technique, the different oocytes stages, as well as the different developmental stages of spermatogenesis were characterized. Surprisingly, 10% of specimens, macroscopically classified as males, showed the presence of previtellogenic and vitellogenic oocytes within the testis.
In addition, the hepatic ultrastructural changes were investigated by histological analyses, while the hepatic lipid changes were analysed by FTIR microspectroscopy. Modifications of hepatic lipids content were evinced among male, female and intersex fish; these changes could be attributed to vitellogenin synthesis.
Concluding, this study contributes to a better understanding of Mediterranean swordfish reproductive process. The existence of intersex in the population here studied let hypothesize the presence of estrogen-mimicking substances in the environment and may therefore increase concern regarding the effects caused by bioaccumulation/biomagnification of toxic compounds in top predators.Acknowledgements: Supported by: The Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture, MiPAAF – Italy.

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A NEW RECIPE FOR BREEDING SUCCESS? RECONSIDERING SOY AND ALFALFA IN SOUTHERN WHITE RHINOCEROS DIETS

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The captive southern white rhinoceros (SWR) population is not currently self-sustaining due in large part to low fertility exhibited by captive-born females. Our research has focused on the potential role of dietary phytoestrogens in this phenomenon. Specifically, we have shown that phytoestrogens are potent agonists of SWR estrogen receptors and that estrogenicity of captive diets is directly proportional to the amount of soy and alfalfa-containing pellets fed. We have also demonstrated a significant negative relationship between the estrogenicity of an institution's diet and the fertility of their captive-born females. We found no such relationship for female SWR imported from the wild and fed high phytoestrogen diets, supporting the hypothesis that developmental exposure to phytoestrogens compromises fertility of female SWR. Recently, our institution developed a low phytoestrogen pellet containing minimal soy or alfalfa-based ingredients, and reduced the total amount of pellet consumed by SWR by 80%. This change resulted in a greater than 90% reduction in the estrogenicity of our SWR diets. Although the goal in modifying captive SWR diets was to reduce potential deleterious effects of developmental phytoestrogen exposure, it was unclear whether the diet change would affect fertility of the current population of non-reproductive captive-born female SWR. However, following the reduction in dietary phytoestrogen exposure, elevations in fecal progestagen levels indicate that three pregnancies have been achieved in two captive-born female SWR that had previously not reproduced. To date, one of those pregnancies has resulted the successful birth of a SWR calf. Taken together, our data strongly suggest that phytoestrogens negatively affect SWR fertility. While we suspect, the primary mechanism is through developmental exposure, our recent observations indicate adult exposure could also be compromising fertility of some individuals, but may be reversible. As a result, we advocate that dietary phytoestrogen levels should be reduced in order to increase fertility of SWR in managed settings.

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SOCIAL FACTORS ARE ASSOCIATED TO ADULT MALE TO FEMALE SEX CHANGE IN CAPTIVE GONOCHORIC LEOPARD GROUPER *MYCTEROPERCA ROSACEA*.

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We investigated the influence of social factors on sex change in the leopard grouper *Mycteroperca rosacea*, when captive fish were separated by sex during the reproductive (April to June), and post-reproductive (July to September) seasons. Monosex female, monosex male, and mixed sex, held in social sextet units, were analyzed for sex steroids throughout the period of confinement, and the gonads through histology at the end of the experiment. Histology of males held in monosex social units, showed that one male did not change sex, six were found in a transitional sexual stage, and 11 were classified as immature females. In spring, estradiol-17ß showed a specific female-profile, whereas 11-Ketosterone a specific male-profile, independently from social units, which suggests that male to female sex change was not triggered during the reproductive season. The low steroid levels in summer made not possible to associate the sex change to a gonad hormonal shifting; therefore, gonad sex steroid profiles could not explain this phenomenon. In September, male to female sex change was concomitant with stress symptoms: loss of eating activity, followed by lethargy, subsequently poorly responsive, and death. These symptoms did not begin in all fish at the same time, but usually one by one, and only three males did not show them, and survived. It was difficult to discern if stress symptoms were a consequence of the process of changing sex, or because of the confinement *per se*, because female monosex, and mixed sex social units, neither had these symptoms nor changed sex. Therefore, male to male behavioral interactions apparently occurred, but at the expense of life. We concluded that social factors were associated to adult male to female sex change in leopard grouper, being the first report in captivity in a gonochoric species, which contributes to the knowledge of sexual plasticity in the subfamily Epinephelinae. Acknowledgements: Supported by: CONACYT MEXICO, Grant 0223157 to DAGT. DRM was the recipient of a doctoral fellowship CONACYT.

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EXPOSURE EFFECTS OF ENVIRONMENTAL GESTAGENS ON A SENTINEL FISH SPECIES FROM RECEPTOR ACTIVATION TO ALTERED REPRODUCTIVE BIOLOGY

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Environmental gestagens include endogenous progestogens, such as progesterone and 17α,20β-dihydroxypregnenone, which bind progesterone receptors and have critically important roles in vertebrate physiology, especially reproduction. They also include synthetic progestins, such as gestodene and levonorgestrel, which are components of contraceptive pharmaceuticals. Gestagens enter the aquatic environment through wastewater treatment plant effluent, papermill effluent, and agricultural runoff. In this talk, we will present environmental concentration data from a large field study. We will also present the results of lab studies, where we tested the in vitro receptor

activation and in vivo exposure effects of a handful of gestagens. These results provide potential mechanisms that explain, in part, findings by our lab and others. Given the rapid and profound effects of gestagens on fish reproduction, this class of contaminants should garner increased attention by researchers and regulators alike. (Funding to EFO by the Morris Animal Foundation-D14ZO-010 and the Department of Interior-USGS/NIWR- 2014MD321G.)

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PRENATAL EXPOSURE TO BISPHENOL-A (BPA) REPROGRAMS THE EXPRESSION OF MICRORNA-378 and MICRORNA-224 INVOLVED IN OVARIAN GRANULOSA CELL ESTROGEN BIOSYNTHESIS AND TARGETS NOVAL OVARIAN TRANSCRIPTS

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Ability of BPA to cross the protective placental barrier and reach the fetus during critical stages of development is still a cause of major concern because of the fact that BPA, similar in structure to estradiol, can bind to multiple targets, acting as an estrogenic endocrine disrupting chemical. Animal experiments have implicated fetal ovary as an important organ that can be programmed by transient changes in the prenatal hormonal environment. These long term consequences in postnatal endocrine and reproductive function was demonstrated with molecular mechanisms involving epigenetic alteration. This study was aimed to determine the effect of prenatal exposure to BPA on the expression of miRNAs (miR-133b, miR-378 and miR-224) which were previously documented to be involved in the regulation of estrogen biosynthesis in ovarian granulosa cells. We have analyzed the expression of miRNAs and their target genes to elucidate the regulatory roles of altered miRNAs employing gene network analysis. The pregnant Wistar rats were given a subcutaneous injection of BPA covering human exposure relevant doses (0.2 µg/kg-bw/day and 2 µg/kg-bw/day) during the gestational days 11-18. Female offspring were sacrificed on postnatal week 12 and granulosa cells were isolated. We have observed miR-378 and miR-224 to be differentially expressed in the BPA exposed and control groups; these miRNAs showed a dose-dependent increase in their expression (miR-378: 2.43 and 7.41 fold increase; miR-224: 1.9 and 5.8 fold increase). Gene network analysis of altered miRNAs predicted the target genes with roles in DNA hypomethylation, insulin and cancer signaling pathways,which were regulated by the dysregulated miR-378. The visualized gene network for the altered miR-224 targeted genes showed predicted pathways of oocyte maturation, MAPK, EGFR1, Ras, BMP and TGF-beta receptor signaling. Taken together, these data suggest the effects of early exposure to BPA on the ovarian granulosa cells. Further, it helps extend our knowledge on the molecular mechanism linking early life exposure of BPA with adult female reproductive disorders. Acknowledgements: Supported by: DBT (India)-Rapid grant for young investigator fund to CL and SWS. Department of Biotechnology, SRM University-Research initiative fund for CL and SB.

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PRIMORDIAL GERM CELL DEVELOPMENT AND EARLY GONAD FORMATION IN THE EUROPEAN SEA BASS DURING THE TEMPERATURE SENSITIVE WINDOW.

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Most vertebrates rely on sex chromosomes to either activate male or female mode of differentiation. However, in teleost fish there is a high degree of plasticity and the activation of sex differentiation process relies on the activation of complex gene cascades that depending on the dosage converge to promote a male or a female differentiation pathways. In the European sea bass, this process is also modified by temperature and at high temperatures (21ºC) female development is at least partly suppressed and male biased populations are obtained. With the aim of clarifying the mechanism behind the masculinizing effect we have isolated transcripts that are differentially expressed in larvae grown at normal and masculinizing temperatures using suppressive subtraction hybridization. We found an enrichment of genes involved in Wnt signaling, in epigenetic signaling and candidate germ cell markers. The characterization of the expression profiles of the transcripts and in situ hybridization allowed for the first time to detect primordial germ cell progression from mouth opening up to the end of metamorphosis and identify candidate genes involved in the early steps of germ cell progression and gonadal formation. In addition, analysis of DNA and histone methylation further suggests the involvement of the latter in the regulation of germ cell progression. Acknowledgements: Supported by: the European Commission FP7 through ERA-NET COFASP project 0002/2015 and Aquaexcel project TNA 0089/05/07/20; the Foundation for Science and Technology of Portugal (FCT) through project UID/Multi/04326/2013 and fellowship SFRH/BPD/111512/2015 to RSTM.

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STEROIDOGENIC GENE EXPRESSION IN MALE MUMMICHOG (*FUNDULUS HETEROCLITUS*) EXPOSED TO MODEL ANDROGENS 5A-DIHYDROTESTOSTERONE OR 17A-METHYLTESTOSTERONE; AN *IN VITRO* INVESTIGATION.

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Elucidating key events in adverse outcome pathways (AOP) is integral to strengthening biological links across multiple levels of organization. Current research has identified decreased plasma sex steroid hormones in mummichog, an estuarine killifish, after exposure to androgenic compounds, in conjunction with decreased egg size and reduction of plasma vitellogenin. However, the molecular cause is currently unknown. Potential impacts on the steroidogenic pathway in mummichog were assessed by exposing testis tissue *in vitro* to control (ethanol only), 10^{-6}, 10^{-9} and 10^{-12} M of the non-aromatizable androgen 5α-dihydrotestosterone (DHT) and the aromatizable androgen 17α-methyltestosterone (MT) for 6, 12, 18 and 24 hours. A suite of genes including steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase (3βHSD) and cytochrome P450 17A1 (CYP17) were analyzed to elucidate androgen impact. Genes were normalized to elongation factor 1-α. Temporal impacts of gene expression occurred at 18 and 24 hour time points, with upregulation of StAR, cytochrome P450 11A1, 17β-hydroxysteroid dehydrogenase, 11β-hydroxysteroid dehydrogenase (11βHSD) and CYP17 in both MT and DHT treatments. 3βHSD expression was altered after 6 hours of exposure, indicating it may be a biomarker for androgenic activity. Downregulation of androgen receptor was found in MT treatments at 24 hours but not in DHT treatments, indicating potential estrogenic effects of MT. Androgen impact on cytochrome P450 19A1 is currently in progress. Non-monotonic responses in StAR at 24 hours, 3βHSD at 6 and 12 hours and 11βHSD at 18 hours were observed in DHT treatments, indicating potential challenges to current AOP testing regimes. Comparison of aromatizable and non-aromatizable model androgen responses will increase knowledge of potential differences in androgen impact due to androgen classification and support AOP development. Linking of gene expression impacts to higher levels of biological organization is the next step in producing a robust AOP for androgenic exposure.

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ASSESSING THE ESTROGENIC POTENTIAL OF LEAD IN THE CALIFORNIA CONDOR (GYMNOGYPS CALIFORNIANUS)

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The most significant challenge facing the recovery of the critically endangered California condor (*Gymnogyps californianus*) is lethal exposure to lead (Pb) acquired from scavenging carcasses containing spent Pb-based ammunition. Due in part to the management of Pb poisoning, nearly the entire wild condor population of approximately 200 individuals is captured semiannually and blood-Pb levels are evaluated. Results of these health assessments have shown that the median blood-Pb level of free-flying condors is greater than 100ng/mL. Similar levels of Pb have been shown to exhibit weak to moderate estrogenic activity through direct interaction with the ligand-binding domain (LBD) of human estrogen receptor-α (ERα). Interestingly, the 5 amino acid residues within the human ERα LBD with which Pb putatively interacts are identical to those in the LBD of condor ERα, suggesting that Pb could be estrogenic in condors as well. To test this possibility, *in vitro* activation of wild-type California condor ERα by Pb was assessed. In addition, site-directed mutagenesis was performed to mutate each of the 5 amino acid residues in condor ERα suspected to bind Pb (C372A, C438A, D529N, H515A, E514Q). Treatment of wild-type and all mutant condor ERα's with 10^{-12} -10^{-7} M 17β-estradiol (E_2) resulted in significant receptor activation. However, treatment with up to 10^{-4} M $PbCl_2$ failed to activate any of the condor ERα's tested. Although these finding suggests chronic exposure to Pb may not present sub-lethal challenges to the current wild condor population by acting as an environmental estrogen, further investigation using different assessments of Pb estrogenicity are warranted.

P81
STERILIZATION OF MATURED TESTIS OF TILAPIA BY HIGH TEMPERATURE

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We already demonstrated that long-term exposure of high temperature of fries of tilapias, around the time of sex differentiation, induced permanent sterilization of both the ovary and the testis. In the present study, we examined the effect of high temperature on matured testes of tilapia. Matured males of tilapia were reared in the water at 37±℃ for 60 days. For the histological observation, we sampled the testes of fish at 20, 40 and 60 days after the onset of treatment (dat). Mortality was high during treatment. Eventually 10 individuals remained at the end of treatment. Active spermatogenic germ cells were seen in the testes of five fish in initial control. Efferent ducts were filed with large amount of sperm. In contrast, healthy germ cells including spermatogonia were not seen in the testes of five fish at 20 days after treatment. Some remains, which seem to be dead germ cells, were seen in the lumens of the all testes. Strong immunopositive reactions against Casparse-3 anti-body (AB), indicating apoptosis, were seen on these remains. Spermatogenic germ cells disappeared completely from the testes of fifteen fish at 40 and 60 dat. Clusters of Leydig cells, which are the site of sex hormone production, were seen in the interstitial area among the lobules in the sterilized testes. Immunopositive reactions in the Leydig cells were seen against P450scc and 3b-HSD. From this result, it was also demonstrated that long-term exposure of high temperature also brings about the sterilization of matured testis of tilapia. The present research was supported by grants for the JSPS KAKENHI (Project no. 23658166 and 16H02984 for MN).

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POST-SPAWNING LIFE HISTORY DIVERSITY IN RECONDITIONED FEMALE CLEARWATER RIVER STEELHEAD TROUT (ONCORHYNCHUS MYKISS) KELTS ASSESSED USING PLASMA ESTRADIOL-17B

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Abundance is declining for Clearwater River steelhead trout (Oncorhynchus mykiss), an important anadromous salmonid and a species of conservation concern. Reconditioning of post-spawn female steelhead trout (kelts) is a recovery tool used in the Columbia River Basin. Wild kelts are captured, held in tanks, fed, and released to spawn in the river. Steelhead trout are iteroparous and have diverse post-spawning life histories. The existence of consecutive and skip spawning life history trajectories has emerged as a significant issue in fisheries management, but has received little attention from fish reproductive physiologists. The purpose of this study was to use plasma estradiol-17B (E2) measurements (assayed by ELISA) to determine kelt life history trajectory in a reconditioning program. Hatchery origin kelts were spawned in 2015 and 2016 upon return to Dworshak National Fish Hatchery, placed in tanks, and blood sampled at 10- week intervals. At 30 weeks after spawning, a bimodal distribution of E2 indicated fish had split into two distinct groups, one with high E2, indicative of a consecutive spawning trajectory (30% in 2015, 40% in 2016), and another with lower E2, representing a skip spawning trajectory. 86% of surviving skip spawners matured the following year. E2 was elevated in rematuring versus non-rematuring fish at 20-weeks post-spawn in consecutive spawners, and ~10-weeks after the week of the initial spawning in skip spawners. E2 measurements in Clearwater River kelts show two distinct life history trajectories, important information for managing reconditioning projects for this population.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF MCH AND αMSH IN THE BRAIN AND PITUITARY AND EFFECTS OF BACKGROUND COLOR ON MCH LEVELS IN THE BRAIN OF MEXICAN CAVEFISH, *ASTYANAX MEXICANUS*.

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Body color of teleost fish is regulated by MCH and αMSH, and it is also affected by environmental factors such as light and tank color. In the present study, we focused on MCH and αMSH in the Mexican cavefish *Astyanax mexicanus*. It is considered that Mexican cavefish don't need to change body color, because the fish live in constant dark environments and have regressed eyes. First, the existence of MCH and αMSH in the brain and pituitary was examined by immunohistochemistry. MCH-immunoreactive (ir) cell bodies were mainly detected in the magnocellular neurons of the lateral hypothalamic nucleus, which project to the pituitary. αMSH-ir cell bodies were observed in the nucleus tuberis lateralis of the hypothalamus, and αMSH-ir cells were mainly detected in the pars intermedia of the pituitary. These results indicate that MCH and αMSH exist in the pituitary of the Mexican cavefish, although body color change is considered to be unnecessary. Next, the effects of background color on MCH levels in the brain were examined. Fish (mean total length 3.9 cm, body weight 0.53g) were reared in white or black tanks for two months under 12 h of light and 12 h of dark in freshwater controlled at 23 ± 1℃. As a result, no significant differences were observed in brain MCH levels, body color, and body weight between the two tanks. Interestingly, body color of fish reared in the two tanks were much darker than fish reared in the constant dark (stock tank). These results suggest that, unlike other teleost fishes, MCH levels in the brain are not affected by background color. Thus, it is suggested that the function of MCH in Mexican cavefish is different from other fish living in natural light environment

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EFFECTS OF BREEDING ON THE REGULATION OF SKIN COLOR CHANGES IN KOI CARP.

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Koi carp (*Cyprinus carpio*), also known as nishikigoi, were aquarium fish bred for color mutations from common carp. Our results indicated that the skin color changes associated with background color adaptation were mainly due to morphological rather than physiological color changes. However, it is unknown whether this feature resulted from phenotypic variation associated with the regulation of skin color by the endocrine system. To clarify this issue, we investigated the effects of white or black background adaptation on scale melanophores and gene expression of hypothalamic melanin-concentrating hormone (MCH); the effects of *in vivo* administration of MCH on scale melanophores in koi carp and common carp were also investigated. First, koi carp and common carp (n = 8) were acclimated on white or black backgrounds for 21 days. The scale colors in the white background-adapted (W) fish were paler than those of black background-adapted (B) fish in both koi and common carp. The melanosomes in the melanophores of the W common carp were more aggregated than those of the B common carp. However, there were no differences in the aggregation levels of the W and B koi carp. The scale color difference in koi carp was mainly due to the densities of melanosomes and melanophores (i.e., morphological color change). The brain content of *mch* mRNA in W fish was higher than that in B fish in both koi and common carp. Interestingly, the *mch* mRNA content of W koi carp was eight times lower than that of W common carp, and that of B koi carp was four times lower than that of B common carp. These results suggested that basal

MCH synthesis might be suppressed in koi carp with respect to its expression in common carp. Second, the scale melanophores were observed one day after intraperitoneal administration of MCH (0.1 and 1.0 μg g^{-1} body weight) to koi and common carp (n = 6) acclimated on a black background. In the two MCH-administrated groups, melanosomes were completely aggregated in both koi and common carp, suggesting that there might be no differences in MCH-sensitivity and/or function of MCH receptors in koi and common carp. Thus, the dominance of morphological color change associated with background color adaptation in koi carp could result from decreased basal MCH synthesis, which possibly occurred during selective breeding for color mutations.

P85
THE ROLE OF PHYSIOLOGICAL INTEGRATORS IN AVIAN RANGE EXPANSIONS

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Range expansions are becoming more common as humans move organisms around the planet and alter the climate, forcing species to adjust to new conditions or move to new areas. Some organisms appear better able to endure new conditions than others. Indeed, some introduced species often become pests in new areas, spreading rapidly across habitats they never occupied previously. In many plants and invertebrates, phenotypic plasticity is a particularly important mechanism influencing range expansion; in vertebrates though, there is less evidence for plasticity as a driver of success in new areas. Here, we discuss the potential role of phenotypic plasticity in range expansions of house sparrows, one of the world's most common birds. In particular, we discuss how variation in the regulatory architecture of glucocorticoids, steroid hormones that affect various organismal functions, might have been important to multiple range expansions of house sparrows (i.e., Kenya, Senegal, and North America). We propose that 'epigenetic potential', the propensity for some individuals to adjust gene expression via DNA methylation and related mechanisms, partly facilitated the colonization success in this species. Specifically, we expect that house sparrows are exceptionally capable of altering the roles of key nodes in physiological regulatory networks, which gives them exceptional abilities to adjust to prevailing conditions, even when such conditions are evolutionarily novel. Acknowledgements: We recognize NSF-IOS 0920475 for support.

P86
NEUROHYPOPHYSIAL HOMOLOGS CONVERGENT MEDIATE HOMEOSTASIS IN CUTTLEFISH EMBRYOS

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Cephalopods were proved to process epithelial acid-base regulation. The identification and characterization of those key acid-base transporters demonstrated that the molluscan pH regulatory machinery shows many evolutionary conserved features to those found in vertebrate and mammalian systems. However, its functional basis of how intracellular pH modulation correlate with hormone signalling is poorly understood in cephalopods so far. In this study, we used cuttlefish Sepia pharaonis embryos to examine expressions of neurohypophysial hormones (pro-sepiatocin and sepiatpcin) and respective receptor (sepiatocin receptor, str) under CO2-induced acidic perturbation. RNA in situ hybridization images appeared that, on one hand, pro-sepiatocin and sepiatocin were both expressed in optic lobe neurons. On the other hands, str was found to be expressed in embryonic epithelium, the dominant sites for acid-base regulation. Moreover in CO2-acidified condition, pro-sepiatocin and str were up-regulated accompanied with those acid-base regulation genes in epithelium (e.g. vha, nbc, nhe3, rhp and nka). The present work inferred that the activated features of neurohypophysial hormone signalling would be beneficial to operate epidermal ion fluxes in cuttlefish; accordingly, in order to cope with acid-base disturbances during their oviparous development, cephalopod embryos have evolved convergent endocrinal pathway regulating intact homeostasis. Comparative studies using a range of marine invertebrates will create a novel and exciting research direction addressing the evolution of pH regulatory and excretory systems.

P87
INSECT PEPTIDE NEUROHORMONE SIGNALING: POTENTIAL INSECTICIDE TARGETS

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Insecticide resistance is a recurring problem for most chemical forms of arthropod control, from agricultural pests to vectors of disease. Peptide neurohormone receptors represent a diverse but relatively unexplored array of targets for the development of new insecticide classes to circumvent current forms of insecticide resistance. In insects, peptide neurohormones control or modulate numerous biological processes, ranging from critical physiological functions to behavior. Several types of receptor classes are known to interact with peptide neurohormones, but most activate the diverse class of receptors known as the G-protein coupled receptors (GPCRs). To investigate these receptors, gene sequences for candidate insecticidal target GPCRs were found through homology searches of available insect genome and transcriptome databases and confirmed by sequencing of PCR amplified cDNA. Receptors were expressed in reporter cell lines and pharmacological profiles were characterized in response to predicted peptide ligands and potential non-peptide analogs to determine the activity at these receptors and to explore their potential to disrupt normal signaling. This work provides a foundation for the exploration of peptide neurohormone receptors as viable insecticidal targets.

P88
OCTOPAMINE AND TYRAMINE MODULATE THE FEMALE REPRODUCTIVE SYSTEM IN THE MEDICALLY-IMPORTANT BUG, *RHODNIUS PROLIXUS.*

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Octopamine and tyramine are neuroactive chemicals involved in a wide range of physiological processes acting as neurotransmitters, neuromodulators and neurohormones. Octopamine and tyramine have been shown to play a crucial role in modulating reproductive processes in insects. Both octopamine and tyramine modulate visceral muscle contractions in various insects. In *Rhodnius prolixus*, octopamine decreased the amplitude of spontaneous muscle contractions and reduced the RhoprFIRFa-induced contraction of the oviducts in a dose-dependent manner, whereas tyramine only reduced the RhoprFIRFa-induced contractions. At the bursa, both octopamine and tyramine reduced the frequency of spontaneous contractions and abolished contractions at higher concentrations. These events are mediated by G-protein coupled receptors with cyclic AMP or calcium acting as second messengers. The cDNA sequences of two distinct receptors, Octβ-R and Tyr-R, has been cloned from *R. prolixus* and the transcript is shown to be expressed in all female reproductive tissues. Injection of octopamine into mated and fed adult females results in a higher number of eggs produced and ovulated when compared to control insects. Overall, it appears that octopamine and tyramine modulate the female reproductive tissues leading to successful ovulation, fertilization and the oviposition of eggs. Acknowledgements: Supported by: NSERC Discovery Grant to ABL.

P89
THE REPRODUCTIVE AXIS IN MICE DEFICIENT IN FIBROBLAST GROWTH FACTOR RECEPTOR 3

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Fibroblast growth factor (FGF) signaling is essential for many aspects of growth and development. For example, FGF signaling supports the development and postnatal maintenance of gonadotropin-releasing hormone (GnRH) neurons, which are critical for reproduction. Mutations on several FGF signaling genes disrupt the integrity of the GnRH neuronal network, leading to compromised fertility in humans and mice. However, the role of FGF signaling in the more upstream *KiSS1* neurons, which activate GnRH neurons, was unclear. The goal of this study was to examine if male mice deficient in FGF receptor 3 (FGFR3), a receptor implicated in the postnatal maintenance of GnRH neurons, suffered significant deficits in the arcuate (ARC) and anteroventral periventricular (AVPV) *KiSS1* systems, and if the reproductive axis downstream of *KiSS1* was also affected. Our results revealed that although *KiSS1* expression in ARC and AVPV was not altered in FGFR3-deficient mice, these animals suffered a significant decline in *GnRH* expression at the onset of puberty. Further, FGFR3-deficient males exhibited a significant reduction in pituitary follicle-stimulating hormone (FSH) at pubertal onset. Comparable endocrine defects were not observed in FGFR3-deficient adult males, suggesting only animals undergoing puberty were vulnerable to FGFR3 deficiency. Lastly, FGFR3-deficient males exhibited a significant delay in puberty as measured by the timing of balanopreputial separation, but not the timing or the size of first litter sired, reaffirming an impact on puberty but not the subsequent reproductive outcome. In sum, our results suggest that although FGFR3 deficiency had no effect on the *KiSS1* systems, it led to a transient disruption of GnRH and FSH accumulation at the onset of puberty. The transient nature of the disruption implicates FGFR3 in the function, rather than development, of the reproductive axis. Supported by: NIH R01 HD083260 to Pei-San Tsai.

P90
GH, IGF-I AND GPE: RELATIONSHIPS AMONG THESE NEUROPROTECTIVE HORMONES

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GH is a pleiotropic hormone produced in practically all organ and tissues, including neural stem cells (NSCs). Its role as neuroprotective and neuroregenerative factor is well known; moreover, GH induces the expression of IGF-I in a number of tissues, including human fetal forebrain. Both, GH and IGF-I, locally expressed or exogenously administered, induce the proliferation, differentiation, migration and survival of NSCs, therefore playing a key role in the human fetal brain development and in brain repair after an injury.
GPE is a N-terminal IGF-I derived tripeptide, produced by proteolytic cleavage of IGF-I in neurons. While strong evidence support a neuroprotective effect of GPE its role on neuroregeneration has not been investigated so far.
We studied the ability of GPE to promote the proliferation and migration of NSCs obtained from 23.5 dpc mouse embryos and the signaling pathways involved in GPE actions. GPE treatment promotes the proliferation and the migration of NSCs *in vitro* through a mechanism that involves the activation of the ERK and PI3K-Akt pathways. Our data also suggest that this effect may be mediated by activation of the NMDA receptor. Interestingly, GPE effects and activation of signaling pathways are similar to those we observed with GH treatment in these NSCs. Therefore, it is likely that GPE is an IGF-I derived peptide that may be useful in promoting neuroprotection and neural regeneration after an injury.

Since GH induces the expression of IGF-I, both in liver and in NSCs, and in turn, IGF-I suffers a protelolytical cleavage in neurons for producing GPE, and these three hormones play a neuroprotective and neuroregenerative role, it is of interest to know whether they form part of a specific system involved in neuroprotection and neuroregeneration after a brain injury. Acknowledgements: This study has been supported by Foundation Foltra (Teo, Spain).

P91
INVOLVEMENT OF IGF SYSTEM DURING THUNNUS THYNNUS LIFE CYCLE: EFFECTS ON GROWTH

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Most of understanding on the biology of Atlantic bluefin tuna (*Thunnus thynnus*) started to emerge in the last decade. Current biological information indicates that a substantial amount of uncertainty still exists regarding Atlantic Bluefin tuna growth.
The insulin-like growth factor (IGF) system is an evolutionarily conserved signalling pathway. Studies in several species suggest that the IGF signalling plays a fundamental role in controlling animal development and growth. The aim of the present study was to improve the knowledge on growth supporting the canonical procedures, conversion factor (SFL-CFL) following a molecular approach, focusing on mechanisms involved in BFT growth. For each specimen, curved fork length (CFL) was measured; this measure was then transformed into straight fork length (SFL) using the formula: SFL = CFL × 0.9596 + 2.0985. This allowed further estimation of round weight (RWT), according to the equation $RWT = 5.496940E^{-05} \times SFL^{2.76094}$ (Lombardo et al., 2016). We organized tuna into three groups A, B and C according to size (weight and CFL). The morphometrical results showed differential growth rate depending on age and gender. Molecular analysis revealed that genes involved in the growth including *igf1, igf1r, ir, mtor* and *ampk* were differentially expressed in relation with the BFT size and sex. Specifically, *igf1* expression and its receptors *igf1r and ir,* highlighted a gender-specific pattern. In male, IGF signalling increase was associated with increase in size while an opposite trend was seen in female.
In males, mTOR gene expression showed similar levels in each group. Females showed an increase between group A and group B, while in group C registered a significant decrease. Regarding *ampk* gene expression, males from group A showed lowest gene expression than females. Together these findings suggest that female differently to male, invests in reproduction rather growth, once reached the ideal size. This study highlight findings on the gender differences of the IGF system during the life cycle of *T. thynnus* Acknowledgements: Supported by Ministero delle Politiche agricole e forestali-MIPAAF

P92
COMPARATIVE IMPACTS OF SALINITY STRESS ON MOZAMBIQUE AND NILE TILAPIA GROWTH

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A tilapia strain showing good growth and tolerance to brackish or seawater is still an aquaculture challenge, particularly in view of the limited freshwater resources. Salinity and production studies have been centred on the Mozambique tilapia (*Oreochromis mossambicus*) (MOZ) and its hybrids due to its high salinity tolerance, although it has a slow growth-rate. The Nile tilapia (*O. niloticus*) (NIL) is the main farmed species presenting good growth-rate but low tolerance to seawater. We performed a comparative study of both species to better analyse the interactions of salinity stress and growth, between freshwater (FW) and seawater-adapted (SW) tilapia. Growth experimentations were performed for 6 weeks on 18 fish/species kept in FW and 18 fish/species adapted to 30 ppt SW by increasing the salinity in a step-wise manner. Blood parameters with increases in glucose and plasma Na^+ levels indicated that SW NIL were only partially stressed. Growth rate of NIL was affected negatively by salinity but it showed a better growth with higher specific growth-rate than MOZ whatever the salinity. Interestingly, plasma growth hormone (GH) levels were elevated for both FW & SW NIL while they were >three-fold lower in SW MOZ. GH/IGFs gene expression profiles showed a down-regulation of *igf1* in SW MOZ liver. We observed down-regulation of *igf1* expression in NIL gills with salinity while *ghr1* increased. Differences in *igf3* expressions were also seen in FW gills. In addition, we found species differences for plasma cortisol levels with elevated amounts for both FW and SW MOZ while they were low in NIL. NIL showed in SW gills a downregulation of the glucocorticoid receptor *gr1* gene expression whereas in the brain important decreases in gene expressions were observed for both *gr2* and the mineralocorticoid receptor *mr* with salinity. Acknowledgements: Funded by a Maimonide French-Israeli Grant

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LIVE CELL IMAGING SHOWS DYNAMIC BEHAVIOR OF SERTOLI CELLS AND OTHER TYPES OF TESTICULAR CELLS IN 3-D CULTURE OF RE-AGGREGATED CELLS FROM NEONATAL MOUSE TESTIS

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Organogenesis of testis comprises complex processes and the underlying mechanism remains elusive. We have previously established 3-D culture of dissociated testicular cells from neonatal mouse, in which seminiferous tubule-like structures are formed in the presence of KnockOut Serum Replacement (KSR) (Zhang et al., 2014). In this study, in order to reveal the mechanism of testis reconstruction, first we examined the effects of TGF inhibitors (SB431542 and ALK5i) and DHH inhibitor {cyclopamine (CP)}, since TGF and desert hedgehog (DHH) signaling has been reported to be indispensable for testis development in mammalian embryos. Both SB431542 (ALK4/5/7 inhibitor) and ALK5i (ALK5 inhibitor) disturbed the reconstruction of cord-like and tubule-like structures, indicating that signaling through ALK5 is indispensable for the reconstruction. On the other hand, CP did not disturb the reconstruction of cord-like or tubule-like structures. Then, to visualize the reconstruction process, we monitored the behavior of fluorescent SCs and non-fluorescent testicular cells in culture of re-aggregates prepared from *Sox9-EGFP* mice. When testicular re-aggregates (>500 m in diameter) were observed, fluorescent SCs and non-fluorescent testicular cells gradually segregated from each other accompanying dynamic motion to form brighter re-aggregates of fluorescent SCs and darker those of non-fluorescent testicular cells in about a week. Interestingly smaller testicular re-aggregates (<100 m in diameter) containing fluorescent SCs moved toward and were incorporated into larger re-aggregates. Thus the fusion of two re-aggregates seemed to be caused by active movement of testicular re-aggregates. In the presence of ALK5i, fluorescent SCs and non-fluorescent testicular cells gradually segregated too, but the fluorescence of the re-aggregates was less bright, and smaller re-aggregates weakly moved, rarely fusing with each other. These results indicate that ALK5 signaling is responsible for the movement and assembly of SCs, leading toward the formation of seminiferous cord-like and tubule-like structures.
Acknowledgements: Supported by: JSPS KAKENHI Grant Number 15K07129 and Kumamoto Health Science University Special Fellowship Grant Number 2015-C-12

P94
IDENTIFICATION OF MOUSE OVARIAN THECA/ INTERSTITIAL CELL-SPECIFIC GENES BY TRANSCRIPTOME ANALYSIS

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In the previous study, we revealed that the mouse ovarian secondary follicle growth required the co-cultivation with ovarian theca/ interstitial cells, and that ovarian theca/ interstitial cells were also involved in the theca cell layer formation on the secondary follicles. These results suggest that the ovarian interstitial cells play an important role in the regulation of the secondary follicle growth. However, the genes and molecules specific to mouse ovarian theca/ interstitial cells have not been fully investigated, and thus, the relevant molecular mechanisms have yet to be verified. In this study, we performed transcriptome analysis of theca/ interstitial cells using a next-generation sequencer-based RNA-seq. Theca/ interstitial cells and granulosa cells were isolated from 3-week old mouse ovaries. Subsequently, we sequenced 58 mega reads for each theca/ interstitial cell samples and granulosa cell samples using the Illumina Hi-Seq 1500 platform. The mapping to the mouse genome sequence (mm10) and fragments per kilo base per mega reads (FPKM) estimation were performed with tophat (v2.1.0) and cufflinks (v2.0.10), respectively. Overall, 80.98% of short reads were mapped to the genome and 12000 genes with higher FPKM values than 0.1. Of those, 258 genes were upregulated in theca/ interstitial cells at more than two-fold expression levels, compared to granulosa cells. Moreover, real-time PCR confirmed that 19 selected genes with high FPKM were prominently expressed in theca/ interstitial cells. These genes were categorized into 3 major groups: extracellular matrix genes, chemokine genes, and cell growth regulator genes. Moreover, *in situ* hybridization verified the specific expression of *Nid1* and *Col3a1* in theca cells and interstitial cells of preantral and antral follicles but not in granulosa cells or oocytes. *Spon1* was expressed specifically in interstitial cells and theca cells of secondary, preantral, and antral follicles. Interestingly, expression of *Aspn* was detected specifically in interstitial cells and outer theca cells but not in inner theca cells of preantral and antral follicles. In addition, these genes were expressed at quite low or no levels in atretic follicles. Functional analysis of these molecules in mouse ovary is currently in progress. Acknowledgements: JSPS grant to MA and HS.

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SECRETOGRANIN-II PLAYS A CRITICAL ROLE IN ZEBRAFISH NEUROVASCULAR MODELING

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Secretogranin II (SgII) is a ~600 amino acid secretory vesicle protein that is proteolytic processed to generate the bioactive neuropeptide secretoneurin (SN). Previous data demonstrated that SN is secreted by neuroendocrine cells to regulate neuronal and pituitary function in adult fish. Peripherally, exogenous SN application or gene therapy was also shown to be angiogenic in adult mammals. Here we report on a novel function of the *secretogranin II* (*sgII*) gene in neurovascular development. There are 2 paralogs of SgII in zebrafish and other teleosts named *sgIIa* and *sgIIb*. We found significant differential expression of these genes: both were largely expressed in the CNS, but only *sgIIb* was in the larval hindbrain. Using transcription activator-like effector nucleases, we have generated zebrafish *sgIIa*$^{-/-}$, *sgIIb*$^{-/-}$, *sgIIa*$^{-/-}$/*sgIIb*$^{-/-}$ mutant lines. Our results showed that both *sgIIb*$^{-/-}$ and *sgIIa*$^{-/-}$/*sgIIb*$^{-/-}$ mutant embryos were defective in hindbrain central artery development, while artery development in *sgIIa*$^{-/-}$ mutant embryos was not affected. Neurons expressing *sgIIb* were aligned with central arteries in hindbrain, demonstrating a close neurovascular association. Hindbrain arterial and venous network identities were not different

in *sgIIb*$^{-/-}$ mutant embryos, and the mRNA levels of Notch and VEGF pathway-related genes were not altered. However, the activation of MAPK and PI3K/AKT pathways were inhibited in *sgIIb*$^{-/-}$ mutant embryos. Injection of a synthetic SNb mRNA partially rescued the central artery developmental defects in the *sgIIb* mutant fish. This study provides the first *in vivo* evidence that *sgIIb* plays a critical role in neurovascular modeling of the hindbrain. Acknowledgements: Supported by: The National Natural Science Foundation of China (Grant No. 31325026 to WH), the Natural Sciences and Engineering Research Council of Canada (to VLT) and the University of Ottawa International Research Acceleration Program (to VLT and WH)

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THE NOVEL MEMBRANE ANDROGEN RECEPTOR ZIP9 MEDIATES PRO- AND ANTI-APOPTOTIC RESPONSES IN AN OVARIAN FOLLICLE SIZE-DEPENDENT MANNER IN ATLANTIC CROAKER GRANULOSA CELLS.

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Nongenomic steroid actions have been reported for all of the major classes of sex steroids, which has prompted the discovery and research on novel membrane steroid receptors unrelated to members of the nuclear steroid receptor superfamily. While membrane progesterone and estrogen receptors were characterized over a decade ago, our research group recently cloned the cDNA for a putative membrane androgen receptor from Atlantic croaker ovarian tissue. The cDNA shows high homology with members of the ZIP9 subfamily of zinc transporters, indicating that croaker ZIP9 possesses membrane androgen receptor activity. Testosterone activation of ZIP9 was found to mediate a G protein-induced apoptotic pathway in croaker ovarian granulosa cells collected from fish at the peak of their reproductive season (gonadosomatic index (GSI) >12). Interestingly, granulosa cells isolated from croaker with lower GSIs have been observed to respond to testosterone in an antiapoptotic fashion, prompting us to question what receptor mediates this response. By isolating granulosa cells from ovarian follicles under 300 μm in diameter, we have found that this antiapoptotic response can be mediated by testosterone-conjugated to BSA but not the nuclear androgen receptor agonist mibolerone. Furthermore, small interfering RNA knockdown of croaker ZIP9 abrogates this antiapoptotic androgen-induced response. In addition, granulosa cells isolated from the follicles with a diameter over 400 μm, show the apoptotic response to testosterone that was originally observed in fish with GSIs >12. Our results demonstrate that ZIP9 can mediate opposite physiological responses in ovarian follicles of different sizes even within a single fish. This is the first report to our knowledge that ZIP9 may mediate multiple functions of androgens within the ovary. ZIP9 has also been shown to mediate androgen-induced apoptosis in a number of human cancer cell lines. Thus, it is of interest to determine the means by which ZIP9 can switch its apoptotic response in croaker granulosa cells in order to better understand the potential mechanisms by which the receptor may operate in other models.

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RAPID CORTISOL-MEDIATED TRANSLOCATION OF GLUCOCORTICOID RECEPTOR TOWARDS THE MEMBRANE IN TROUT HEPATOCYTES

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Cortisol rapidly activates cell signalling in trout hepatocytes; however, the mechanisms are far from clear. In our present study we tested the hypothesis that intracellular glucocorticoid receptor (GR) translocation may be part of the rapid cortisol-mediated nongenomic response in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. GR distribution in hepatocytes was confirmed by immunofluorescence labeling with antibody specific to trout GR. We identified rapid changes in GR redistribution in trout hepatocytes upon exposure to stressed levels of cortisol. For instance, there was a rapid translocation of intracellular GR to the cell periphery within minutes after cortisol addition. This GR translocation appeared to be in part mediated by actin filaments, as latrunculin B (blocks actin polymerization) disrupted this movement. We also performed co-localization studies with GR and caveolin -1 to identify if the membrane translocation of GR was associated with caveolin-1. Co-localization of GR with caveolin -1 around the hepatocyte membranes suggests a yet unidentified role for cortisol in rapid cell signaling and membrane receptor regulation. Overall, cortisol rapidly alters GR dynamics in trout hepatocytes, and we propose a role for GR redistribution in mediating rapid cell signaling. Acknowledgements: the Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV supported this study.

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A PROPOSED UNIVERSAL NOMENCLATURE FOR THE OXYTOCIN AND VASOTOCIN LIGAND AND RECEPTOR FAMILIES

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Oxytocin and vasopressin are important neurotransmitter ligands that function through specific receptors to control a diverse set of brain functions. Due to differential naming of the ligands according to small amino acid differences across species and to sequence identity between the oxytocin and vasopressin receptors, there is often confusion about their orthology and paralogy, making it difficult to translate findings across species. Here we performed sequence identity (BLAT/BLAST) and synteny (CoGe and SynMap) analyses on putative oxytocin and vasopressin ligands and their receptors in the genomes of 26 species that span all major vertebrate lineages. These included newly re-sequenced species with long-read technology that filled in gaps and corrected errors in previous shorter-read assemblies. Our

findings indicate that oxytocin and vasopressin are adjacent paralogous genes that formed as a local genomic duplication event near the origin of vertebrates, with vasopressin being the parental gene of oxytocin. What has been called mesotocin, isotocin, or oxytocin-like in non-mammalian species are all the same gene, namely oxytocin. Vasotocin in all non-mammalian vertebrates is the same as vasopressin in mammals. Thus, following the standard practice in molecular biology, we propose that these two genes be given the same orthologous names across vertebrates and paralogous names relative to each other, namely oxytocin and vasotocin. We identified five receptors among vertebrates, of which four formed from the two rounds of whole genome duplications at the origin of vertebrates, and the fifth as a local duplication of one of the four giving rise to the oxytocin receptor (OTR) and what we call vasotocin receptor 1A VTR1A (previously AVPR1A). The VTR2A receptor (previously AVPR2) was lost in birds due to a local genomic deletion of the human X chromosome, the VTR2B receptor (previously VT1) was pseudogenized in mammals, whereas some vasotocin receptors were further duplicated in some fish lineages. This is the first study to propose a universal nomenclature for the Oxytocin and Vasotocin ligands and their receptors. This new nomenclature should prevent further confusion and errors, allow easier translation of findings across vertebrates, and foster more informative design of functional experiments across species.

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CRHR2π: A NEW SPLICE VARIANT OF THE TYPE 2 CORTICOTROPIN-RELEASING HORMONE RECEPTOR IN NONMAMMALIAN VERTEBRATES.

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Corticotropin-releasing hormone (CRH) is a neuropeptide that exerts its role through binding one of two CRH receptors (CRHR1 and CRHR2), which are each encoded by a different gene. In mammals, up to three different splice variants of CRHR2 have been described, but nonmammalian species were believed to possess only a single form of CRHR2. We have investigated the existence of a novel splice variant of CRHR2 that was predicted in the green anole *(Anolis carolinensis)*, Adelie penguin *(Pygoscelis adeliae)*, emperor penguin *(Aptenodytes foster)*, and chicken *(Gallus gallus domesticus)*, which we have named CRHR2π. Utilizing a range of bioinformatics tools and PCR techniques, we investigated (1) whether this previously unknown splice variant is truly expressed in nonmammalian species and in which organs, using the chicken as a model; (2) where the coding sequences for this new splice variant are located within the structure of the *CRHR2* gene; and (3) whether this splice variant could also be expressed in other vertebrate taxa. We successfully confirmed the presence of CRHR2π in chickens with effective cloning indicating a length of the π-specific sequence of 159 bp. The π-specific sequence displayed 46% identity with exon 1 of chicken *CRHR2*. CRHR2π mRNA was detected in chicken cerebellum, optic lobe, brainstem and diencephalon. The coding sequences of the new splice variant, comprising of two exons in chickens, are positioned approximately 40 kb upstream of exon 1. There is also evidence suggesting that CRHR2π is present in a range of other nonmammalian species that date back to primitive fishes. In derived avian species, three exons encode the CRHR2π-specific sequence; however, these exons were lost in snakes and mammals.

P100

GENETIC ANALYSIS OF NUCLEAR ESTROGEN RECEPTORS IN THE ZEBRAFISH

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In most vertebrates including teleost, estrogens signal through both nuclear and membrane receptors with most reported effects of estrogens being mediated via the nuclear estrogen receptors (nERs). Although much work has been reported on nERs in zebrafish, there is still a lack of direct genetic evidence for their functional roles and importance in reproduction. To address this issue, we undertook this study to disrupt all three nERs in the zebrafish, namely *esr1* (ERα), *esr2a* (ERβII) and *esr2b* (ERβI), by genome-editing technology CRISPR/Cas9. Using this loss-of-function genetic approach, we successfully created three mutant zebrafish lines with each ER knocked out. In addition, we also generated all possible double and triple knockouts of the three ERs. The phenotypes of these mutants in reproduction were analyzed in all single, double and triple ER knockouts in both females and males. Surprisingly, all three single ER mutant fish lines display normal reproductive development and function in both male and female, suggesting partial functional redundancy among these three ERs. Further analysis of double and triple knockouts showed that nERs, especially Esr2a and Esr2b, were essential for female reproduction, and loss of these two ERs led to an arrest of folliculogenesis at previtellogenic (PV, stage II) stage followed by sex reversal from female to male. In addition, the present study also revealed unique roles for *esr2a* in follicle cell proliferation and trans-differentiation, follicle growth, chorion formation and even early embryonic development. Taken together, this study provides the most comprehensive genetic analysis for differential functions of *esr1, esr2a* and *esr2b* in teleosts. Acknowledgements: This work was funded by grants from University of Macau and The Macau Fund for Development of Science and Technology.

P101

DEVELOPMENTAL VITAMIN DEFICIENCY IMPACTS NEURODEVELOPMENT/DEGENERATION IN ZEBRAFISH.

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Vitamin D (1α, 25-dihydroxyvitamin D_3**)** is a steroid hormone traditionally associated with mineral ion homeostasis. Accumulating evidence however suggest a wider biological role for vitamin D and its importance in immune function, xenobiotic metabolism, cell differentiation

and neural development. Like other members of steroid hormones, the biological effects of vitamin D are mediated through the binding of 1α, 25-dihydroxyvitamin D_3 (ligand) to its hormone receptor, VDR. VDR is a member of the nuclear receptor superfamily, comprised of a large group of ligand-activated transcription factors, which are highly conserved across vertebrate evolution. In recent years the vitamin D signaling axis has emerged as crucial player driving embryonic neurodevelopment, neuroprotection from xenobiotics and its deficiency has been implicated as a risk factor for development of neurodegenerative diseases such as Parkinson's disease, Schizophrenia, and Multiple sclerosis. In this study we describe a zebrafish vitamin D deficiency model and associated neurogenic, adipogenic and skeletal phonotypes. We demonstrate that VDR is expressed ubiquitously within the zebrafish brain and may directly impact critical aspects of neurodevelopment, axonal synthesis and neural degeneration. In order to evaluate the impacts of vitamin D deficiency during early development we elucidate the molecular mechanisms of VDR during early neurogenesis and assess it putative role(s) in neurodegenerative disease prediction and prevention.

P102
MEMBRANE AND NUCLEAR ESTROGEN RECEPTORS IN SEA BASS PROVIDE INSIGHT TO EXPLORE GENOMIC AND NON-GENOMIC ESTROGENIC ACTIONS: THE MINERALIZED SCALE EXAMPLE

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The numerous estrogen functions across vertebrates have been classically explained by binding to nuclear estrogen receptors (ERs) regulating the transcription of responsive genes. It is now known that estrogenic compounds can also produce rapid non-genomic actions initiated by binding to estrogen membrane receptors, such as the recently identified G protein-coupled estrogen receptor1 (GPER). Sea bass (*Dicentrarchus labrax*) express three ER subtype genes, one *esr1* and two *esr2* genes that appear to have arisen from the original *esr2* gene during the teleost-specific whole genome duplication. We have recently identified two genes for GPER in the sea bass genome and phylogenetic analyses also suggests they are teleost-specific gene duplicates. Quantitative PCR revealed the five receptors have a wide tissue distribution in both male and female sea bass and that expression occurs across the reproductive cycle in brain and pituitary, although with subtype-specific and seasonal differences. When analyzing the sea bass scales, mineralized structures previously shown to be estrogen-responsive, the receptor repertoire and their regulation was different from liver, a classical target tissue. In juvenile sea bass scales, the main forms of estrogen receptors expressed were *esr2a* and *gperb*, which were also up-regulated after injection with the natural estrogen estradiol (E2) and the phytoestrogen genistein (Gen). Both rapid (30 min) and slow (1 day or more) changes in the activities of enzymes related to mineral turnover were detected in fish scales in response to E2, Gen and xenoestrogens and the gene networks activated 1-5 days after injection of E2 and Gen revealed both common and compound-specific effects at the transcriptional level. Functional characterization of the three sea bass ER subtypes and two GPERs is revealing the specific signaling pathways that are activated by different estrogenic compounds. These studies reveal how estrogen regulates fish scale function and how the phytoestrogens and other xenoestrogens may disrupt scale function and the relative importance of genomic and non-genomic mechanisms in these actions. Acknowledgements: Supported by FCT (Portugal) projects PTDC/AAG-GLO/4003/2012 and CCMAR/Multi/04326/2013 and fellowships to PISP and RF.

P103
PUTATIVE CORTICOSTEROID RECEPTORS ON THE PLASMA MEMBRANE OF RAINBOW TROUT LIVER

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Cortisol, the primary glucocorticoid in teleost fish, exhibits both genomic and nongenomic physiological effects. The nongenomic effects are rapid (seconds to minutes) and involve changes to second-messenger signalling pathways that are independent of mRNA transcription and protein synthesis. Although there is considerable literature examining genomic cortisol actions mediated by the classical glucocorticoid and mineralocorticoid receptors (GR and MR, respectively), studies delineating rapid cortisol signalling remain poorly characterized. Studies have shown that cortisol treatment rapidly alters plasma membrane fluidity and activates downstream signalling cascades in rainbow trout (*Oncorhynchus mykiss*) liver. However, a membrane cortisol receptor(s) has not been identified in any model organism. In this study, we isolated cortisol-binding membrane proteins from rainbow trout liver using various protein purification techniques. Our results suggest the presence of the intracellular GR and MR on the plasma membrane of trout liver and we propose a role for these receptors in rapid nongenomic cortisol signalling. Together, the findings highlight a role for GR and MR in rapid stress signaling in trout liver, which may have huge implications in our understanding of stress coping mechanisms.
Acknowledgements: This study was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV.

P104
PHYSIOLOGICAL PROCESS DURING SILVERING OF THE NOCTURNAL EEL, *ANGUILLA JAPONICA* : EXPRESSION OF STEROID HORMONE RECEPTORS MRNA IN FEMALE EEL

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Mature stage of Japanese eels (Silver eel), *Anguilla japonica*, begin to sexually mature and their eye size increase while they migrate to ocean. However, concrete physiological process about sexual maturity in Japanese eel is still unknown during spawning migration. Generally, steroid hormones are synthesized and secreted from gonad, and steroid hormone receptors are distributed in various tissues. The present study aimed to investigate expression of sex steroid hormone receptors in neuro tissue (brain) and peripheral tissues (retina, pituitary and gonad) of the Japanese eel during the sexual maturation. Fishes were treated for sexually artificial maturation with injection of SPE (Salmon pituitary extract). For our experiment, we divided into four stages (Immature stage, stage1; primary sexual maturation stage, stage2; secondary sexual maturation stage, stage3; final sexual maturation stage, stage4) of sexual maturity through histological observation. Experiment fish (n=41) were sampled at 24:00h. Plasma levels of steroid hormone, E2, were measured by EIA. The mRNA expressions of steroid hormone receptors (ARα, ARβ, ERα, and ERβ) in sexually immature and mature eel were observed compared sexually immature and mature eel using qPCR. E2 levels in plasma increased with progression of sexual maturity than immature stage. The expression of ARα and ARβ mRNA decreased with progression of sexual maturity in pituitary. While ARα, ARβ, ERα and ERβ mRNA expression increased from stage3 than stage1 and 2 in gonad. ERα mRNA expression increased with progression of sexual maturity in brain, pituitary, retina and gonad, but ERβ mRNA expression not showed a significant change in brain and pituitary compared expression in retina and gonad. ERα and ERβ mRNA expression especially increased at stage3 and 4 than stage1 and 2. These results suggest that estrogen involved in sexual maturation through by ERα principal activation, and increasing of E2 level by stages may related to change of eye size during spawning migration in eel retina. Acknowledgements: Supported by: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A6A3A04041089).

P105
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS), PHTHALATES AND REPRODUCTION IN ZEBRAFISH (*DANIO RERIO*)

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Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that upon activation modify the expression of target genes involved in various physiological processes. PPARs play an important role in mammalian reproduction through direct effects on the ovary where they affect oocyte maturation, steroidogenesis and ovulation. Five different isoforms of PPARs (αA, αB, βA, βB and γ) have been identified in the zebrafish (*Danio rerio*) but unlike mammals, the presence of these isoforms in the ovary and their functions are largely unknown. In our study, using real time quantitative PCR, we have shown that genes for all five isoforms of PPARs are present in the zebrafish ovary. In addition, we have further shown that that the mRNA expression of certain PPARs (*pparαA*, *ppar βb* and *pparγ*) are higher in earlier follicular stages compared to more mature follicles suggesting a potential role of PPARs in ovarian follicle development. In separate experiments, injections of zebrafish with the known PPAR activators, di-2-ethyl-hexyl phthalate (DEHP; 50 and 500 mg kg^{-1}) and mono (2-ethylhexyl) phthalate (MEHP; 35.6 and 356 mg kg^{-1}) led to a significant dose dependent reduction in the numbers of eggs that were spawned. Fish treated with both phthalates had reduced mRNA expression of the nuclear progestin receptor (*npr*) in the ovary. Fish injected with MEHP also had reduced mRNA expression of a disintegrin and metalloproteinase with thrombospondin motifs1 (*ADAMTS-1*). *npr* and *ADAMTS*-1 are key regulators of the final stages of follicle development and ovulation in zebrafish. In summary, our results demonstrate the presence of PPARs in the zebrafish ovary and that the activation of these transcription factors may play an inhibitory role in ovarian follicle development and ovulation. Acknowledgements: Supported by a NSERC Discovery Grant to GVDK.

P106
THYROID DISRUPTION IN RAINBOW TROUT AND BROOK TROUT EXPOSED TO SELENIUM AND MERCURY: DOES SELENIUM PROTECT AGAINST MERCURY?

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Selenium (Se) is an essential element; however, at slightly above homeostatic concentrations Se becomes toxic. Mercury (Hg) is a non-essential heavy metal that biomagnifies in the food chain and accumulates in fish. There is some evidence that Se has antagonistic interactions with mercury (Hg) and that at low concentrations at molar ratio of Se/Hg >1, Se protects against the toxicity of Hg. In this study, we investigated the effects of mixtures of Se and Hg on physiological endpoints, including thyroid status, and tissue burdens of Se and Hg in rainbow trout (RT) and brook trout (BT) under controlled laboratory conditions. Fish were exposed to Se (0, 14, 37 µg Se/g food) through diet for 14 days prior to a single Hg ($HgCl_2$) injection at a controlled dose and 14 days following the Hg injection. Tissue concentrations of Se and Hg, tank water Hg concentrations, liver GSH and LPO, and plasma T3, T4 and cortisol were measured on Day 0 and Day 28 of the experiment. To test the hypothesis that Se prevents accumulation of Hg in tissues and protects against Hg toxicity, fish were exposed to Se alone, Hg alone, or a combination of both. Differences between RT and BT in the retention and tissue distribution of Hg in co-exposures with Se were observed, with higher Hg burdens in liver of BT than RT and no effect of Se. High Se protected against Hg-induced changes in GSH, LPO and AChE activity. Higher liver GSH and lower LPO in BT than RT suggested BT may be less sensitive to Hg than RT. Plasma T3 and T4 titers were lower in both RT and BT exposed to Hg compared to controls, and co-exposure to high Se prevented this effect of Hg. This study provided evidence for a species-specific interaction between Hg and Se in RT and BT. It also demonstrated that Hg interferes with the thyroid axis and that Se may protects against the Hg-induced thyroid dysfunction. The mechanisms of this protective effect remain to be investigated. Acknowledgements: Supported by: NSERC Canada operating grants to AH.

P107

EFFECTS OF THE NEONICOTINOID PESTICIDE, IMIDACLOPRID, ON THE REGULATION OF HEPATIC GLUCOSE PRODUCTION IN MAMMALIAN CELLS

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There has been increasing concern that many of the pesticides currently used to increase crop yields may be adversely affecting metabolic homeostasis in mammals. One such class of pesticides is the neonicotinoids. Neonicotinoids account for approximately 25% of the pesticides used worldwide. Neonicotinoids were previously thought to act solely as agonists of insect nicotinic acetylcholine receptors (nAChR), however, recent studies have shown that neonicotinoids can also interact with mammalian nAChRs to affect metabolic pathways. For example, in rodents imidacloprid, one of the most commonly used neonicotinoids, has been shown to disrupt glucose homeostasis, resulting in hyperglycemia. The mechanisms by which neonicotinoids can affect glucose homeostasis are unknown but may involve direct effects of these compounds on the liver to alter gluconeogenesis and/or glycolysis. The goal of this study was to examine the direct effects of imidacloprid on hepatic gluconeogenic and glycolytic pathways. Treatment of rat MCA-RH7777 hepatoma cells with imidacloprid significantly inhibited glycolysis, glycolytic capacity and glycolytic reserve. In addition, imidacloprid increased expression of the key gluconeogenic enzyme glucose 6-phosphatase (G6Pase). This was concomitant with an increase in intracellular glucose concentrations. Importantly the effects of imidacloprid to affect hepatic glucose production do not appear to be mediated solely via the nAChR as equimolar concentrations of nicotine, a full nAChR agonist, did not inhibit glycolytic function or increase glucose production. Moreover, the ability of imidacloprid to increase G6Pase expression persisted in the presence of the non-selective nAChR antagonist mecamylamine. These data support the hypothesis that imidacloprid can directly impact hepatic glucose production in mammals through a mechanism that does not exclusively involve the nAChR. Acknowledgements: Supported by NSERC Discovery Grant to ACH.

P108

PERINATAL EXPOSURE TO COMMERCIAL MIXTURE OF POLYBROMINATED DIPHENYL ETHERS, DE-79, AFFECTS VASOPRESSINERGIC SYSTEM IN ADULT MALE RATS.

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The polybrominated diphenyl ethers (PBDEs) are extensively used as additive flame retardants in different daily products. PBDEs are lipophilic and bioaccumulate in animals and in the environment. They are classified as endocrine disruptors causing adverse effects in human health. We investigated the effects of perinatal exposure to DE-79, an octabromodyphenyl ether (octaBDE) commercial mixture, on the vasopressinergic system of adult male rats. Pregnant Wistar dams were given orally DE-79 at doses of 0 (control), 1.7 and, 10.2 mg/kg/day dissolved in corn oil from gestational day 6 to postnatal day 21. Two groups containing animals of all doses were formed with 3 months old male offspring. One group was processed for vasopressin (AVP) immunofluorescence in coronal brain sections of the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON). AVP immunoreactivity (AVP-IR) was quantified by integrated optical density (IOD). Another group was processed to obtain AVP mRNA by RT-PCR assay in PVN and SON punches. Both groups included dehydrated (drinking 2% saline ad libitum for 4 days) and euhydrated rats (tap water ad libitum). Plasmatic AVP and osmolality were measured. Results: IOD values showed an increased AVP-IR in euhydrated animals at 1.7 dose and a decrease with 10.2 dose compared to control, significant differences were observed only in SON. There was no expected physiological increase due to the osmotic activation in groups of 1.7 and 10.2 doses. The AVP mRNA significantly increased with 10.2 dose compared to control and after dehydration did not present the expected physiological increase. The osmolality showed an increased doserelated tendency in dehydrated animals. This may be explained by preliminary results of plasmatic AVP levels, which showed a diminishing tendency in dehydrated animals of 1.7 and 10.2 doses compared with euhydrated groups. These results suggest that perinatal exposure to octaBDEs affects AVP content and mRNA expression in adult rats, which compromises osmoregulation. Acknowledgements: Dr. Gainer (Bethesda, USA) for antiAVP donation, Dr. Kodavanti (USEPA) for DE-79 donation, Mr. Feliciano Camacho for technical assistance. Supported by: CONACyT/UNAM (294229 MYAG), INPRFM NC093290.0 (MLO).

P109

EARLY LIFE EXPOSURE TO COMMERCIAL NAPHTHENIC ACIDS DISRUPTS GROWTH, DEVELOPMENT AND THYROID HORMONE GENE EXPRESSION IN THE LARVAL WESTERN CLAWED FROG *SILURANA TROPICALIS.*

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Naphthenic acids (NAs) are a complex mixture of carboxylic acids that occur naturally within bitumen deposits across Canadas's oil sands region. During the extraction process, NAs are released into the aqueous phase as potentially toxic water soluble organic components in oil

sands process-affected waters. NAs are also used commercially as paint driers, wood preservatives, fuel additives and surfactants. Previous studies have demonstrated the toxicity of NAs to fish, but there is little information on effects in amphibian tadpoles. Egg masses from *S. tropicalis* were allowed to develop until Nieuwkoop and Faber (NF) stage 8-10 (embryonic) or 26 (hatchling) before being exposed to 0-24 mg L^{-1} of a commercial naphthenic acid mixture from Merichem Co. Animals were photographed 96hr post exposure to determine morphometric parameters, abnormalities and survival rate. Embryonic *S. tropicalis* exposed to 3-12 mg L^{-1} NAs were significantly smaller in size, had altered retinal development, uncoiling of the intestines and thickening of the intestinal lumen ($p<0.003$). Hatchlings exposed to the same NA concentrations exhibited similar but more severe morphological alterations. The lethal concentration (50% mortality) was calculated to be 10 mg L^{-1} for embryonic tadpoles and ~2-fold higher for hatchlings, suggesting a critical window of exposure. Anuran development is dependent on thyroid hormone (TH) and TH receptors (TR), so we sought to link developmental abnormalities with TH-related gene expression. We found that 12 mg L^{-1} of NAs induced mRNA level of TR ✔ relative to controls ($p<0.005$) at NF8-10. Similarly, 6 mg L^{-1} induced TR ✔ mRNA, however 8 and 12 mg L^{-1} caused a downregulation of the gene ($p<0.001$). In contrast, NA exposure did not affect the expression deiodinase Type II and III. These are the first results to demonstrate the potentiating ability of NAs to disrupt thyroid related processes. Acknowledgements: Supported by: NSERC Strategic Grants (VLT, JMB) and ECCC (JVH).

P110
PHTHALATE DISRUPT OVARIAN FUNCTION AND AGGRAVATE OVARIAN FAILURE

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Phthalates are chemicals used to improve the plasticity of industrial polymers and used in commercial products such as toys, paints, packaging materials, medicals devices and personal care items. Phthalates, endocrine disrupting chemicals, have been documented to cause adverse effects to the human health such as breast cancer in female, reduced uroogenital distance and changed the expression of steroidogenesis and folliculogenessis. In this study, we investigated the impact of di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), and butyl benzyl phthalate (BBP) on loss of ovarian function through folliculogenesis and steroidogenesis. 4-Vinylcyclohexene diepoxide (VCD), a disruptor of ovarian small pre-antral follicles, was used as a positive control. Female Spargue-Dawley rats (8 weeks of age, 160-180g bodyweight) were administered VCD (80mg/kg) by intraperitoneal, DEHP (25mg/kg), BBP (250mg/kg) and DBP (250mg/kg) by oral gavage in 0.3ml of corn oil at LOAEL during 6 weeks. Vaginal smear was collected at 9 a.m every day to check estrus cycle. Blood, pituitary, uterine and ovaries were collected after 24 hours final injection. There was significantly increased in body weight of DEHP groups compared to other groups. Estrus cycle in DEHP and DBP groups showed no difference comparing with vehicle group. However, diestrus phase in VCD and BBP groups drew out compared to vehicle group. The transcriptional levels of folliculogenesis-related genes (Foxl2, Kitl and Amh) and steroidogenesis-related genes (Star and Cyp11a1) were changed. Our findings suggest that these phthalates can induce premature ovarian failure by disturbance in folliculogenesis and steroidogenesis and failure in hormone regulation.

P111
SILVER NANOPARTICLES INDUCE OOCYTE MATURATION IN ZEBRAFISH (*DANIO RERIO*).

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Public concern regarding silver nanoparticles (AgNPs) in the environment has been increasing since they can cause adverse effects in some aquatic species. However, few data are actually available on the effects of AgNPs on the germ cells. In the present study, we used the zebrafish ovarian follicle as a model to assess the potentially adverse effects of AgNPs on oocyte maturation (germinal vesicle breakdown, GVBD) in vitro. Similar to the maturation inducing hormone (17α, 20β-dihydroxy-4-pregnen-3-one), AgNPs induced GVBD, and reduced the total cyclic adenosine monophosphate (cAMP) concentration in zebrafish ovarian follicles. The results from transmission electron microscope observation and Hoechst 33342 staining clearly indicated that AgNPs induced apoptosis in ovarian follicle cells surrounding the oocyte. Similar to AgNPs, AgNO3 also induced GVBD, decreased cAMP concentration and induced apoptosis of ovarian follicle cells. However, the results from gene expression analysis showed that transcript levels of oxidative stress related genes were more sensitive to AgNPs than AgNO3. Further more, H2O2 has an ability to induce zebrafish oocytes maturation by induction of apoptosis in ovarian follicle cells. Taken together, the results from our study indicated that oxidative stress appeared to be one of important mechanisms in AgNP induced apoptosis in ovarian follicle cells, which further triggered the GVBD. Acknowledgements: Supported by: Natural Science Foundation of Fujian Province and National Natural Science Foundation of China to CSX and HWS.

P112
TRANSGENERATIONAL HEALTH EFFECTS CAUSED BY DEVELOPMENTAL EXPOSURE TO BISPHENOL A AND 17 ✔-ETHINYLESTRADIOL IN MEDAKA

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Bisphenol A (BPA) is a compound used primarily to manufacture polycarbonate plastics and epoxy resins. Due to extensive use of BPA in commercial products, the threat to human health posed by BPA-containing waste in the environment is a potential concern. 17a-

ethinylestradiol estradiol (EE2) is a female contraceptive pill component. Millions of women continue to take contraceptive pill before realizing that they are pregnant exposing developing fetuses to a substantial amount of estrogen. Consequences of fetal exposure to BPA and EE2 in human is currently unknown; however, animal and fish data suggest both compounds can cause adverse health outcomes in later life stages due to early life exposure. We tested the ability of embryonic exposure to BPA or EE2 to cause adverse health outcomes at later life stages and transgenerational health abnormalities in medaka. Exposures of F0 medaka embryo to either BPA (100 µg/L) or EE2 (0.05 µg/L) during the first 7 days of embryonic development, when germ cells are differentiating, did not cause any apparent phenotypic abnormalities in F0 or F1 generations, but led to a significant reduction in the fertilization rate in offspring two generations later (F2) as well as a reduction of embryo survival in offspring three generations later (F3). Cross breeding between male and females from control and treated lineages suggested that the defect was mediated by male germ line. Then, we examined gene expression and epigenetic changes in both whole testis and isolated germ cells of F0 and F2 male medaka. BPA induced subtle changes in DNA methyltransferase enzyme expression in germ cells of the F0 adults exposed during embryonic development, and expression of Dnmt genes increased 2- to 10-fold in germ cells of the F2 males, accompanied by 2.5-fold increase in global DNA methylation. Elevated DNA methylation levels were maintained at the CpG island of androgen receptor (AR) core promoter of both testicular germ cells and somatic cells in the F2 generation. As expected, the expression of the AR gene in testicular somatic cells was significantly decreased, which confirms an inverse relationship between DNA methylation and gene expression. Together, these findings provide insights into transgenerational inheritance of BPA-induced epigenetic marks by germ cells and soma at the F2 generation.

P113
REPRODUCTIVE IMPAIRMENT OF FATHEAD MINNOWS IN THE BOW RIVER DOWNSTREAM OF WASTEWATER TREATMENT PLANT IN THE CITY OF CALGARY

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The objective of this study is to investigate the adverse effects of contaminants present in the Bow River, within the City limits of Calgary, which receives municipal wastewater effluents (MWWEs) from three wastewater treatment plants (WWTP) on fathead minnows (*Pimephales promelas*) gonadal development. These treated effluents are a complex mixture of environmental contaminants that includes natural and synthetic hormones, pharmaceuticals, industrial chemicals, with hormone-like activity which can disrupt normal physiological function at low environmentally relevant concentrations (parts per trillions) by interfering with the hypothalamic-pituitary-gonadal axis (HPG axis). Aquatic receiving environments have long been used to dilute municipal wastewater effluents (MWWE) which are the largest discharge by volume into the aquatic environment in Canada. In the present study, adult fathead minnows (male and female) were caged and exposed to Bow River water upstream and downstream of WWTPs for 28 days. After the exposure period, fish were euthanized for morphological, physiological and biochemical evaluation. We investigated various reproductive parameters in both male and female fish. Increased levels of caspase 3 activity, which is indicative of gonadal apoptosis, were observed in the gonads of both males and females downstream of the WWTPs. This was supported by the increases in caspase 3 mRNA expression occurring in conjunction at the same locations in both sexes. Histological examination and flow cytometry analysis revealed proportional increase in spermatogonia /spermatids population ratio, suggesting suppression of spermatogenesis. The present results suggest that current treatment process in the Bonny brook WWTP does not remove harmful contaminants with hormone-like activity and highlights a need to improve wastewater treatment process.
Acknowledgements: This study was supported by the Natural Sciences and Engineering Research Council of Canada Grants to HRH and MMV.

P114
ADVERSE EFFECTS OF SULFOLANE EXPOSURE ON ZEBRAFISH (*DANIO RERIO*) EMBRYONIC DEVELOPMENT

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Sulfolane, a polar, aprotic solvent initially developed to increase efficiency of the refining process in the oil and gas industry, has spread to multiple applications in numerous industries. With increased global utilization and with over 150 sulfolane production sites worldwide, some operating under no environmental regulations, sulfolane has been introduced into the environment through problems with storage, leaks, spills, and leeching from disposal sites. Sulfolane has been detected by chemical analysis in groundwater and creeks around the world including Alberta, Canada (800mgL^{-1}) and Louisiana, USA (2900mgL^{-1}). Previous research on terrestrial vertebrates demonstrated hyperactivity, shortness of breath, convulsions, and internal molecular changes following sulfolane exposure. However, relatively little information is available on the adverse impacts of sulfolane on aquatic organisms. This study focuses on the effect of sulfolane exposure on embryonic development, using a morphometric and behavioral approach on zebrafish larvae as model organisms. A wide range of doses (0 – 5000 mgL^{-1}) were tested and various growth (embryo length, eye diameter, yolk sac utilization, condition factor), morphometric (survival, hatching, hemorrhaging, spinal malformations, pericardial and yolk sac edemas) and behavioral (touch reflex, total activity) parameters were quantified. Negative impact trends for growth and morphometric abnormalities were observed in doses higher than 800 mgL^{-1} and significant differences from control were observed in doses higher than 3000 mgL^{-1} of sulfolane. Changes in activity levels were also observed post sulfolane exposure. These results provide novel information on adverse effects of sulfolane in aquatic vertebrate species, and provide a framework for better understanding of health risks associated with environmental levels of sulfolane in fish and other vertebrates.
Acknowledgements: Supported by NSERC Canada and Bonavista Energy Corporation grants.

P115
EFFECTS OF THE MYCOTOXIN ZERALENONE ON REPRODUCTION ENDOCRINE DISRUPTION IN ZEBRAFISH

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Zearalenone (ZEN) is a mycotoxin from *Fusarium* species commonly found in many food commodities and are known to cause reproductive, genotoxic and immunosuppressive disorders. There is limited information about its exotoxicological effects on fish. The present study focuses on estrogenic potency of ZEA at different concentration 0.5, 1, 5, 10 µg /L on zebrafish for 21 days. After the treatment period, there was a significant decrease in GSI which was in correlation to the caspase 3 activity, which is indicative of gonadal apoptosis. High abundance of p53 transcript was also observed, which highlights the increased apoptotic activity in female gonads. We also observed high abundance of estrogen receptor measured in brain, liver and gonads, compared with the control fish. Observed increased expression of VTG in liver follow exposure with ZER indicates that ZEA has estrogen like activity in zebrafish. We also observed significant variations in the expression of a number of other genes involved in steroidogenesis, including StAR, 3β-HSD, 17βHSD and Cyp19a1, fshr, lhr, Cyp19a1b, supporting the hypothesis that ZER is an endocrine disrupting chemical that can disrupt reproduction in fish. Supported by grants from NSERC.

P116
SELECTIVE SEROTONIN REUPTAKE INHIBITORS ALTER PITUITARY GONADOTROPIN EXPRESSION *IN VIVO* AND *IN VITRO* IN SALMON AND TROUT.

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Selective serotonin reuptake inhibitors (SSRIs) are receiving increased attention for their high occurrence in wastewater treatment plant effluent, in the aquatic environment and in fish tissues as well as for their ability to disrupt reproduction in fish. Previous work in our lab using salmonids has shown that SSRIs are selectively taken up by several tissues including the pituitary gland and that specific SSRIs, such as fluoxetine (FLX), can decrease follicle stimulating hormone (Fsh) beta subunit (*fshb*) mRNA levels and increase plasma Fsh levels *in vivo*. To better understand the effects of SSRIs on pituitary gonadotropin (Gth) levels, we undertook a series of cell culture experiments to examine direct effects of SSRIs on pituitary Gth mRNA expression and protein secretion. Primary pituitary cells from previtellogenic rainbow trout were exposed to 0, 80, 200, 4000, or 10000 nM FLX, sertraline (SERT) or citalopram (CIT) with or without estradiol (E2) for 3 days. Luteinizing hormone beta subunit (*lhb*) mRNA levels were increased 8-fold in response to 3 nM E2 treatment but co-treatment with at least 400 nM FLX or SERT decreased E2-stimulation of *lhb* by approximately 50%. Cells co-exposed to CIT, on the other hand, showed no inhibition of E2-induction of *lhb* mRNA levels, but 2000 nM CIT or higher increased *fshb* mRNA levels. In contrast to our previous *in vivo* results, FLX did not decrease *fshb* mRNA levels or increase Fsh release at the examined doses, nor did any of the other SSRIs. These differences suggest that some of the *in vivo* effects of SSRIs could be due to actions on the hypothalamus. In conclusion, we have shown that SSRIs may act directly on pituitary cells to alter *lhb* and/or *fshb* mRNA levels in rainbow trout primary pituitary cell cultures. However, further research is needed to explain different actions of specific SSRIs and the discrepant effects of SSRIs *in vivo* and *in vitro*. This project was supported in part by EPA-Star project R835167.

P117
GROWING UP IN THE ATHABASCA OIL SANDS REGION: INTEGRATIVE HEALTH ASSESSMENT OF EARLY LIFE STAGES OF WOOD FROGS

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Oil sands mining in northern Alberta has drawn scrutiny for its potential impacts on wildlife and the ecosystem. The release of potentially hazardous chemicals such as polycyclic aromatic compounds (PAHs) and naphthenic acids (NAs), by land disturbances, tailings pond seepage, and atmospheric deposition are among the concerns raised. To characterize physiological effects of oil sands on the health of wildlife, we examined wood frogs from three ponds in the Fort McMurray area, one of which is an opportunistic pond formed on a reclaimed overburden site. The water at this site has higher conductivity, NA, and PAH levels than the other ponds. Similarly, tadpole tissues have higher levels of NAs and PAHs. We collected eggs from ponds immediately after laying, reared them in mesocosms, and compared health indices of individuals to those of individuals collected from field populations. Eggs collected from this pond had a 4-fold lower survival rate, larvae (Gosner stages 36-38) had higher intensities of ranavirus (RV) infections, higher whole-body corticosterone (CORT) concentrations, and exhibited bloating relative to those collected from other ponds, possibly related to osmotic stress. Tadpoles from this population also had higher CORT levels and RV infection intensities when reared in mesocosms, suggesting a parental effect or the effect of exposure to water at the time of laying. At 90 d after metamorphosis, individuals from this pond (field-collected and lab-reared) had

similar growth rates compared to other ponds, but prevalence and intensity of RV infection were still higher, suggesting that the ability to clear infections was compromised. These findings suggest that in addition to greater egg mortality, there are non-lethal, potentially transgenerational physiological effects expressed by larvae developing in ponds affected by oil sands-related contaminants and that these effects may extend after metamorphosis. Supported by Carl H. Elling Endowment Award to TS, WSU School of Biological Sciences and College of Arts and Sciences research awards to KD, WSU ADVANCE Transition Award to EJC, Keyano College Research Innovation Award to DMS. Water and tissue contaminants data provided by the Joint Canada-Alberta Oilsands Monitoring Program

P118
MICROARRAY APPLICATIONS TO INVESTIGATE THE IMPACTS OF EXPOSURE TO ENVIRONMENTAL CONTAMINANTS IN MALE FATHEAD MINNOWS

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Chronic or acute exposure to environmental contaminants may result in impairment of reproduction, metabolism and development in wild life and humans. Efficient, specific, and robust biomarkers will be needed to develop effective screening tools. Our main objective was to investigate the mechanisms may involved in adverse effects a number of chemicals present in the Alberta Rivers [Nonylphenol, BPA, DEHP and mixture of the three chemicals] using microarray approach. IPA core and toxicity analysis, and gene ontology revealed a distinct mode of actions for the individual chemicals and their mixture in the liver of male fathead minnows. A number of canonical pathways were significantly affected by these contaminants, including cell cycle & proliferation, inflammatory, innate immune response, stress response, and drug metabolism. In the present study, we identified a number of genes as potential biomarkers for monitoring all contaminants as well as the individual chemicals. In general, the results of this study provide novel information on mechanisms by which contaminants disrupt normal health based on pathway analysis, and identified a number of specific new biomarkers that can be used for screening the presence of contaminants in the aquatic environment.
Study was funded by NSERC grants.

P119
KRÜPPEL-LIKE FACTOR 9 IS A NOVEL NEGATIVE REGULATOR OF THE CELLULAR CIRCADIAN CLOCK THAT PARTICIPATES IN THE MODULATION OF PERIPHERAL CLOCKS BY CORTICOSTEROIDS

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Circadian rhythms in mammals arise from a cell-autonomous transcription-translation feedback loop (TTFL). Here we report that Krüppel-like factor 9 (Klf9) is a direct output gene of this TTFL that feeds back on clock gene expression and modulates clock output. A genome-wide screen on the mouse hippocampus-derived cell line HT22 showed that Klf9 associated with genomic regions of many clock- or clock-output genes, including *Per1*, *Per3*, *Dbp*, *Tef*, *Bhlhe40*, *Bhlhe41*, *Nr1d1*, and *Nr1d2*. Furthermore, *Klf9* is a direct CLOCK/Bmal1 target gene, whose mRNA level shows circadian oscillation in synchronized HT22 cells, and in mouse hippocampus and liver *in vivo*. Klf9 shows circadian association in liver chromatin *in vivo* with *Tef, Nr1d1* and *Klf16*, supporting a role for Klf9 in regulating the rhythmic expression of these genes. We analyzed transcription of *Dbp* in tissue culture cells as a model for Klf9 regulation of clock-output genes. Forced expression of Klf9 reduced *Dbp* mRNA level and repressed transcriptional activity of a reporter construct containing the *Dbp* genomic region. Klf9 strongly inhibited transcriptional activation of *Dbp* by CLOCK/Bmal1. Taken together, these results support Klf9 as a novel regulator of the circadian TTFL that represses transcription of clock and clock-output genes.
Peripheral circadian clocks, are partly synchronized by diurnal variations in adrenal glucocorticoids (GCs) that act via by the GC receptor (GR). However, although many clock and clock output genes are direct GR targets, exposure to an acute stressor does not disrupt circadian rhythms. We previously found that GCs strongly regulate Klf9 via two conserved GC response elements. We hypothesized that Klf9 acts as a feed forward repressor induced by GCs that governs the transcriptional response of clock and clock output genes to elevated GCs. Forced expression of Klf9 inhibited induction by GCs of the clock gene Per1 in HT22 cells. In addition, mice null for Klf9 showed impaired Per1 induction in response to an acute restraint stress, supporting a role for Klf9 in regulating the transcriptional response to GCs.
Supported by NSF (IOS 0922583) and National Institute of Neurological Disorders and Stroke, NIH (1 R01 NS046690-01A2) grants to RJD. JRK was supported by a MCubed grant and by NIH training grant in Reproductive Sciences 1T32HD079342-01.

P120
COMPARATIVE ASPECTS OF PHOTOSENSITIVE MOLECULE OPSIN 5 IN BIRDS AND MAMMALS.

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It has been known for many decades that nonmammalian vertebrates detect light by deep brain photoreceptors that lie outside the retina and pineal organ to regulate seasonal cycle of reproduction. However, the identity of these photoreceptors has remained unclear. Expression

analysis of genes coding photosensitive proteins, the opsin superfamily, identified as *Opsin 5* (*OPN5*) mRNA in the paraventricular organ (PVO) of the quail mediobasal hypothalamus (MBH). Heterologous expression of quail OPN5 in *Xenopus* oocytes resulted in short-wavelength light-dependent activation of membrane currents. Immunohistochemistry identified OPN5 in cerebrospinal fluid (CSF) -contacting neurons of the PVO, as well as fibers extending to the external zone of the median eminence adjacent to the pars tuberalis of the pituitary gland, which translates photoperiodic information into neuroendocrine responses. CSF-contacting neurons extend knob-like dendrites into the ventricular cavity, where they form ciliated terminals. The dendritic structures of photoreceptor cells in the developing retina and the pineal organ resemble those of CSF-contacting neurons. Interestingly, the OPN5-positive CSF-contacting neurons in the quail PVO were suggested to show an intrinsic photosensitivity electrophysiologically. We also found that short-wavelength light induced the gonadal development even in eye-patched, pinealectomized quails, while knock down of OPN5 in the PVO prevented from inducing the photoperiodic expression of thyroid stimulating-hormone in the pars tuberalis of the pituitary gland, which is a center for seasonal reproduction. Thus, OPN5-positive CSF-contacting neurons in the PVO appears to be one of the deep brain photoreceptors that regulates seasonal reproduction in birds. In contrast to birds, eyes are believed to be the only photoreceptive organ in mammals. We are also analyzing the expression and the photosensitivity of OPN5 in mice. Acknowledgements: Supported by: JSPS KAKENHI "Grant-in-Aid for Specially Promoted Research" (26000013), and by the Human Frontier Science Program (RGP0030/2015).

P121

SEX DEPENDENT VARIATION IN THE MEMBRANE PROGESTIN AND SEX STEROID RECEPTORS IN OLFACTORY REGION OF BRAIN AND GONADS OF MULLET, *MUGIL DUSSUMIERI*

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The comparative analysis of sex steroid receptors in olfactory region of brain and the membrane progestin receptors (mPR)(maturation inducing steroid (MIS) receptor) in gonads of male and female in *Mugil dussumieri* has been investigated to know the role of olfactory region on gonadal response towards final maturation. Sexual differentiation of mPR has been studied in the reproductively active mature gonads of testis and ovary. Fresh tissues were fixed in RNAlater, trizol method employed to isolate total RNA, M-MuLV-RT enzyme used to reverse transcribed to get cDNA, gene specific primers were used to get the amplified sequence of the transcripts. It was found that the mPRβ expression is significantly less in both male and female gonads when compare with mPRα. mPRγ expression also noticed in testis and ovary in almost same level of expression. It is now understood that the mPRα playing a role in final maturation of oocytes in *M. dussumieri*. Further the sequence analysis of the mPRα, mPRβ and mPRγ has been studied to compare with other species to authenticate the mPRs in which it is confirmed that the mPRα is expressed high level during the final maturation. Further, the present study unravelled androgen receptor (AR), Estrogen receptors: alpha (ERα) and beta (ERβ) expression in the olfactory region of the estuarine mullet, although ERα has been noticed in brain tissues of many species. This indicates that the olfactory region does not show any difference in the expression of the sex steroid receptors. In addition, kisspeptin-I receptor (kiss1r) is identified in brain and gonads of the mullet. The kiss1r expressed higher in ovary than in testis, but there is no difference in its expression in the olfactory region of brain. The receptor study signifies that the olfactory region of brain do not interfere with the expression of the MIS receptor in the species during the final maturation.

P122

REVEALING THE LOCALIZATION AND PHYSIOLOGICAL FUNCTION OF ION TRANSPORT PEPTIDE IN THE MOSQUITO, *AEDES AEGYPTI*

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Hydromineral balance in the haemolymph of insects is primarily regulated by the excretory system, which in turn, is ultimately under the control of diuretic and antidiuretic hormones. Ion Transport Peptide (ITP) was the first characterized antidiuretic factor shown to act on the hindgut of the locust, which was later uncovered in other insects including the mosquito, *Aedes aegypti*. My research aims to elucidate the function of ITP within the hindgut of the mosquito, *A. aegypti* – the vector responsible for spreading a range of diseases, such as dengue and yellow fever. In order to generate a functional ITP recombinant peptide, we utilized an endocrine-derived cell culture system involving mouse anterior pituitary (AtT-20) cells to express *A. aegypti* ITP (AeaegITP). Protein extracts were isolated from AtT-20 cells transiently expressing AeaegITP and samples were processed through western blot analysis. Using a primary antibody against the C-terminal region of AeaegITP, a band size of approximately 9 kDa was detected.
The central nervous system of four-day-old adult *A. aegypti* was examined for ITP-like immunoreactivity using wholemount immunohistochemistry. Preliminary findings reveal ITP-immunoreactive cells located medioposteriorly and ventrally on each abdominal ganglia of the ventral nerve cord. Using Scanning Ion-selective Electrode Technique to measure ion transport across the hindgut epithelia, the influence of recombinant AeaegITP and its supposed second messengers, cAMP and cGMP, were investigated. Results thus far indicate that application of cAMP generally promoted hemolymph directed Na^+ flux (i.e absorption), while it generally inhibited K^+ absorption. Conversely, cGMP generally promoted lumen-directed Na^+ flux (i.e secretion).
Acknowledgements: Research supported by an NSERC Discovery Grant and Petro Canada Young Innovator Award to JPP.

P123
IDENTIFICATION AND CHARACTERIZATION OF THE ADIPOKINETIC HORMONE/CORAZONIN-RELATED PEPTIDE (ACP) RECEPTOR IN *AEDES AEGYPTI*

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The adipokinetic hormone/corazonin-related peptide (ACP) is an insect neuropeptide that is structurally intermediate between the corazonin (CRZ) and adipokinetic (AKH) hormones, all of which demonstrate homology to the vertebrate gonadotropin releasing hormone (GnRH). Various studies have characterized the corazonin and AKH signalling systems within many insect species, and putative cardioacceleratory and energy mobilization functions, respectively, have been proposed. In contrast, the ACP peptide and its receptor, ACPR, have only been identified in few insect species and its function remains unknown. Despite ACP/ACPR being structurally related to AKH and CRZ and their receptors, studies have shown it to be functionally unrelated to the two later signalling pathways. Here, we aim to identify and functionally characterize the ACP/ACPR signalling system in the dengue and yellow fever mosquito, *Aedes aegypti*. Thus far, three ACP receptor variants have been identified, one functional receptor (ACPR-I; 577 residues, 7 TM domains) and two nonfunctional truncated receptor isoforms (ACPR-II and ACPR-III; 328 residues, 5-TM domains and 243 residues, 3-TM domains, respectively). Functional assays testing ACP, AKH, CRZ, and other *A. aegypti* peptides have demonstrated the specificity of ACPR-I for ACP with an EC50 value in the low nanomolar range. Spatial expression profiles reveal ACPR-I transcript enrichment in the ventral nerve cord and carcass of adult male and female mosquitoes. Outcomes of this research will improve our understanding of the ACP/ACPR signalling pathway to elucidate its function.
Acknowledgements: Research supported by an NSERC Discovery Grant and Petro Canada Young Innovator Award to JPP.

P124
TRIIODOTHYRONINE MODIFIES THE DIFFERENTIAL GENE EXPRESSION OF Na^+/K^+ AND Ca^{2+}-DEPENDENT ATPASES ISOFORMS IN RESTRAINT-STRESSED MICE BRAIN

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Thyroid hormones (TH) are critical for the differentiation and maintenance of brain functions. It is likely that triiodothyronine (T3) could interact with cortisol, a prominent stress hormone that would modify the neuronal functions. We, thus, tested the action of T3 on ion transporter functions and examined whether TH are involved in stress response in mice brain. Gene expression of neuronal-specific Atp1a1, Atp1a3 and Atp1b1 isoforms of Na^+/K^+-ATPase (NKA) and Atp2b2 and Atp2b3 isoforms of plasma membrane Ca^{2+} ATPase (PMCA) and the transporter kinetics were quantified in the brain of restraint-stressed mice. Molecular analyses of the isoforms expression in cortex, hippocampus and cerebellum of eight weeks old mice brain showed differential regulation after T3 challenge and that indicates a direct role of T3 in Na^+ and Ca^{2+}signaling. These molecular markers further showed spatial and temporal distributions in restraint-stressed mice brain, providing clues on the vital role of these transporters in neuronal functions during stress response. Analysis of the pattern of the tested isoform expression and transporter kinetics of restraint-mice treated with T3 showed a marked modification in the transporter function. Taken together, these data provided evidence for a critical stress modifier role for T3 in Na^+ and Ca^{++} signaling during restraint stress in mice brain (supported by grants from iCEIB, IIGovt. of Kerala and UGC SAP DRS and UoK).

P125
MELATONIN REGULATES NA^+ HOMEOSTASIS DURING STRESS RESPONSE IN FISH IONOCYTES

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The role of melatonin in ion transport particularly on Na^+ transport has not yet adequately addressed in fish. As transporters that provide the driving force for many other transport systems, Na^+/K^+-ATPase, H^+/K^+ATPase (HKA) and H^+-ATPase (HA) are vital for Na^+/K^+, and H^+ homeostasis. We, therefore, examined the *in vivo* action of melatonin on these ion transporter functions in the ionocytes of osmoregulatory epithelia of an air-breathing fish kept either in stressed or non-stressed conditions. Analysis of mRNA expression showed differential regulation of nka α1 isoforms viz., nkaαla, nkaα1b and nkaα1c in these tissues. Furthermore, localization of NKA in these tissues provided evidence that melatonin could modify the pattern of NKA immunoreactivity in hypoxia-stressed fish. Likewise, the NKA protein abundance also showed varied response pattern to melatonin challenge. Overall, our data indicate that melatonin regulates Na^+ homeostasis during stress response by exerting spatial and differential actions on the varied ionocytes in air-breathing fish (supported by grants from iCEIB project, Gov. of Kerala, UGC-SAP DRS II and DST project on fish).

P126
TRANSGENERATIONAL EFFECTS ON THE REPRODUCTIVE AXIS OF MEDAKA FISH CAUSED BY EARLY DEVELOPMENTAL EXPOSURE TO BISPHENOL A

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Embryonic exposure to bisphenol A (BPA) causes transgenerational health defects in diverse species. Impaired fertilization efficiency, increased mortality of embryos, heart defects, and altered social interactions are the currently reported phenotypes induced by ancestral exposure to BPA. Mechanisms underlying such dramatic effects in the individuals that were not directly exposed to environmental stressors are not clearly understood. In the present study, one-time developmental exposure of the first generation (F0) interestingly resulted in no adverse health effects within the same or succeeding generation (F1), but reduced fertility and embryo survival were found respectively in fish two generations (F2) and three generations (F3) later. To understand underlying mechanisms of BPA-induced transgenerational inheritance, compared gene expression profiles in the reproductive organs (brain, pituitary, and gonads) of fish from F0 and F2 generations. Quantitative real-time PCR and ΔΔCt method were used to measure and calculate gene expression, respectively. Embryonic BPA exposure caused significant increase in expression of GnRH II and *Kiss* genes and their receptors in the brain of F2 generation fish, which was accompanied by significant reduction in expression of DNA methyltransferase enzyme gene 1 (Dnmt1). Pituitary *Fsh*♂ and *LH*♂ genes were hyperactivated at F0 generation which decreased to control level at F2 generation, while *Fshr* in the testis was significantly reduced at F0 generation but was not changed at F2 generation. In the testis, androgen synthesizing enzyme *Cyp11b1* was not changed at F0 generation but significantly increased at F2 generation. Taken together, this differential expression pattern in the reproductive axis suggests that exposures during embryonic development can induce unique changes in grandoffspring generation and provide significant insights into current understanding of transgenerational reproductive effects of BPA exposure impacting the health of future generations.

P127
TARGETED GENE DISRUPTION OF SECRETOGRANIN II IN ZEBRAFISH REDUCES REPRODUCTIVE SUCCESS AND COURTSHIP BEHAVIOURS

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Secretogranin II (SgII) is a precursor protein in secretory vesicles that is processed into several secreted bioactive peptides, however only the 31-44 amino acid central domain called secretoneurin (SN) is evolutionary conserved in vertebrates. There are two paralogs of teleost SgII, SgIIa and SgIIb that are processed to produce SNa and SNb, respectively. Our previous studies have shown that SNa stimulates the synthesis and release of luteinizing hormone, indicating hormone-like activities to regulate reproduction. Here, we examined the function of SgIIa/SgIIb in zebrafish reproduction using TALENs to produce *sgIIa*$^{-/-}$, *sgIIb*$^{-/-}$ and *sgIIa*$^{-/-}$/ *sgIIb*$^{-/-}$ knockouts (KO). Spawning success of pairwise within-line matings between wild-type (WT) fish was 62% while only 37% (P=0.06), 44% (P=0.017) and 6% (P<0.0001) of *sgIIa*$^{-/-}$, *sgIIb*$^{-/-}$ and *sgIIa*$^{-/-}$/*sgIIb*$^{-/-}$ animals spawned, respectively. Reciprocal crosses indicated a female specific effect; spawning success of *sgIIa*$^{-/-}$/ *sgIIb*$^{-/-}$ females crossed with WT males was 8% (P<0.0001) relative to WT. Surprisingly, *in vitro* % fertilization was high (>87%) in WT and all mutant lines, so we tested the hypothesis that impaired spawning is linked to disruption of courtship behaviour. Courtship in zebrafish consists of sequential distinctive male behaviours in response to female sex pheromones that include chasing, tail-nose nudging and encirclement of the female. Once swimming alongside the female, the male oscillates his body (quiver), and sperm is released simultaneously with oviposition by the female. The duration and number of all behaviours (P<0.05) was reduced in *sgIIa*$^{-/-}$/ *sgIIb*$^{-/-}$ within line crosses. There was an overall reduction of courtship (P<0.05) in *sgIIa*$^{-/-}$/ *sgIIb*$^{-/-}$ females crossed with WT males. We investigated the potential role of altered sex pheromone production in SgII-KOs. Exposure of *sgIIa*$^{-/-}$/ *sgIIb*$^{-/-}$ females crossed with WT males to 'spawning water' (water collected after a normal WT spawning event) increased spawning success from 0% to 20% (P=0.023), indicating partial rescue of the behavioural deficit. These data indicate that SgII is an important regulator of reproductive behavior and spawning success.
Acknowledgements: Supported by: NSERC Canada and uOttawa grants to VLT and NSFC China and CAS grants to WH.

P128
PEFFECTS OF FIBER－RICH FOOD IN LEPTIN RECEPTOR DYSFUNCTION RATS

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The SHR/NDmcr-cp/cp (SHR-cp) rat shows Leptin receptor dysfunction and it is a new model for metabolic syndrome such as obesity, hypertension, hyperlipidemia, and insulin resistance. Calorie restriction has been known as the method for extending lifespan. In the present study, we examined the lifespan of SHR-cp rats and effects of fiber-rich diet on it. Male SHR-cp rats from 5 weeks of age were fed a pellet diet (MF, Oriental Yeast Co, Tokyo, Japan) ad libitum throughout the experiment as the control (AL group). While the ones in the fiber-rich (FR) group were given a fiber-rich diet (containing 30% cellulose) ad libitum. We monitored body weight, food consumption, blood parameters, and the survival of all rats. Members of the two groups that died spontaneously were autopsied. All experimental procedures were approved by a local committee for animal research of Faculty of Medicine, Shimane University. The average of lifespan of the FR group was significantly longer than that of the AL group (Log-rank test, p<0.01). The mean maximum body weight of the AL group was 927.9±46.0g, while that of the FR group was 673.0±49.2g (P<0.01). Increase of body weight was significantly less in rats in the FR group when compared with AL group. The FR group rats began to reduce body weight significantly after 3 weeks from the start compared with the AL group (P<0.01). There were no significant differences in the systolic blood pressure, except for 240 days of age. Energy intake of the FR group was not significantly different compared with that of AL group. FR group did not showed polyuria and urine glucose compared with AL group. Plasma total cholesterol and triglycerides levels as well as liver weights in the FR group were significantly lower (P<0.05) than those of the AL group. Fiber-rich diet reversed the symptoms of metabolic syndrome in SHR-cp such as urine glucose, obesity, fatty liver, and hyperlipidemia, but not hypertension. Fiber-rich diet may prevent many health problems and may also increase lifespan without calorie restriction from the mouth. Although uptake of energy from the mouth was not different significantly, uptake of the

calorie from the bowl may be different as body weight gain reduced significantly in FR group.

P129
EXPRESSION OF INSULIN SUPERFAMILY GENES IN JAPANESE MEDAKA (*ORYZIAS LATIPS*) DURING DEVELOPMENT AND UNDER CONDITIONS OF PROLONGED FASTING

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The relaxin peptides are a relatively recently discovered group of peptides hormones that belong to the insulin superfamily and appear to play diverse roles in vertebrate neuroendocrine regulation and reproduction. One of the least well understood members of the peptide family is Insulin-like peptide 5 (INSL5). Mammals have a single copy of *INSL5* that signals via its cognate receptors Relaxin Family Peptide Receptor 4 (*RXFP4*), while Japanese medaka, like other teleosts, have duplicated copies of both insl5 (known as insl5a and insl5b) and a greatly expanded repertoire of possible receptors for insl5, including *rxfp3-2,* rxfp3-3 and rxfp4. Recent studies suggest that INSL5 influences glucose homeostasis and a recent study using a mouse model suggested that INSL5 appears to be one of the few gut hormones that stimulates appetite (such as ghrelin). To date, no experimental work has been performed on the physiological function of insl5 in teleosts. Given the highly expanded repertoire of insl5 genes and candidate receptors in fish, a first goal of this study was to look for evidence of co-expression of relaxin ligand and receptors in Japanese medaka to gain insight into possible ligand-receptor. To this end, we performed an analysis of the promoter regions of relaxin family genes to develop hypotheses about the stages and sites of possible gene expression and then performed quantitative reverse-transcription (qRT-PCR) on the entire suite of relaxin family genes in 7 embryo stages and 9 tissues from adult female and male medaka. This indicated that the peptides insl5a and insl5b were highly expressed in intestine, a result that was confirmed for insl5a using *in situ* hybridization of transverse colon sections using a fluorescently labeled insl5a-specific probe, while many of the candidate receptors were expressed in gut but to a greater extent in brain. Secondly, to test the hypothesis that insl5a and/or insl5b are orexigenic hormones in teleosts, we examined whether levels of insl5 RNA increased when fish were subjected to a prolonged fast and decreased following re-feeding. To test this hypothesis, fish were fasted for six days, and RNA-sequencing performed on intestine and brain samples of control, fasted and re-fed fish to identify changes in gene regulation in response to food-deprivation. Following this, qRT-PCR was performed on our focal genes (insl5a/insl5b and their receptors) and a suite of hormones known to influence appetite/satiety and/or glucose metabolism that additionally showed expression changes in the RNA-seq data (e.g. cck, pyy, gcga). The analysis of this data is underway. To our knowledge this is the first study of the function of insl5 teleosts; we propose that the highly subfunctionalized insl5-receptor system in teleosts render fish a good model for teasing apart the multiple functions of this novel gut hormone.

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NOVEL NASAL ADMINISTRATION OF GNRH ELICITS SPERMIATION IN ANAXYRUS FOWLERI

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Today, over a third of all amphibian species are considered threatened or endangered; captive breeding programs have thus been established for declining species as a hedge against extinction. For many species, natural reproductive success is low, requiring administration of exogenous hormones to elicit synchronized reproductive behaviors and gamete production. Currently, intraperitoneal injections (IP) of gonadotropin releasing hormone (GnRH), human chorionic gonadotropin (hCG), or combination of the two are used to activate the hypothalamic-pituitary-gonadal (HPG) axis for steroid induced gamete production. These hormones require minimally invasive injections and can be costly in the high doses required. One method for avoiding these challenges is to use an Intranasal Hormone Delivery (IHD) system, which may elicit the same reproductive behaviors and gamete production, but at lower dosages. GnRH receptors, found within the olfactory bulb and preoptic area of the hypothalamus, could be activated through IHD by directly targeting the receptors by a shorter pathway.
We investigated the effects of intranasal GnRH on spermiation parameters in male Anaxyrus fowleri based on a summer breeding (July-Aug) and winter non-breeding (Nov- Jan) period. Treatment dosages of 1, 5, and 10 µg GnRH suspended in 20 µl PBS were administered nasally. Spermic urine samples were collected hourly for seven consecutive hours. The percentage of males responding to each treatment, latency to spermiation response, duration of spermiation, and percent motile sperm were recorded. IHD administration of 1, 5, and 10 µg dosages of GnRH elicited spermiation in 0%, 71%, and 86% of males (n=7/TRT), respectively, in summer months. By comparison, the winter group (n=6/TRT) had 0%, 83%, and 17% of male responders to these same treatment groups. Across dosage groups, latency to spermiation was higher in winter than in summer, whereas motility was lower. This study demonstrates the effectiveness of IHD in eliciting sperm production in male A. fowleri. Previous literature reports that IP injection of GnRH results in fewer male responders and increased latency to spermiation than reported here. Lastly, IHD may be preferential to IP administration of GnRH for breeding synchronization and maximizing gamete production in select anuran species.

STRESS RESPONSE IN FATHEAD MINNOWS CAGED DOWNSTREAM OF MUNICIPAL WASTEWATER TREATMENT PLANTS IN THE BOW RIVER, CALGARY, ALBERTA

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Municipal wastewater effluent (MWWE) is a significant point-source of emerging contaminants to aquatic ecosystems. MWWE contains nutrients, pharmaceuticals and personal care products that are not completely eliminated during wastewater treatment processes. The occurrence of emerging contaminants in our surface waters is concerning because they are designed to be biologically active at low concentrations, can generate synergistic effects when present in mixtures, and their effect(s) on non-target organisms remain poorly understood. The objective of this study was to determine whether exposure to MWWE compromised the adaptive response to a secondary acute-stressor in fish. To test this, adult fathead minnows (*Pimephales promelas*) were caged for 26 days at multiple sites upstream and downstream of municipal wastewater treatment plants along the Bow River, Calgary, Alberta. After the exposure period, half of the fish were sampled immediately, while the other half were sampled 60 minutes after secondary stressor exposure (one minute of air exposure). Whole-body cortisol, glucose, glycogen and lactate levels, markers of the primary and secondary stress response, were measured in these fish. The results suggest changes in the hormonal and metabolic response to stressors in wild fish exposed to MWWE in the Bow River. Studies conducted in natural systems will be essential in validating the use of stress performance indicators as biomarkers of sub-lethal effects due to MWWE exposure. Acknowledgement: This study was supported by the Natural Sciences and Engineering Research Council of Canada Strategic Grant to MMV, and by the Alberta Conservation Association through the ACA Grant in Biodiversity to Analisa Lazaro-Côté.

Cancelled presentation

Moriyama, Shunsuke (Japan)

IDENTIFICATION AND CHARACTERIZATION OF NEUROPEPTIDE HOMOLOGS FROM PACIFIC ABALONE, *HALIOTIS DISCUS HANNAI*.

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Pacific abalone, *Haliotis discus hannai*, is one of the most important aquaculture species in the Tohoku and Hokkaido areas of Japan. However, little information is available on the regulations of growth, metabolism, and reproduction by neuropeptides and their receptors in this species. In an attempt to understand the neuroendocrine controls of somatic growth, metabolism, and reproduction in the abalone, we attempted to determine neuropeptide cDNAs from the cerebral ganglion cDNA library of abalone. By the method of expressed sequence tag analysis, we found and/or cloned neuropeptide homolog cDNAs for the following neuropeptides: neuropeptide Y (NPY), APGWamide, Adipokinetic hormone, Cerebrin, Small cardioactive peptide, Enterin, Schistosomine, FMRFamide peptide, NdWFamide peptide, insulin-related peptides (IRP1 and 3), and gonadotropin-releasing hormone (GnRH). Moreover, we found the cDNAs for the following receptors: NPY-R, IRPRs, GnRH-R, TRH-R, PrRP-R, and SST-R by a next-generation sequencing system. Higher NPY mRNA levels in the cerebral ganglion were observed in fast growing larger and larger-sized abalone than in slow growing one. Intramuscular injections of synthetic NPYs in juvenile abalone resulted in an increase in food intake and body mass. On the other hand, higher GnRH content was observed in the cerebral ganglion of mature abalone compared with that of immature abalone. These finding suggest that a number of neuropeptides are produced in the central ganglion and that these peptides are involved in the regulation of somatic growth, metabolism, and reproduction of the Pacific abalone. Acknowledgements: Supported by: Grants-in-Aid for Scientific Research and Japan Science and Technology Agency to SM.

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