3rd BIENNIAL CONFERENCE OF THE NORTH AMERICAN SOCIETY FOR COMPARATIVE ENDOCRINOLOGY

June 21-25, 2015, Ottawa, Ontario, Canada

FINAL PROGRAM

Sunday, June 21st
Summary of events

	Events	**Location**
14h00-17h30	Registration	90 University (residence)
17h30-18h30	**Opening ceremony** MCs: Vance Trudeau & Nafissa Ismail Speakers/performers: Carlos Arámburo, NASCE President & Robert Dores, NASCE Vice President Thierry Giordano, Vice-Dean Governance and International affairs, Faculty of Science, uOttawa Marcel Merette, Dean, Faculty of Social Sciences & Pierre Gosselin, Director, School of Psychology, uOttawa Mona Nemer, Vice-President Research, uOttawa Thomas Louttit, Elder of the Moose Cree First Nation of James Bay Célina Cada-Matasawagona, Sheshegwaning Ojibway First of Manitoulin Island	UCU AUD
18h30-19h30	**Plenary: Randy J Nelson** Day length, reproduction, and immune function	UCU AUD
19h45-	Opening reception – Summer Solstice Party	Tabaret Hall

Monday, June 22nd
Summary of events

	Events	**Location**
08h00-16h30	Registration	DMS lobby
09h00-10h00	**Plenary: Alexander Raikhel** Interplay between juvenile hormone, ecdysone and microRNAs in mosquito reproduction	DMS 1160
10h00-10h30	Coffee	DMS lobby
10h30-12h15	Presidential symposium: MicroRNAs and endocrine biology Co-chairs: Carlos Aramburo & Peter Lobie	DMS 1160
12h15-14h00	Lunch	
12h30-13h45	NASCE Council meeting	FSS 4004
14h00-15h45	Symposium 1: Regulation of Energy balance Co-chairs: Suraj Unniappan & Peggy Biga	DMS 1160
14h00-15h45	Symposium 2: Comparative endocrinology of terrestrial arthropods Co-chairs: Jean-Paul Paluzzi & Patricia Pietrantonio	DMS 1140
14h00-15h45	Symposium 3: Environmental gestagens: effects on reproductive biology of aquatic wildlife Co-chairs: Edward Orlando & Werner Kloas	DMS 1120
15h45-16h15	Coffee	DMS lobby
16h15-17h15	**Gorbman-Bern Memorial Lecture: Glen Van Der Kraak** The multiple regulatory networks controlling ovulation in teleosts	DMS 1160
17h30-19h00	**Bio-Rad workshop**: Introduction to Droplet Digital PCR (ddPCR) and application to molecular medicine Sean Taylor, Bio-Rad Field Application Manager Andrew Gumley, Bio-Rad Instrument Specialist/Territory Manager	DMS 1160

Presidential symposium: MicroRNAs and endocrine biology
Location: DMS 1160
Co-chairs: Carlos Aramburo & Peter Lobie

	Speaker
10h30-11h05	miRNA regulation of endocrine dependent cancer **Lobie, P.E.** and Zhu, T.
11h05-11h40	Endocrine crosstalk with microRNAs in the control of energy metabolism: insights from the rainbow trout **Mennigen, J.A.**, Panserat, S., Skiba-Cassy S.
11h40-12h15	Small details, big consequences: microRNAs, hormonal signaling and insect metamorphosis **Belles, X.**

Symposium 1: Regulation of Energy balance
Location: DMS 1160
Co-chairs: Suraj Unniappan & Peggy Biga

	Speaker
14h00-14h25	The effects of glucose concentration on muscle cell proliferation and regulation **Biga, P.R.**, Froehlich, J.M., Larimer, M.N., Seiliez, I., Gabillard, J.C.
14h25-14h50	Regulation of Drosophila food intake and lifespan **Ja, W.W**., Bruce, K.D., Deshpande, S.A., Hoxha, S. and Carvalho, G.B.
14h50-15h15	Comparative Endocrinology of Nesfatin-1: Update 2015 **Unniappan, S.**
15h15-15h30	Effects of deficiency of acylated ghrelin signaling on locomotor activity and energetic metabolism by daytime restricted feeding in rats **Arellanes-Licea, E**., Khazall, R., Díaz-Muñoz, M. and Abizaid A.
15h30-15h45	Novel function of leptin as a stimulator of glycolysis **Douros, J.D.**, Baltzegar, D.A., Reading, B. and Borski, R.J.

Symposium 2: Comparative endocrinology of terrestrial arthropods
Location: DMS 1140
Co-chairs: Jean-Paul Paluzzi & Patricia Pietrantonio

	Speaker
14h00-14h25	Impact of the feminizing Wolbachia wVulC strain on the androgenic hormone gene expression which is involved in male differentiation of the terrestrial isopod *Armadillidium vulgare* **Grève, P.**, Geniez, S., Moumen, B., Saint-Jean, A., Bertaux, J., Bouchon, D., Foster, J., Slatko, B.E. and Cerveau, N.

14h25-14h50	The fire ant (*Solenopsis invicta*) short neuropeptide F and insulin-like peptides signaling systems: toward elucidating their role in reproduction, colony nutrition and worker division of labor **Pietrantonio, P.V**, Bajracharya, P., Castillo, P.
14h50-15h15	To pee or not to pee – investigation of an anti-diuretic hormone in the adult mosquito, *Aedes aegypti* Al Dhaheri, A., Uyuklu, A. Teymouri, K., Bhatt, G. and **Paluzzi J.P.**
15h15-15h30	The presence and the functions of insulin-like peptides in the Chagas' disease vector *Rhodnius prolixus* **Defferrari, M.S.**, Orchard, I. and Lange, A.B.
15h30-15h45	Identification and characterization of the adipokinetic hormone/corazonin-related peptide (ACP) signalling system in Rhodnius prolixus: a novel role for truncated receptor variants **Zandawala, M.**, Haddad, A., Hamoudi, Z. and Orchard, I.

Symposium 3: Environmental gestagens: effects on reproductive biology of aquatic wildlife
Location: DMS 1120
Co-chairs: Edward Orlando & Werner Kloas

	Speaker
14h00-14h25	Environmental gestagens: Sources, concentrations, and exposure effects from receptor activation to reproductive behaviour **Orlando, E.F.**, Frankel, T.F., Cardon, M., Chambers, I.G., Ellestad, L.E., Hartig P., and Wilson V.
14h25-14h50	Reproductive, histological and transcriptional effects of synthetic progestins medroxyprogesterone acetate and dydrogesterone and their binary mixtures in zebrafish (*Danio rerio*) **Zhao, Y.**, Castiglioni, S. and Fent, K.
14h50-15h15	Environmental gestagens disrupt endocrine systems associated with reproduction and metamorphosis in amphibians **Kloas, W.,** Lorenz, C., Zikova, A., Hoffmann, F. and Lutz, I.
15h15-15h30	Is the combined contraceptive pill as oestrogenic to fish as we think? **Katsiadaki I.**, Antczak, P.,Williams, T.D., Sebire, M., Elphinstone-Davis, J., Viant, M.R., Scott, A.P. and Falciani, F.
15h30-15h45	Effects of progesterone and norethindrone on female fathead minnow (*Pimephales promelas*) steroidogenesis **Petersen, L.H.**, Hala, D., Martinovic, D. and Huggett, D.B.

Tuesday, June 23rd
Summary of events

	Events	**Location**
08h00-10h30	Registration	DMS lobby
09h00-10h00	**Plenary: Kathleen Gilmour** Regulation of the stress axis during chronic social stress	DMS 1160
10h00-10h30	Coffee	DMS lobby
10h30-12h15	Symposium 4: Neuroendocrine Disruptors Co-chairs: Cheryl Rosenfeld & Nancy Denslow	DMS 1160
10h30-12h15	Symposium 5: Cellular and molecular aspects of the hypothalamo-pituitary-adrenal/interrenal axis Co-chairs: Matt Vijayan & Robert Dores	DMS 1140
10h30-12h15	Symposium 6: Growth factor signalling in vertebrate reproduction Co-chairs: Chun Peng & Juan Fernadino	DMS 1120
12h15-14h00	Lunch	
12h30-13h45	GCE Editorial Board meeting	FSS 4004
14h00-15h45	Symposium 7: Vertebrate reproductive neuroendocrinology Part I Co-chairs: Stacia Sower & Hamid Habbibi	DMS 1160
14h00-15h45	Symposium 8: Practical applications of comparative endocrinology Co-chairs: Vance Trudeau & Gabriela Mastromonaco	DMS 1140
14h00-15h45	Symposium 9: Ocular hormones Co-chairs: Steve Harvey & Carlos Aramburo	DMS 1120
15h45-16h15	Coffee	DMS lobby
16h15-17h15	**Gorbman-Bern New Investigator Lecture: Deborah Lutterschmidt** Sex or candy? Neuroendocrine regulation of seasonal life-history transitions	DMS 1160

17h45-20h45	Poster session (supper provided)	Minto Sports Complex & The Draft pub
20h30-	Social	The Draft pub

Symposium 4: Neuroendocrine Disruptors
Location: DMS 1160
Co-chairs: Cheryl Rosenfeld & Nancy Denslow

	Speaker
10h30-10h55	Integrative molecular responses of estrogens in the teleost brain **Denslow, N.D.**, Lavelle, C., Smith, L.C., Garcia-Reyero, N., Sabo-Attwood, T. and Martyniuk, C. J.
10h55-11h20	The sexually dimorphic vasotocin system as target for neuroendocrine disruption in birds **Panzica G.C**. and Gotti S.
11h20-11h45	A multi-taxa comparative animal model approach to provide mechanistic insight into neuroendocrine disruption **Rosenfeld, C.S**
11h45-12h00	Naphthenic acids from petroleum extraction disrupt larval development and metabolism in *Silurana (Xenopus) tropicalis* **Gutierrez-Villagomez, J.M.**,Vazquez-Martinez, J., Xing, L., Gan, A., Langlois, V.S., Martyniuk, C.J. and Trudeau, V.L.
12h00-12h15	Investigating how developmental exposure to bisphenol-A (BPA) and ethinyl estradiol (EE2) affect F1 parental care and F2 pup parameters **Johnson, S.A**, Javurek, A.B., Painter, M.S., Adams, G. F., Manshack, L.K., Ellersieck, M.R, Roberts, R.M. and Rosenfeld, C.S.

Symposium 5: Cellular and molecular aspects of the hypothalamo-pituitary-adrenal/interrenal axis
Location: DMS 1140
Co-chairs: Matt Vijayan & Robert Dores

	Speaker
10h30-10h55	Ontogeny of the hypothalamus-pituitary-interrenal axis functioning in zebrafish **Vijayan, M.M.**, Best , C., Faught, E. and Nesan, D.
10h55-11h20	Analyzing the activation of the melanocortin-2 receptor (MC2R): projecting a role for Transmembrane Domain 4 (TM4) and Extracellular Loop 2 (EC2) in the activation of *Xenopus tropicalis* MC2R **Davis, P.**, Liang, L. and Dores, R.M.

11h20-11h45	The optic tectum: An emerging target for CRF action **Carr, J.A.**, Prater, C. and Harris, B.N
11h45-12h00	Corticotrophin-releasing hormone (CRH) regulates depressive-like behaviors and BDNF expression in the mesocorticolimbic system following global cerebral ischemia **de la Tremblaye, P.B.**, Narvaez Linares, N. and Plamondon, H.
12h00-12h15	Krüppel-like factor 9 is a circadian transcription factor that regulates clock output genes and participates in corticosteroid modulation of peripheral circadian oscillators **Knoedler, J.R.** and Denver, R.J.

Symposium 6: Growth factor signalling in vertebrate reproduction
Location: DMS 1120
Co-chairs: Chun Peng & Juan Fernandino

	Speaker
10h30-10h55	IGF regulation of Y-Box Binding Protein 1 (YB-1; Ybx1) in the ovary: A potential mechanism of how the growth axis influences ovarian follicle activation **GE, W.**, Lau, S.-W., Zhang, L. and Zhu B.
10h55-11h20	Mechanisms of sex determination in South American silversides: from TSD to GSD **Hattori, R.S.**, Zhang, Y., Sarida, M., Yamamoto, Y., Strüssmann, C.A., Fernandino, J.I. and Somoza, G.M
11h20-11h45	Nodal and its modulating microRNAs in placental development **Peng, C.**, Nadeem, U., Nadeem, L., Brkic, J., Fu, G., Luo, L., Dunk, C. and Lye, S.
11h45-12h00	Growth hormone (GH) effects on proliferation of ovarian granulosa cells in the hen **Ahumada-Solórzano, S.M.**, Carranza, M., Ávila-Mendoza, J., Luna, M. and Arámburo, C.
12h00-12h15	Follicle stimulate hormone and vascular endothelial growth factor increase the production of sphingosine-1-phosfate in cultured of bovine granulosa cells **Hernández-Coronado C.G**., Guzmán A., Romano-Pardo M.C., Gutiérrez C.G., Rodríguez A, Mondragón J.A. and Rosales-Torres A.M.

Symposium 7: Vertebrate reproductive neuroendocrinology Part I
Location: DMS 1160
Co-chairs: Stacia Sower & Hamid Habbibi

	Speaker
14h00-14h25	Characterization of novel neuropeptides modulating fish reproduction **Levavi-Sivan, B.**

14h25-14h50	Plasticity in the postnatal gonadotropin-releasing hormone system **Tsai, P.-S.**, Rochester, J.R., Gonzalez, P.K., Link, C.D., Kavanaugh, S.I.
14h50-15h15	Evolution of the GnRH and GnRH Receptor Families: Insight from a Basal Vertebrate and Mollusk **Sower, S.A.**, Tsai, P-S., Kavanaugh, S.I. and Plachetzki, D.C.
15h15-15h30	Expression and Localization of Two Glycoprotein Hormone Receptors in the Ovary and Thyroid Implies that Hypothalamic-Pituitary-Ovary & -Thyroid Axes Overlap in the Sea Lamprey, *Petromyzon marinus* **Hausken, K.N.**, Scialabba, R.L. and Sower, S.A.
15h30-15h45	GnRH-selective regulation of PIP_3-dependent signaling contributes to the differential control of LH and GH secretion in goldfish, *Carassius auratus* **Pemberton, J.G.**, Stafford, J.L. and Chang, J.P.

Symposium 8: Practical applications of comparative endocrinology
Location: DMS 1140
Co-chairs: Vance Trudeau & Gabriela Mastromonaco

	Speaker
14h00-14h25	Applied comparative endocrinology and novel hormone entities: Finding a pathway to commercialization **Lovejoy, D.A.**
14h25-14h50	Novel substrates for hormone analysis in wildlife. **Mastromonaco, G.F.**, Gilman, C., O'Handley, S.
14h50-15h15	Applying the principles of evolutionary endocrinology to develop spawning induction methods in amphibians **Trudeau, V.L.**, Vu, M., Xia, X., Thoney, D., McGinnity, D. and Dancosse, J.
15h15-15h30	Identification of California condor (*Gymnogyps californianus*) estrogen receptors 1 and 2 and their activation by endocrine disrupting chemicals **Felton, R.**, Steiner, C., Milnes, M., Durrant, B. and Tubbs, C.
15h30-15h45	Optimisation of ovulation and oviposition in the Barred Frog (*Mixophyes fasciolatus*) by induction with human chorionic (hCG) gonadotropin with pregnant mare serum gonadotropin (PMSG) priming **Clulow, J.,** Clulow, S., Guo, J., French, A.J., Mahony, M.J. and Archer, M.

Symposium 9: Ocular hormones
Location: DMS 1120
Co-chairs: Steve Harvey & Carlos Aramburo

	Speaker
14h00-14h25	Ocular growth hormone: a comparative perspective

	Harvey, S., Martínez-Moreno, C.G., Luna, M. and Arámburo, C.
14h25-14h50	Growth hormone is expressed in the neuroretina of green iguana **Ávila-Mendoza, J.**, Carranza, M., Luna, M., and Arámburo, C.
14h50-15h15	Vascular and neuroprotective actions of vasoinhibins and prolactin in the retina Clapp, C., **Arnold, E**., Thebault, S., Díaz-Lezama, N., Arredondo-Zamarripa, D., Vázquez-Membrillo, M., Adán-Castro, E. and Martínez de la Escalera, G
15h15-15h30	Neuroprotective effect of growth hormone against glutamate/BSO-induced cell death in QNR/D cells **Martínez-Moreno C.G.**, Ávila-Mendoza J., Wu, Y., Arellanes-Licea E., Arámburo C. and Harvey, S.
15h30-15h45	Growth hormone (GH) internalization in embryonic retinal ganglion cells: A synaptogenesis modulator? **Fleming, T.**, Martínez-Moreno, C.G., Mora, J., Luna, M., Arámburo, C. and Harvey, S.

Poster session
Location: Minto Sports Complex & The Draft pub
**Presenters for posters with odd numbers should be present at their poster 17h45-19h15; those with even numbers should be present 19h15-20h45.

Poster #	Presenter
Presidential symposium: MicroRNAs and endocrine biology	
1	Effects of silencing and overexpressing GH upon chicken embryonic cerebellar cell viability **Carranza-Salas, M.**, Ávila, J., Martínez-Moreno, CG., Luna, M., Harvey, S. and Arámburo, C.
Symposium 1: Regulation of Energy balance	
2	Fasting increases GH but not IGF-1 expression in the green iguana **Ávila-Mendoza, J.**, Urban, V., Carranza, M., Luna, M. and Arámburo, C.
3	Defining an ancient neuromuscular-endocrine interaction in the Metazoa: TCAP and its role in glucose metabolism in skeletal muscle **D'Aquila, A.L.**, Chen, Y., Xu, M., and Lovejoy, D.A.
4	Glycemia in free-ranging birds: No evidence for regulation by glucocorticoids during acute stress **Deviche, P.**, Bittner, B., Davies, S., Gao, S., Carpentier, E. and Valle S.
5	Teneurin C-terminal associated peptide (TCAP): An independently transcribed peptide involved in stress and cellular metabolism and a possible ancestor to the ligands of the Secretin GPCR family **Husić, M.**, de Lannoy, L., Michalec, O., D'Aquila, A.L., Lovejoy D.A.
6	The Orexigenic Peptide Ghrelin and its Relationship with Metabolic Challenges Mammals Face During Reproduction **Hyland, L.**, Murray, E., Woodside, B., and Abizaid, A.
7	Nesfatin-1 modulates ghrelin and leptin in mouse insulinoma (MIN6) cells **Pasupulleti, V.K**. and Unniappan, S.

8	Adrenergic regulation of hepatic glycogenolysis in the European eel *Anguilla anguilla* at different silvering stages. **Kiwan, A.**, Franzellitti, S., Valbonesi, P. and Fabbri, E.
10	*In vitro* regulation of hepatic Leptin A synthesis and secretion by glucose and stress hormones in a teleost fish, the tilapia (*Oreochromis mossambicus*) **Mankiewicz, J.L.**, Taylor, J.D., Douros, J.D., Baltzegar, D.A. and Borski, R.J.
11	Serum lipid levels and oxidative stress parameters in streptozotocin-induced diabetic rats administered aqueous preparation of *Kalanchoe pinnata* leaves **Menon, N.**, Sparks, J. and Omoruyi, F.O.
12	Regulation and function of endogenous nesfatin-1/NUCB2 in mice **Mohan, H.**, Ramesh, N., Le, A., Mortazavi, S., Pasupulletti, V., Iwakura, H., Tsushima, R., Ceddia, R. and Unniappan, S.
13	Enteric hormone regulation of NUCB2/nesfatin-1 expression in mice **Mortazavi, S.** and Unniappan, S.
14	Characterization of appetite-regulating factors in platyfish, *Xiphophorus maculatus* (Cyprinodontiformes Poeciliidae) **Pitts, P.** and Volkoff, H.
15	Tectal corticotropin-releasing factor (CRF) neurons respond to fasting and a reactive stressor in the African clawed frog, *Xenopus laevis* **Prater, C.**, Harris, B.N., Garcia, C. and Carr, J.A.
16	Does nesfatin-1 regulate enteric hormone secretion in mice? **Ramesh, N.**, Mortazavi, S. and Unniappan, S.
17	Insulinotropic actions of a nesfatin-1-like peptide encoded in nucleobindin-1 **Ramesh, N.**, Mohan, H. and Unniappan, S.
18	Nucleobindin-1 encoded nesfatin-1 like peptide (NLP) decreases food intake and downregulates preproghrelin mRNA expression in fish **Sundarrajan, L.** and Unniappan, S.
19	Behavioural aspects of locomotion in rodents with teneurin C-terminal associated peptide (TCAP) administration **Shrestha, T.C.**, Lovejoy, D.A., Ralph, M.R.
20	Sex steroid hormones modulate NUCB2/nesfatin-1 and ghrelin in goldfish **Unniappan, S**., Bertucci, J.I. and Canosa, L.F.
21	Circadian pattern of the ghrelinergic system and NUCB2/nesfatin-1 in goldfish **Unniappan, S**., Imperiali, A.B. and Delgado Saavedra, M.J.
22	Macronutrient composition of diet modifies tissue specific abundance of endogenous ghrelin and NUCB2/nesfatin-1 in goldfish (*Carassius auratus*) **Unniappan, S**., Imperiali, A.B. and Delgado Saavedra, M.J.
23	An ancient modulator of stress-related metabolism: role of Teneurin C-terminal associated peptide-1 (TCAP-1) and its receptor latrophilin (LPHN) **Woelfle, R.**, Chen, Y., Trubiani, G., Casatti, C., Barsyte-Lovejoy, D. and Lovejoy, D.A.
24	Neuropeptidergic control of feeding in starfish: characterization of the SALMFamide signaling system **Zandawala, M.**, Egertová, M., Slade, S., Rowe, M., Anderson, S., Semmens, D., Scrivens, J. and Elphick, M.
Symposium 2: Comparative endocrinology of terrestrial arthropods	

25	The sulfakinin-signalling pathway in *Rhodnius prolixus* **Al-Alkawi, H.**, Orchard, I. and Lange, A.B.
26	Allatostatin-like neuropeptides in *C. elegans* regulate locomotion and reproduction **Bendena, W.G.**
28	Ovarian ecdysteroid rhythms: An ovarian clock or response to rhythmic neuropeptides? **Durant, A.D.**, Vafopoulou X. and Steel, C.G.H.
29	Isolation and characterization of the corazonin receptor in the kissing bug, *Rhodnius prolixus* **Hamoudi, Z.**, Orchard, I. and Lange, A.B.
30	Starfish gonadotropin, relaxin-like gonad-stimulating peptide: the gene and its expression Haraguchi, S., Ikeda, N., Abe, M., Tsutsui, K. and **Mita, M.**
31	Fusion of testis is beneficial to male reproduction in *Spodoptera litura* (Lepidoptera) **Liu, L.**, Ma, Q., Liu, Y., Feng, Q.
32	Expression analysis of the heterodimeric glycoprotein hormone receptor, LGR1, in the dengue fever vector, *Aedes aegypti* **Rocco, D.A.**, Kim, D.H. and Paluzzi J.P.
33	Characterization and cloning of the long neuropeptide F (NPF) receptor in the Chagas disease vector, *Rhodnius prolixus* **Sedra, L.** and Lange, A.B.
Symposium 3: Environmental gestagens: effects on reproductive biology of aquatic wildlife	
34	Exposure effects of two progestins, gestodene and levonorgestrel, on the reproductive behavior and fitness of the fathead minnow (*Pimphales promelas*) **Frankel, T.E.**, Gillis, A.B. and Orlando, E.F.
35	Transgenerational reproductive effects of pharmaceuticals in zebrafish (*Danio rerio*) raised from chronically exposed parents **Galus, M.**, Rangaraanjan, S., Lai, A., Shaya, L., Balshine, S. and Wilson, J.Y.
36	Comparing rapid screening bioassays to assess the impacts of progestins on reproduction in the zebrafish **McDermid, C.** and Van Der Kraak, G.
37	Uncovering novel mechanisms of sex-steroid induced previtellogenic ovarian follicle growth in coho salmon (*Oncorhynchus kisutch*) using high-throughput sequencing and pathway analysis **Monson, C**., Harding, L., Goetz, G., Swanson, P. and Young, G.
38	Physiological roles, molecular regulation, and disruption of the progesterone receptor signaling pathways in amphibians **Thomson P.** and Langlois, V.S.
Symposium 4: Neuroendocrine Disruptors	
39	An integrated, landscape-based approach to link landuse, endocrine disrupting chemicals and biological effects **Bertolatus, D.**, Vajda, A.M. and Barber, L.B.
40	Evaluation of the histological and functional effects of estrogen and atrazine on the thymus gland of *Xenopus laevis* tadpoles Reilly C., Morante, K., Quinde, J., Monhart, M., Marina H., Chavez, A., **Fateye, B.** and Schreiber, A.M.

41	The role of Triiodothyronine in sex reversal and delay of hatching in medaka embryos Castañeda Cortes, D.C., Langlois, V.S. and **Fernandino, J.I.**
42	Fluoxetine bioaccumulation and concentration in *Daphnia magna* – studying toxicity in planktonic food webs **Halliwushka, K.** and Heyland, A.
43	Pituitary gonadotropins are targets of endocrine disruption in salmon and trout **Harding L.B.**, Schultz I.R., Young G. and Swanson P.
44	Early-life bisphenol-A (BPA) exposure impairs adult leptin sensitivity and predisposes to obesity **MacKay, H.**, Patterson, Z.R. and Abizaid, A.
45	Does developmental exposure to the endocrine disrupting chemicals, bisphenol A (BPA) and ethinyl estradiol (EE2) affect growth and learning memory in painted turtles (*Chrysemys picta*)? **Manshack, L.K.**, Johnson, S.A., Conard, C.M., Tillitt, D.E., Deem, S.L., Holliday, D.K. and Rosenfeld, C.S.
46	Fish endocrine disruption responses along complex land-use gradients: opportunities and limitations for mitigation by regulation and treatment technology **Vajda, A.M.**
47	Larval exposure to fluoxetine suppresses the stress response in adult zebrafish **Vera Chang, M.N.**, Moon, T.W. and Trudeau, V.L.
48	Differential hepatic gene expression profile of male fathead minnows exposed to environmental pollutants individually and in mixture **Zare, A.**, Henry, D., Chua, G. and Habibi, H.R.
Symposium 5: Cellular and molecular aspects of the hypothalamo-pituitary adrenal/interrenal axis	
49	Regulation of hypothalamic-pituitary-interrenal axis function by serotonin in rainbow trout (*Oncorhynchus mykiss*) **Bélair-Bambrick, M-E.**, Dionne-Wilson, L.E. and Gilmour, K.M.
50	Maternal stress-induced behavioural reprogramming in offspring **Cortez Ghio, S**., Boudreau-Leblanc, A., Audet, C. and Aubin-Horth, N.
51	DNA methylation patterning in the promoter region of the V1a/oxytocin-type vasotocin receptor in the sea lamprey (*Petromyzon marinus*) **Mayasich, S.A.** and Clarke, B.L.
52	Phylogeny of the corticotropin-releasing factor (CRF) family of peptides: The structure of urocortins 2 and 3 as a window into early evolution of the ancestral CRF gene **Michalec, O.** and Lovejoy, D.A.
53	Demonstration of an ovarian corticotropin releasing factor system in the zebrafish, *Danio rerio* **Partch, T.H.**, Bernier, N.J. and Van Der Kraak, G.
54	Effects of maternal social stress on offspring survival, anxiety behaviour, and responsiveness to stress in zebrafish (*Danio rerio*) **Redfern, J.C.**, Brown, A. and Gilmour, K.M.
55	Cortisol effects on local GH/IGF signaling in rainbow trout myogenesis **Timber Wulff, K.**, Latimer, M.N., Galt, N. and Biga, P.R.

Symposium 7: Vertebrate reproductive neuroendocrinology	
56	Corticosterone and the regulation of parental care behavior in captive birds **Bittner, B.**, Hutton, P. and Deviche, P.
57	Identifying brain and gonadal gene expression patterns during natural and induced sex change in black sea bass (*Centropristis striata*) **Breton, T.S.**, Kenter, L.W., Luckenbach, J.A., Goetz, F.W. and Berlinsky, D.L.
58	Secretoneurin as a potential regulator of radial glial cell function in neurosteroidogenesis and neurogenesis **Da Fonte, D**., Xing, L. and Trudeau, V.L.
59	Physiological and behavioral correlates of reproductive "decisions" in female red-sided garter snakes (*Thamnophis sirtalis parietalis*) **Dayger, C. A.** and Lutterschmidt, D. I.
60	Sex steroid regulation of pituitary gonadotropins during primary oocyte growth in coho salmon **Harding L.B.**, Monson C.A., Young G. and Swanson P.
61	Interactions of nesfatin-1-GnRH-kisspeptin in murine hypothalamic cells i*n vitro* **Hatef, A.** and Unniappan, S.
62	Brain and plasma oxytocin levels under basal and mating conditions in the female rat **Langett, M.L.** and Cameron, N.M.
63	Identifying molecular mechanisms underlying crosstalk between the thyroid hormone and androgen axes in the frog *Silurana tropicalis* **Langlois, V.S.** and Campbell, DEK
64	Seasonal life-history transitions between reproduction, migration, and foraging in red-sided garter snakes: The role of neuropeptide Y and arginine vasotocin **Lucas, A.R.** and Lutterschmidt, D.I.
65	A proto-pluritropic cell type and its expression of novel glycoprotein hormones in the pituitary of the basal vertebrate, the sea lamprey **Marquis, T.J.**, Nozaki, M., and Sower, S
66	Examining the functional roles of gonadotropin-releasing hormone II (GnRH2) in the zebrafish (*Danio rerio*) **Marvel, M.M.**, Spicer, O.S., Stubblefield, J.D., Wong, T-T., Xia, W., Zmora, N. and Zohar, Y
67	Estrogen receptor sequence from the brain and gonads of *Chirostoma humboldtianum* (Atheriniformes: Atherinopsidae). **Muñoz-Osnaya G. A**, Chavez M; Cárdenas-Reygadas., R.
68	Teneurin C-terminal associated peptide (TCAP) expression and function in the male reproductive system of chordates **Pavlović T.**, Chand D., Casatti, C., Colacci, M., Hsieh, A. and Lovejoy D.A.
69	Investigation of a novel endocrine axis in male-pregnant syngnathid fish: the hypothalamic-pituitary-brood pouch axis **Scobell, S.K.**, Low, M.P., Forlano, P.M. and Wilson, A.B.
70	Expression and functional roles of gonadotropin-inhibitory hormone (GnIH) in the zebrafish (*Danio rerio*) **Spicer, O.S.**, Golan, M., Gothilf, Y., Levavi-Sivan, B., Stubblefield, J.D., Wong, T-T., Zmora, N. and Zohar, Y.

71	Primary radial glial cell culture as a model for dopaminergic regulation of neuroestrogen synthesis in the fish forebrain **Xing, L.**, McDonald, H., Da Fonte, D., Manuel Gutierrez Villagomez and Vance L Trudeau
Symposium 8: Practical applications of comparative endocrinology	
72	Assessment of testicular histology in male Gulf killifish from Barataria Bay Louisiana one year after the Deep Water Horizon oil spill. **Carr, D.L.**, Smith, E.E., Thiyagarajah, A., Davis, A., Dong, M., Garcia, C., Heintzman, L.,Vaughn, K., Snodgrass, P. and Carr, J.A
73	The stress response suppresses innate but not adaptive immunity in the house sparrow **Gao, S.**, Vandrie, A. and Deviche, P.
74	The antagonistic roles of teneurin C-terminal associated peptide (TCAP) and corticotropin-releasing factor (CRF) in stress-related behaviours in the vase tunicate, *Ciona intestinalis* **D'Aquila, A.L.**, Hsieh, A., De Almeida, R., Lovejoy, S.R., Sephton, D., Vercaemer, B. and Lovejoy, D.A
75	Do environmental conditions matter? Gonadal androgens have neither direct nor indirect effect on male growth in a captive gecko **Kubička, L.**, Starostová, Z. and Kratochvíl, L.
76	Timing of stress, not cortisol magnitude, is an important egg viability determinant in female rainbow trout (*Oncorhynchus mykiss*) **Medeiros, L.R.**, Elliott, M. and Nagler, J.J.
77	Gut melatonin response to pathogenic stress: A study in relation to the activity of digestive enzymes and antioxidant defense system in carp *Catla catla* **Pal, P.K.** and Maitra, S.K.
78	Estrogenicity of captive southern white rhinoceros diets and their association with fertility **Tubbs, C.**, Moley, L., Ivy1, J., Gerrard, R., Durrant, B. and Milnes, M.
79	Effects of thyroid hormone and dexamethasone on thymocyte cell death and proliferation in *Xenopus laevis* tadpoles **Yee, S.**, Monhart, M., Quinde, J., Mauch, E., Fateye, B. and Schreiber, A.M.
Symposium 10: Molecular networks in endocrinology	
80	Nicotine replacement therapy – what's the harm? **Boucher, J.**, Forbes, H.R., Gill, N.M.K., Anand, S., Holloway, A.C. and Konkle, A.T.M.
81	In silico pathway analysis linking perturbed steroidogenesis with gonad growth in fathead minnows (*Pimephales promelas*) exposed to 17α-ethynylestradiol **Hala, D.**, Petersen, L.H., Martinović, D. and Huggett, D.B.
82	Jab1 is a coregulator of the thyroid hormone receptor beta 1 **Hernández-Puga G**., Mendoza A., Villalobos P. and Orozco, A.
83	Characterization of the gonadotropin-releasing hormone receptor in *Aplysia californica* **Kavanaugh, S.I.** and Tsai, P.-S.
84	Anti-apoptotic effects of growth hormone (GH) and IGF-I in chicken cerebellar cell cultures during the hypoxia injury.

	Luna, M., Armenta, M.E., Granados, E., Alba-Betancourt, C., Carranza, M. and Arámburo, C
85	Sub-network enrichment analysis as a tool to characterize hormone signaling pathways in the teleostean gonad **Martyniuk, C.J.**, Feswick, A. and Bahamonde, P.
86	Transcript variability for genes in the fathead minnow ovary: Implications for molecular reproductive studies **Martyniuk, C.J**., Loughery, J.R., Wood, R.K., Chishti, Y., Feswick, A. and Cowie, A.M.
87	Structural and functional disruptions of the suprachiasmatic nucleus in fibroblast growth factor-deficient mice. **Miller, A.V.**, Kavanaugh, S.I. and Tsai, P.-S.
88	T3 and 3,5-T2 regulate expression of different gene sets in the thalamus-pituitary of tilapia (*O. Niloticus*) **Olvera, A**., Navarrete-Ramírez, P., Villalobos, P. and Orozco, A.
89	Chronic social defeat paradigm effects on GHSR and NR3C1 mRNA expression in the PFC, HIPP and VTA of C57/BL6 male mice **Park, S**., Rodrigues, T., Patterson, Z.R., Mackay, H. and Abizaid, A.
90	Molecular mechanisms of hormone synergy at the Krüppel-like factor 9 nuclear receptor synergy module **Raj, S.**, Bagamasbad, P.D., Bonett, R.M., Sachs, L., Buisine, N., Ruan, Y., Ruan, X., Knoedler, J., Kyono, Y. and Denver, R.J.
Symposium 11: Genetic analysis of endocrine signaling in animal development	
91	Specific DNA methylation and mRNA expression patterns of srd5α1, srd5α2, srd5α3, and srd5β during early development in the frog *Silurana tropicalis* **Bissegger, S.** and Langlois, V.S.
92	A gene expression screen for genes synergistically regulated by thyroid hormone and glucocorticoid in mouse hippocampal neurons. **Harden, A.**, Bagamasbad, P.D., Knoedler, J.R. and Denver, R.J.
93	Secretogranin IIb plays an important role in zebrafish cerebral artery development **Hu, W.**, Tao, B., Hu, H., Trudeau, V. and Zhu, Z.
94	Developmental expression of TRα and TRβ in the limb of the direct-developing frog *Eleutherodactylus coqui* **Laslo, M.** and Hanken, J.
95	Structural and transcriptomics analysis of IGF ligand, receptor, and binding proteins in a short-lived fish **Liu, C.**, Vila, A.S., Allard, J., Kamei1, H. and Duan, C.
96	Antiapoptotic effects of growth hormone are mediated by PI3K/Akt pathway in the chicken bursa of Fabricius **Luna-Acosta, J.L.**, Alba-Betancourt, C., Martínez-Moreno, C., Ramírez, C., Carranza, M., Luna, M. and Arámburo, C.
97	Zebrafish lhβ and fshβ transgenic lines for the analysis of hormone-regulated pituitary development **Mitchell. K.**, Esau, C., Hu, W. and Trudeau, V.L.
98	Genetic analysis of gonadotropin functions in the zebrafish by TALEN-mediated targeted disruption of follicle-stimulating hormone (FSH) and luteinizing hormone

	(LH) subunit genes (fshb and lhb) **Zhang, Z.**, Zhu, B. and Ge, W.
Symposium 12: Puberty: A critical period for sex and environmental influences on the brain	
99	Effect of LPS on dopamine and Parkinsonian-like behaviours **Girard-Joyal, O.** and Ismail, N.
100	Age and sex differences in the effect of chronic partial sleep disruption on the corticosterone response to a novel stressor in mice **Latus, O**., Trudeau, V. and Ismail, N.
101	Age and sex differences in serum cytokine levels following exposure to a bacterial endotoxin **Rooke, J.**, Kolmogorova, D., Weng, R., Kane, L., Liang, J. and Ismail, N.
102	Age and sex differences in c-fos expression following Poly I:C treatment in pubertal and adult CD1 mice **Sarr, F.**, Sharma, R. and Ismail, N.
103	Effects of Poly(I:C) on thermoregulation in CD1 mice **Sharma, R.** and Ismail, N.
104	Age and sex differences and programming effects of LPS treatment on thermoregulation, sickness behaviour, cytokine levels, and arginine-vasopressin expression. **van Mil, S**., Cai, K.C. and Ismail, N
Symposium 13: Neuroendocrine regulation of ionic, osmotic, and acid-base balance in fish	
105	Acidic water acclimation stimulates ionocyte proliferation by activating the IGF signaling pathway via the Trpv5/6 channel in larval zebrafish **Bai, Y.**, Dai, W., Liu, C., Liu, J. and Duan, C.
106	Can dietary 18β-glycyrrhetinic acid supplementation ameliorate the effects of an abrupt ionoregulatory challenge in a freshwater teleost fish? **Chen, C.C.**, Kolosov, D. and Kelly, S.P
107	A role for cortisol in altering the molecular physiology of the tight junction complex of adult rainbow trout integument **Gauberg, J.**, Kolosov, D. and Kelly, S.P
108	Involvement of parathyroid hormone in calcium regulation and gill cartilage development in zebrafish **Kwong, R.W.M.** and Perry, S.F
109	Differential stimulation of the hypothalamus-pituitary-interrenal axis during smolt development of landlocked and anadromous Atlantic Salmon **McCormick, S.D.**, Regish, A.M. and Bernier, N.J.
110	The role of cortisol in osmoregulation and thermal tolerance in brook trout (*Salvelinus fontinalis*) during seawater acclimation **Shaughnessy, C. A.** and McCormick, S.D.
111	The effects of 11-deoxycortisol on the molecular physiology of the tight junction complex in an extant agnathan **Zimina, A.**, Kolosov, D., Wilkie M.P. and Kelly S.P.
Symposium 14: Mechanism of evolution for hormone mediated phenotypes	
112	Habitat-associated behavioural, hormonal and transcriptional evolutionary divergence in sticklebacks **Di-Poi, C**., Bélanger, D., Amyot, M., Rogers, S. and Aubin-Horth, N.

113	The synthesis of steroids by *Taenia crassiceps* WFU cysticerci and tapeworms is related to the developmental stages of the parasites Patricio-Gómez, J.M., Valdez R., Aguilar-Vega, 2, Zurabian, R., **Romano, M.C.**
114	Postnatal vanishing testis-like syndrome in a 38XX/38XY agonadic hog (*Sus scrofa*) **Ramos, L.**, Chávez, B., Paredes, A. and Vilchis, F.
115	Hormone regulation of maternal care in the mouth brooding cichlid *Astatotilapia burtioni* **Renn, S.C.P.** and O'Rourke, C.
Other	
116	Multiplex detection of KRAS mutations in colorectal cancer FFPE samples using droplet digital PCR Yang, W., Shelton, D.N., Berman, J.R., Zhang, B., Cooper, S., Tzonev, S., Hefner, E., Regan, J.F. (Presented by **Sean Taylor**)

Wednesday, June 24th
Summary of events

	Events	**Location**
09h00-10h00	**Plenary: Yun-Bo Shi** Chromatin remodeling and histone modification underlying the regulation of *Xenopus* growth and development by thyroid hormone receptor	DMS 1160
10h00-10h30	Coffee	DMS lobby
10h30-12h15	Symposium 10: Molecular networks in endocrinology Co-chairs: Valérie Langlois & Chris Martyniuk	DMS 1160
10h30-12h15	Symposium 11: Genetic analysis of endocrine signalling in animal development Co-chairs: Yun-Bo Shi & Cunming Duan	DMS 1140
10h30-12h15	Symposium 12: Puberty: a critical period for sex and environmental influences on the brain Co-chairs: Melissa Holmes & Russ Romeo	DMS 1120
12h15-	Excursions/free afternoon	

Symposium 10: Molecular networks in endocrinology
Location: DMS 1160
Co-chairs: Valérie Langlois & Chris Martyniuk

	Speaker
10h30-10h55	Sniffing out endocrine disruption from complex mixtures with a new genome, metagenomics, and systems approaches **Helbing, C.C.**, Birol, I., Brinkman, F., Hall, E.R., Lesperance, M.L., Parker, W., Pyle, G., van Aggelen, G., Behsaz, B., Brown, L.Y., Griffiths, E., Hammond, S.A., Heerema, J., Kuçuk, E., Manek, A., Miliano, R.C., Unverferth, C., Van Rossum, T., and Veldhoen, N.
10h55-11h20	A global ovary gene regulatory network derived from a large-scale analysis of chemical effects on the fathead minnow **Garcia-Reyero, N.**, Habib, T., Villeneuve, D., Escalon, L., Ankley, G., Perkins, E.J.
11h20-11h45	Toxicogenomics analysis of liver responses in rats and mice exposed to the food contaminant furan: applications in risk assessment **Yauk, C.L.**, Dong, H., Webster, A.F., Curran, I.H., Recio, L., Williams, A., Kuo, B., Gill, S., Wade, M.G.
11h45-12h00	The molecular basis of skin-specific modifications associated with metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus*) **Power, D.M.**, Alves, R.N., and Gomes, A.S.
12h00-12h15	Mineralocorticoid receptor is associated with cellular membranes and may mediate cortisol-stimulated CREB phosphorylation in rainbow trout brain **Dindia, L.** and Vijayan, M.M

Symposium 11: Genetic analysis of endocrine signalling in animal development
Location: DMS 1140
Co-chairs: Yun-Bo Shi & Cunming Duan

	Speaker
10h30-10h55	Efficient targeted gene disruption in *Xenopus* embryos using TALEN and CRISPR/Cas9 **Zhao, H.**, and Chen, Y.
10h55-11h20	Unliganded thyroid hormone receptor regulates developmental timing **Buchholz, D.R.**, Choi, J., Suzuki, K.-I.T.
11h20-11h45	Dissecting oxygen-dependent and -independent actions of hypoxia-inducible factors in early development using CRISPR/Cas9 gene editing **Zhang, P.** and Duan, C
11h45-12h00	Generating and characterizing nuclear progestin receptor (Pgr) knockouts in zebrafish **Zhu, Y.**, Liu, D., Wan, C.L., Chen, S. and Hong, W.
12h00-12h15	Disruption of zebrafish FSH receptor (fshr) but not LH receptor (lhcgr) gene by

	TALEN leads to failed follicle activation in females followed by sexual reversal to males **Zhang, Z.**, Lau, S.-W. and Ge, W.

Symposium12: Puberty: a critical period for sex and environmental influences on the brain
Location: DMS 1120
Co-chairs: Melissa Holmes & Russ Romeo

	Speaker
10h30-10h55	Hypothalamic-pituitary-adrenal (HPA) responses to stressors in pre- and post-pubertal adolescent rats compared with adult rats **McCormick, C.M.**, Green, M.R. and Hodges, T.E.
10h55-11h20	Adolescence and the shaping of the stress response **Romeo, R.D**
11h20-11h45	Hypothalamic gene expression and gonadal steroid hormone levels during transitions in reproductive status in naked mole-rats **Holmes, M.M.**, Swift-Gallant, A., Mo, K. and Monks, D.A.
11h45-12h00	The impact of pubertal immune stress on learning and memory between the sexes **Kolmogorova, D.**, Paré, C., Kostuck, S. L. and Ismail, N.
12h00-12h15	Programming effects in the adult immune response following a pubertal immune challenge **Melanson, B.**, Rooke, J., Liang, J., Schwarz, J. and Ismail, N.

Thursday, June 25th
Summary of events

	Events	**Location**
09h00-10h00	**Plenary: Olivier Kah** Neuropeptides in vertebrate reproduction: many players at the table	DMS 1160
10h00-10h30	Coffee	DMS lobby
10h30-12h15	Symposium 7: Vertebrate reproductive neuroendocrinology Part II Co-chairs: Stacia Sower & Hamid Habbibi	DMS 1160
10h30-12h15	Symposium 13: Neuroendocrine regulation of ionic, osmotic, and acid-base balance in fish Co-chairs: Katie Gilmour & Steve McCormick	DMS 1140
10h30-12h15	Symposium 14: Mechanism of evolution for hormone mediated phenotypes Co-chairs: Nadia Aubin-Horth & Suzy Renn	DMS 1120
12h15-14h00	Lunch	
12h30-13h45	NASCE Council meeting	FSS 4006
14h00-15h00	**Plenary: Raúl Paredes** Neurogenesis and sexual behaviour	DMS 1160
15h00-15h15	Best presentation awards	DMS 1160
17h30-	Closing ceremony and banquet (Museum visits from 17h00)	Canadian Museum of Nature

Symposium 7: Vertebrate reproductive neuroendocrinology Part II
Location: DMS 1160
Co-chairs: Stacia Sower & Hamid Habbibi

	Speaker
10h30-10h55	Gonadotropin-Inhibitory Hormone, stress and reproduction in birds **Bentley, G.**, Ernst, D. and Dickens, M.
10h55-11h20	5α-dihydrotestosterone in female sablefish (*Anoplopoma fimbria*) **Guzmán, J.M.**, Luckenbach, J.A., Da Silva, D.A.M., Middleton, M.A., Masse, K., Jensen, C., Curles, E., Ylitalo, G.M., Young, G., Goetz, F.W., Swanson, P.
11h20-11h45	A closer look into the roles played by Kiss1, Kiss2 and NKB in the network controlling reproduction in the striped bass, *Morone saxatilis* **Zmora, N.**, Wong, T.T., Stubblefield, J., Levavi-Sivan, B., Millar, R.P. and Zohar, Y.
11h45-12h00	Characterization of nestin protein in the goldfish brain **Venables, M.**, Navarro-Martín, L., Zhang, D., Basak, A., and Trudeau, V.L.
12h00-12h15	Maternal care programs estrous cycle-mediated affective behaviours in the female rat Borrow, A.P. and **Cameron, N.M**

Symposium 13: Neuroendocrine regulation of ionic, osmotic, and acid-base balance in fish
Location: DMS 1140
Co-chairs: Katie Gilmour & Steve McCormick

	Speaker
10h30-10h55	Neuroendocrine control of ionic balance in larval zebrafish (*Danio rerio*) **Perry, S.F.**, Kumai, Y. and Kwong, R.W.M.
10h55-11h20	Prolactin and cortisol direct branchial aquaporin 3 expression in euryhaline tilapia **Breves, J.P.**, Inokuchi, M., Yamaguchi, Y., Seale, A.P., Watanabe, S., Lerner, D.T., Kaneko, T. and Grau, E.G.
11h20-11h45	Endocrine regulation of ion transport in marine fish: intestinal mineralization supports osmoregulation **Fuentes, J.**
11h45-12h00	Autocrine regulation of prolactin release from tilapia prolactin cells: modulation

	of hormonal responses by extracellular osmolality **Yamaguchi, Y.**, Moriyama, S., Lerner, D.T., Grau, E.G. and Seale, A.P.
12h00-12h15	Claudin-8 tight junction protein isoforms and cortisol-mediated alterations of teleost fish gill epithelium paracellular permeability **Kolosov, D.** and Kelly S.P.

Symposium 14: Mechanism of evolution for hormone mediated phenotypes
Location: DMS 1120
Co-chairs: Nadia Aubin-Horth & Suzy Renn

	Speaker
10h30-10h55	Answering big questions in evolutionary endocrinology: combining field and lab research, high-throughput molecular genetics tools, and modeling. **Swanson, E.M.** and Snell-Rood, E.C.
10h55-11h20	Exploring the genetic and neuroendocrine mechanisms underlying behaviour and reproductive success in a species with male alternative reproductive tactics **Nugent, B.M.**, Stiver, K.A., Hofmann, H.A. and Alonzo, S.A.
11h20-11h45	The regulation of metamorphic development in a marine environment revisited – Exogenous cues and endogenous modulators **Heyland, A.** and Lutek, K
11h45-12h00	Prolactin and parental care in the zebra finch (*Taeniopygia guttata*) **Smiley, K.O.** and Adkins-Regan, E.
12h00-12h15	Phylogenetic-based analyses of endocrine pathway evolution: inferences and challenges **Bonett, R.M.**

NASCE 2015 ABSTRACTS

Abstracts are in alphabetical order by first author; the presenting author is underlined. [P#] indicates poster number, [S#] indicates symposium number.

Growth hormone (GH) effects on proliferation of ovarian granulosa cells in the hen
[S6, contributed]

Ahumada-Solórzano, S.M., Carranza, M., Ávila-Mendoza, J., Luna, M. and Arámburo, C.
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, México

Pre-ovulatory follicular development is mainly regulated by gonadotropins (FSH, LH), but other intraovarian hormones and factors (steroids, GH, IGF-l) are also involved. It is now known that GH participates in the differentiation, proliferation, steroidogenesis and cell survival processes in the ovary. The ovarian expression of GH has been described in some mammals, including the human, but little is known about its local expression and activity in this organ in birds. Recently, we showed that ovarian GH is involved in the regulation of steroidogenesis in the hen's granulosa cells (GC). Here we studied the effect of GH on the proliferation of GCs. We used F4 follicles, (1-2 cm of diameter, at the beginning of the hierarchical stages) of adult (25-35 weeks old) hens. GH treatment (0.01, 0.1, 1, 10 nM) increased the proliferation of cultured GCs, as determined by the ^{3}H-thymidine incorporation assay and measurement of PCNA (1.6-, 2.5-, 4-fold) or the MTT assay (1.5-, 2-, 3-, 3.5-fold), respectively. This GH-dependent proliferative effect was substantially decreased when a GH-specific siRNA or a specific anti-GH antibody were employed. The addition of conditioned media (CM), containing the GH produced locally by cultured GCs, was capable to stimulate proliferation of freshly cultured GCs (2-, 4-, 8-fold at 0.01, 0.1, 1 nM GH, respectively) and this effect was suppressed by co-incubation with the anti-GH antibody. AIso, the CM stimulated (10-fold) the expression of PCNA in cultured GC, and this decreased in a dose-dependent manner with the addition of anti-GH antibody. Several GH molecular variants were found in the CM by western blotting, with the 17 kDa isoform being the most abundant one. Also, GH stimulated the phosphorylation of ERK in proliferating GC cultures. Both exogenous and locally produced GH increased the release of IGF-1 from cultured GCs. These data suggest that GH expressed in the ovary may be involved in follicular development by stimulating the proliferation of GCs through paracrine/autocrine mechanisms.
Supported by PAPIIT-DGAPA-UNAM IN 208812, IN206813, IN206115; CONACYT 178335. A-SSM received a posdoctoral fellowship (5165). We acknowledge the technical support from C. Courtois.

The sulfakinin-signalling pathway in *Rhodnius prolixus*
[P25]

Al-Alkawi, H., Orchard, I. and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

Neuropeptides play influential roles in feeding and digestion-related activities in insects, including the blood-sucking hemipteran *Rhodnius prolixus*. Sulfakinins (SKs) are one family of such neuropeptides that have been shown to influence digestive, diuretic, and myotropic activities in a variety of insects. These SKs, which are functionally and structurally homologous to the mammalian gastrin/cholecystokinin [CCK] neuropeptides, have only been identified in crustaceans and insects. In this study, the presence of two sulfakinin peptide sequences, namely Rhopr-SK-1 and Rhopr-SK-2, was confirmed by sequencing the open-reading frame (ORF) of the *R. prolixus* cDNA. Immunohistochemical staining of SK-like peptides was observed in cell bodies and processes in the CNS as well as in processes on the posterior midgut and anterior hindgut of 5th instar and adult *R. prolixus.* Quantitative real-time PCR revealed that the Rhopr-SK-1 transcript is primarily localized to the brain and subesophageal ganglion in 5th instar *R. prolixus*. Bioassays revealed dose-dependent increases in contraction of the hindgut in response to Rhopr-SK-1. A heart contraction assay is being used to examine the effects of Rhopr-SK-1 on heartbeat frequency. To further understand the role of the SK-signaling pathway in *R. prolixus,* the G protein-coupled receptors [GPCRs] will be cloned and spatial expression will be analyzed. In addition, dsRNA for both the peptide and its receptor will be used for RNA interference to determine the functional role of the SK-signaling pathway in *R. prolixus*.
This work was supported by NSERC.

To pee or not to pee – investigation of an anti-diuretic hormone in the adult mosquito, *Aedes aegypti*
[S2, invited]

Al Dhaheri, A., Uyuklu, A. Teymouri, K., Bhatt, G. and Paluzzi J.P.
Department of Biology, York University, Toronto, ON, Canada

Insects utilize an array of hormones to maintain ionic and osmotic homeostasis of their haemolymph. These hormones act upon elements of the gut, including the excretory system, which is composed of the Malpighian tubules (MTs) and hindgut. One iono/osmoregulatory regulator which has received significant attention is a peptidergic factor belonging to the CAPA family that is produced following processing of the precursor polypeptide encoded by the *capability* gene. In the mosquito *Aedes aegypi*, an important vector of various diseases including dengue and yellow fever, the CAPA peptides were structurally identified but discovery of their physiological roles has been sluggish. Nonetheless, earlier studies on adult stage *A. aegypti* had established a diuretic function for CAPA peptides at doses within or greater than the mid-nanomolar range. Interestingly in the larval stage, recent studies have indicated an anti-diuretic function for the CAPA peptides at femtomolar doses, inhibiting serotonin-stimulated fluid secretion by MTs. To better understand this potentially complex regulatory mechanism, we have investigated control of MTs using an endogenous CAPA peptide (AedesCAPA-PVK1, GPTVGLFAFPRVamide) at both high and low doses in the adult *A. aegypti* unfed female. In addition, we have determined the distribution of CAPA-like cells and processes in the central nervous system as well as peripheral sites including gut tissues and associated innervations in the adult stage mosquito. Finally, we have isolated and are characterizing a putative CAPA receptor,

which should shed light on the full complement of physiological functions regulated by the CAPA peptides in the mosquito, *A. aegypti*.

Effects of deficiency of acylated ghrelin signaling on locomotor activity and energetic metabolism by daytime restricted feeding in rats
[S1, contributed]

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Acylated ghrelin (AG) is an orexigenic signal that regulates energy expenditure and in vivo it's a modulator of hypophyseal growth hormone (GH) secretion. There is one known functional receptor of AG that is the growth hormone secretagogue receptor-1a (GHSR). When access to food sources is restricted to a few hours per day (2 to 6 h), the subsequent behaviour and metabolism are reorganized as a consequence of nutrients availability. Under this paradigm, food becomes a zeitgeber that causes the emerging of the food entrained oscillator (FEO). We've shown that mice with genetically targeted deletion of the GHSR-1a (KO), had an attenuation of the behavioural arousal before feeding time, the food anticipatory activity (FAA). Our goal in this study, was to explore the role of AG signaling in the FEO using adult male GHSR KO rats and their wild type (WT) littermates. Animals were subjected to daytime restricted feeding (dRF) from geographical time 12:00 to 14:00 h for 3 weeks or Ad libitum conditions. On the last day, rats were sacrificed at three different time points starting at 08:00, then 11:00, and finally 14:00 h to examine a number of metabolic and hormonal parameters in anticipation of, and two hours after meal presentation. We compared the total locomotor activity and FAA, food intake, body growth, somatometry, metabolism of glucose and triglycerides, along with endocrine parameters, and the gene expression by RT-qPCR of limiting enzymes of hepatic lipid metabolism acetyl-coA carboxylase (ACC) and carnitin-palmitoyl transferase-1 (CPT-1). Results showed that locomotor activity increased in scheduled fed rats of both genotypes versus Ad lib conditions. We found that the GHSR KO animals, however, had lower FAA in the first 10 days and an overall mean reduction of 22 % throughout the 21 days. Interestingly, there was a decrease of liver weight at 11:00 h in both genotypes, but GHSR KO rats had lower levels of triglycerides, and decreased expression of acc expression and cpt-1 compared to WT littermates. As expected, AG levels peaked at 11:00, one hour prior to the scheduled meal in both GHSR KO and WT rats. Interestingly, leptin levels rose only in KO rats postprandially. Overall, this results confirm the importance of GHSR in FAA and suggest a link between AG signaling and liver lipid metabolism during FEO for regulation of nutrient handling.
Supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and scholarship from CONACYT 317690 to Arellanes-Licea, E.

Growth hormone is expressed in the neuroretina of green iguana
[S9, invited]

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Growth hormone (GH) is expressed in several extrapituitary tissues, including the nervous system and the ocular tissue, where it is involved in autocrine/paracrine actions. In the eye, the presence of GH has been described in fish, amphibians, birds, and mammals, where it has been shown to play a protective effect and an antiapoptotic role. Little is known, however, in reptiles, so in this study we characterized the expression and distribution of GH in the ocular tissue of the green iguana. It was found by western blotting (WB) that GH was expressed as a family of distinct molecular weight variants in the eye, and the most abundant isoform corresponded to a protein of 15 kDa, although the monomeric form of 22 kDa was also present. The small variant might be the result of post-translational modifications since, by RACE5', we could not find mRNAs with alternative splicing that might lead to this variant. Similarly determined by WB, an immunoreactive band of 70 kDa using an antibody against chicken growth hormone receptor (GHR) was located in the ocular tissue. GH, GHR and IGF-1 were mainly distributed in the ganglionar retinal cells (GRC) in the neuroretina, where it co-localized, by immunocytochemistry, with the nuclear neuronal marker NeuN, but not with glial marker GFAP. Also, GH mRNA was amplified in the retina and its sequence was identical to pituitary GH mRNA. On the other hand, GHR, IGF-1, GHRH, PACAP, TRH and SST mRNAs were also expressed in the retina, suggesting that the synthesis and secretion of retinal GH may be regulated through autocrine/paracrine mechanisms by these neuropeptides in this tissue. Taken together, these studies suggest that GH may be expressed locally to regulate processes of cell survival, differentiation and proliferation, since as described in other studies reptilian retina has the ability to regenerate after damage.
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Fasting increases GH but not IGF-1 expression in the green iguana
[P2]

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Growth hormone (GH) has pleiotropic activities. In addition to regulate development and postnatal growth of vertebrates, it also participates in the modulation of energetic metabolism. Its actions are performed either directly or mediated through insulin-like growth factor 1 (IGF-1). Fasting is an environmental condition that can influence the functioning of this axis. Little is known, however, on the effects of fasting upon this endocrine system in reptiles, a group of vertebrates with a temperature-dependent and slow metabolism. In this work, we evaluated the effect of acute (1 to 2 days) and chronic (10 days) fasting on gene expression of GH and IGF-1 and its possible effect(s). Overall, the results show that GH serum concentration increased from 27.6±2.8 ng/ml in iguanas fed *ad libitum* to 50.73±5.5 ng/ml after two days of fasting, while

during chronic fasting GH levels were similar to the control. Accordingly, pituitary GH mRNA expression increased 10.95-fold under fasting conditions. This effect was opposed to that observed in IGF-1 secretion, as its serum concentration decreased gradually from 27.46±2.4 to 13.8±2.5, 9.18±2.2 and 6.81±1.1 ng/ml at 1, 2 and 10 days of fasting, respectively. The same effect was observed for the hepatic expression of IGF-1 mRNA which decreased in all fasting conditions up to 2.7-fold over control. The decrease in expression and secretion of IGF-1 could be caused by a decrement in the expression of liver GH receptor (GHR) mRNA, which exhibited the same behaviour as IGF-1 mRNA, diminishing 1.4 times. Results suggest that, during fasting GH may target directly the adipose tissue, as insinuated by the metabolic parameters evaluated in this study: glucose concentration declined from 1.98±0.1 mg/ml to 1.3±0.1 mg/ml after 10 days of fasting, while free fatty acids and ketone bodies increased, both, by up to 300%. Taken together, these results may indicate that during fasting conditions GH exerts its effects directly upon lipid metabolism, and these are not mediated by IGF-1.
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Acidic water acclimation stimulates ionocyte proliferation by activating the IGF signaling pathway via the Trpv5/6 channel in larval zebrafish
[P105]

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Acidification of freshwater habitats is a major environmental problem worldwide. The ecological effects of chronic aquatic acidification on local fish populations are widely recognized. In addition to the chronic aquatic acidification problem, many more habitats encounter "spring acid shock", i.e., brief periods in the spring during which water pH drops dramatically due to runoff from melting snow. Springtime is the spawning season for many fish species. How fish embryos and larvae sense and respond to the episodic acidification is poorly understood. In this study, we investigated the effect of "acid shock" on larval epidermal tissue using zebrafish. Zebrafish has emerged as a suitable model for studying acid-base balance and osmoregulation in fish. Zebrafish larval yolk sac skin contains several types of ionocytes, including NaR cells, HR cells, and NCC cells. NaR cells are Ca^{2+}-transporting epithelial cells. HR cells are involved in Na^{+} uptake/acid secretion, while NCC cells are important for Cl^{-} uptake. Exposure of zebrafish larvae to acidic water resulted in major increases in the number of NaR cells. BrdU labeling experiments suggest that acid shock stimulates NaR cell proliferation. These ionocytes specifically express Igfbp5a, a high-affinity and specific binding protein for insulin-like growth factors (IGFs). Biochemical analyses revealed that acidic water treatment activated the IGF-PI3K-Akt-TOR signaling pathway in NaR cells. Inhibition of IGF1 receptor, Akt , and TOR abolished the low pH-induced NaR proliferation. NaR cells also express the *trpv5/6* gene, which encodes the Ca^{2+} channel Trpv5/6. Because TRPV5/6 channels can be inhibited by extracellular acidic pH, we speculated that acidic water treatment might activate IGF signaling in NaR cells through the inhibition of Trpv5/6 channel activity. This idea was tested using two TRPV5/6 inhibitors/blockers, ruthenium red, and cadmium. Indeed, treatment of zebrafish larvae with these inhibitors resulted in a dose-dependent increase in IGF signaling and NaR cell

proliferation. These results suggest that acid shock can induce the activation of IGF-PI3K-Akt-TOR signaling and NaR cell proliferation through altering the action of Trpv5/6 channels.

Regulation of hypothalamic-pituitary-interrenal axis function by serotonin in rainbow trout (*Oncorhynchus mykiss*)
[P49]

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There is evidence of serotonergic regulation of the hypothalamic-pituitary-interrenal (HPI) axis in both unstressed and chronically stressed teleost fish. However, the mechanisms underlying serotonergic regulation of the HPI axis are not clear. We hypothesized that serotonin (5-HT) plays a paracrine role in regulating the HPI axis at the level of the head kidney. To test this hypothesis, the mRNA abundance of 5-HT receptors in head kidney tissue of rainbow trout (*Oncorhynchus mykiss*) was probed by real-time RT-PCR. Head kidney tissue contained detectable levels of 5-HT_{1A}, 5-HT_2 and 5-HT_4 receptor mRNA. Next, the ability of selective 5-HT receptor agonists to elicit cortisol production by head kidney tissue preparations *in vitro* was assessed. Cortisol production was significantly increased over background levels in preparations incubated with 5-HT, or with the 5-HT_4 receptor agonist cisapride, and this elevated cortisol production was blocked by the 5-HT_4 receptor antagonist GR125487. Neither the 5-HT_{1A} receptor agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) nor the 5-HT_2 receptor agonist α-methyl-5-hydroxytryptamine maleate (-methyl-5-HT) stimulated cortisol production by head kidney preparations. To investigate possible sources of 5-HT that could act in a paracrine fashion, immunohistochemistry experiments using antibodies against 5-HT and tyrosine hydroxylase (TH) (an enzyme used to identify chromaffin cells) were conducted on head kidney sections. Serotonin was detected in chromaffin cells of the head kidney tissue, but also in a separate, as yet unidentified, population of cells. In conclusion, our data support the possibility that 5-HT could act in a paracrine fashion at the head kidney level to regulate cortisol production, probably via the 5-HT_4 receptor.

Small details, big consequences: microRNAs, hormonal signaling and insect metamorphosis
[Presidential symposium]

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Studies carried out in our laboratory had shown that RNAi depletion of Dicer-1, the enzyme that catalyzes the final step of miRNA biosynthesis, prevents metamorphosis in the German cockroach *Blattella germanica*. This species follows a hemimetabolan mode of metamorphosis, where the juvenile stages have the adult body plan, thus making the transition from nymph to adult gradual. The most dramatic changes (especially wing and genitalia complete development) occur in the transition from the last nymphal instar to the adult. In insects, the regulation of

metamorphosis is basically ensured by two hormones, the molting hormone (generally 20-hydroxyecdysone), that triggers non-metamorphic and metamorphic molts, and the juvenile hormone (JH) that represses the metamorphic character of the molts. JH bound to its receptor induces the expression of Krüppel homolog 1 (Kr-h1), a master repressor of adult morphogenesis. Kr-h1 expression decreases in the last instar nymph, which is crucial for metamorphosis progression. The decrease is abrupt, and this abruptness is hardly explained only by mechanisms controlling Kr-h1 transcription, and might be driven by miRNAs. Interestingly, RNAi of Dicer-1, in addition to inhibit metamorphosis, results in abnormally high levels of Kr-h1 mRNA in the last instar nymph, whereas RNAi of Kr-h1 carried out in Dicer-1-depleted specimens rescues normal metamorphosis. Moreover, the 3'UTR of Kr-h1 mRNA contains functional binding sites for miR-2 miRNAs, and that depletion of miR-2 impairs the decrease of Kr-h1 expression that normally occurs in the last nymphal instar. Finally, administration of miR-2 mimic rescues normal metamorphosis in Dicer-1-depleted insects. Taken together, the data suggests that miR-2 plays the key role of radically removing Kr-h1 mRNA in the pre-metamorphic stage, as a prerequisite for the activation of the genes repressed by Kr-h1 that are the ultimate inducers of metamorphosis. The elegant and new concept emerging is that a single miRNA family leads insect metamorphosis to its correct conclusion, a concept that can be extended at least to all hemimetabolan species.

Allatostatin-like neuropeptides in *C. elegans* regulate locomotion and reproduction
[P26]

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Allatostatins are neuropeptides identified as having roles in inhibiting juvenile hormone biosynthesis and muscle contraction in insects. There are three different neuropeptide structures that function as allatostatins in differing insects. In cockroaches, allatostatins have a conserved sequence at the carboxy-terminus, Tyr (Xaa) Phe Gly Leu –amide. In *C. elegans*, two genes (nlp-5 and nlp-6) specifying neuropeptides have been identified that would express peptides with sequence similarity to cockroach-like allatostatins. *C. elegans* normally moves on food with a bias for movement in the forward direction with periodic reversals, however we have found that a nlp-5 null mutant results in locomotion abnormalities. A decrease in roaming behaviour is demonstrated in the presence of food, yet, the animal appears to have normal locomotory behaviour in the absence of food. There is currently no mutant available for nlp-6, however, when nerve cells expressing nlp-6 were inhibited by targeted expression of halo-rhodopsin, locomotory behaviour was also altered. The mutant nlp-5 exhibits egg laying defects which can be rescued by creating a transgenic animal expressing nlp-5 as an extrachromosomal array. Egg laying defects were also apparent when animals were grown on E. coli expressing nlp-5 and nlp6 RNAi . This work supports the notion that inhibition of muscle contraction may have been the ancestral function of the allatostatins.
Supported by an NSERC Discovery grant.

Gonadotropin-inhibitory hormone, stress and reproduction in birds

[S7, invited]

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Gonadotropin−inhibitory hormone (GnIH) acts to inhibit reproduction at all levels of the hypothalamo−pituitary−gonad (HPG) axis. GnIH expression and/or immunoreactivity in the hypothalamus and gonads change with season and respond to stress in birds and mammals in vitro and in vivo. Thus, GnIH seems to be involved in stress-induced reproductive inhibition. Here, I discuss regulation of GnIH in the avian brain and gonads in response to different stressors and compare our findings to those in mammals. Our data suggest that although GnIH responsiveness to stress appears to be conserved across species, the response of specific tissues and the direction of GnIH regulation can vary according to species, stressor, time of year and ecological context.

An Integrated, landscape-based approach to link land use, endocrine disrupting chemicals and biological effects
[P39]

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The elucidation of cause and effect relationships between putative endocrine disrupting chemicals and adverse outcomes in wildlife is confounded by the complexity of mixtures and temporal variation present in ecosystems. We have developed a landscape-based model in an attempt to link complex chemical mixtures to adverse outcomes across multiple levels of biological organization. We hypothesize that landuse patterning in a watershed will correlate with the chemical profile of the water, which in turn will correlate with biological effects at the molecular, cellular and organismal level. The Potomac River watershed represents an ideal microcosm for testing this hypothesis. Beginning in 2002, widespread fish kills have occurred in the Potomac and its tributaries, including the Shenandoah River. The cause of these fish kill has yet to be established, although high rates of intersex fish in the area have lead to a focus on endocrine disruption as a contributor to mortality. In August 2014, we deployed in-situ, flow-through aquaria at four locations in the Shenandoah Valley with distinct landuse patterning, including an agriculturally dominated site, a WWTP effluent site, a downstream mixed-use site, and a pristine reference site. Fathead minnows (Pimephales promelas) were exposed to native water sources and sampled at 7 and 21 days of exposure. Water was sampled every seven days for chemical profiling. Gonads were prepared for histological examination and analyzed for cellular and tissue abnormalities. In males, serum concentrations of vitellogenin were measured by ELISA as a biomarker of exposure to estrogenic substances. To link these traditional biomarkers to molecular changes, RNA sequencing will be used to profile transcriptomic changes and identify differentially expressed genes [DEGs] following chemical exposure. Gene set enrichment analysis and sub-network enrichment analysis will identify biological processes

and molecular pathways that are statistically over represented among DEGs. To link the transcriptomic profile back to landscape patterning, a principle components analysis will be preformed on the gene expression data. A significant clustering of samples by site will indicate that transcriptomic changes do indeed vary based on watershed. Together, these data will provide novel insight into the relationship between landuse, chemical contamination and biological effect.

The effects of glucose concentration on muscle cell proliferation and regulation
[S1, invited]

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As a group, teleost fish exhibit glucose intolerance, which leads to persistent hyperglycemia and coincides with transient hyperinsulinemia. Insulin-deficiency cannot explain this phenomenon as teleosts normally exhibit high plasma insulin levels. It is most likely due to evolutionary diet adaptation or the peripheral utilization of glucose (Moon, 2000). We recenlty demonatrated that inbred strains of zebrafish exhibit varying degrees of metbaolic and endocrine responses to fasting, where a few strains appeared to be highly tolerant to extended periods of fasting while others exhibited decreased blood glucose levels and changes in muscle myostatin expression. Due to these unique responses, and the persistant hyperglycemia observed, we are interested in how glucose levels affect local tissue responses. A primary myoblast culture, that recapitulates myogenesis *in vitro*, is regularly utilized to study the endocrinology of muscel growth and atrophy. Interestingly, this culture has historically utilized a classical 'mammalian-based' media to culture the myogenic precursor cells. Ih this study, myogenic precursor cells (MPCs) from teleost species were cultured in media with high glucose (similar to that used for the mammalian cell line C2C12) or low glucose to assess the effects of glucose concentration on cell proliferation and regulation. Samples were collected over an 8-day period; D2: myoblasts, D4: differentiating myoblasts, D8: myotubes. Glucose concentration did not affect cell proliferation, as demonstrated by PCNA expression, but myogenin expression was increased by low glucose media suggesting an increase in cellular diffferentiation. Glucose concentrations did alter myostatin levels suggesting that glucose concentration likely regulates myogenic progression.

Specific DNA methylation and mRNA expression patterns of *srd5α1*, *srd5α2*, *srd5α3*, and *srd5β* during early development in the frog *Silurana tropicalis*
[P91]

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Steroids such as androgens are essential in biological functions, including development and reproduction. In most vertebrates, the main circulating androgen testosterone is converted into the more potent androgen 5 alpha-dihydrotestosterone or 5 beta-dihydrotestosterone by the steroidogenic enzymes steroid 5-alpha reductase (srd5α) and steroid 5-beta reductase (srd5β), respectively. Three isoforms of srd5α (srd5α1, srd5α2, and srd5α3) are known to be of crucial importance during gonadal differentiation, whereas the only known isoform of srd5β is a critical enzyme involved in bile acid and neurosteroid synthesis, steroid hormone clearance, erythropoiesis, and vasorelaxation. Srd5α and srd5β enzymes have been shown to play a significant role in human diseases, including prostate cancer and hepatic disorders making these enzymes important in human physiology and pathology. 5-reductases are known to be present in lower vertebrates, but less is known about their exact functions, in particular during early development. Thus, as a first step, we are investigating the tissue distribution of 5-reductases during early development in the frog *Silurana tropicalis* using whole-mount *in situ* hybridization to localize the mRNAs. All enzymes were present during every studied stage. A distinct expression pattern was found at gastrulation, organogenesis, tailbud, and early tadpole stage. During early tailbud stage, high expression of *srd5α1*, *srd5α3* and *srd5β* was present in the brain, otic vesicle, and in the cardiac, respiratory, and detoxifying systems. In contrast, s*rd5α2* is expressed at a lower level in the brain, otic vesicle, and in the respiration system. In addition to localizing the gene transcripts, this study also assesses the DNA methylation pattern in specific promoter regions of the 5-reductases to determine if there is epigenetic regulation of these enzymes during early development in *S. tropicalis*. The expression patterns of 5-reductases, together with specific DNA methylation will bring valuable insight to further our understanding of their biological functions outside of sexual differentiation in amphibians.

Corticosterone and the regulation of parental care behaviour in captive birds
[P56]

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Many animals provide parental care to their offspring. However, even when young depend on parental provisioning and brooding, individual parents vary in behaviour. This variation may affect lifetime fitness by influencing the success of individual breeding attempts and how resources are allocated between breeding seasons. However, surprisingly little is known about how individual variation in parental care is controlled in birds. Hormones, and particularly the stress hormone corticosterone (CORT), are good candidates for this control. Avian studies suggest that at baseline (non-stressed) levels, CORT helps breeding individuals meet predictable energetic demands by stimulating appetite and mobilizing energy stores. After exposure to an acute stressor, plasma CORT increases rapidly and at stress-induced (SI) levels CORT may increase self-maintenance but suspend parental care. We investigated variation in female nestling provisioning and brooding behaviour in relation to baseline and SI (response to capture and restraint for 30 min) plasma CORT in captive Zebra Finches, *Taeniopygia guttata*. We recorded parental behaviour and on subsequent days, collected blood samples to measure baseline and SI plasma CORT. Baseline plasma CORT did not correlate with chick provisioning, brooding, or number. However, the frequency of female provisioning behaviour, hence referred to as

regurgitations, was positively correlated with the increase in plasma CORT during stress ($p=0.0136$). This correlation may reflect the fact that maintaining a high level of regurgitation results in enhanced sensitivity to stressors. Alternatively, a high SI CORT response may result in enhanced ability to escape stressors and to continue breeding during stressful events. In either case, no negative relationship between parental behaviour and baseline or SI plasma CORT was observed. Male brooding behaviour was positively correlated with the frequency of female regurgitations ($p=0.0201$.) Female brooding did not correlate with male brooding, nor did female regurgitation correlate with male regurgitation. This observation suggests the degree of male care may influence how the female Zebra Finch allocates her time. Taken together, the data suggest that high baseline plasma CORT may not be necessary to maintain routine parental care and that stress-associated plasma CORT may not be as detrimental as usually thought to be the case.

Phylogenetic-based analyses of endocrine pathway evolution: inferences and challenges
[S14, contributed]

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Molecular endocrine mechanisms that regulate phenotypes have been primarily detailed in laboratory model organisms. Comparatively few studies have analyzed endocrine pathways across more closely related species to understand how deviations have contributed to phenotypic diversification. Analyzing the evolution of mechanisms that underlie phenotypic diversity presents special challenges, such as the comparability of data and collection methods across species. At the same time, some evolutionary questions require phylogenetic comparative methods and an even greater sampling of taxa. Integrating these components (pathway variation and phylogeny) will ultimately lead to a more profound understanding of how the evolution of endocrine pathways has resulted in phenotypic diversity. It will also allow for the incorporation of other associated variables such as ecological parameters. The timing of metamorphosis and maturation vary extensively across salamanders (including direct developing, biphasic, and paedomorphic species) making them an ideal system for analyzing the evolution of endocrine mechanisms in a phylogenetic context. In this presentation I will use the potential endocrine variation that underlies the developmental diversity in salamanders as a framework to discuss the inferences and challenges of phylogenetic-based analyses of endocrine pathway evolution.

Maternal care programs estrous cycle-mediated affective behaviours in the female rat
[S7, contributed]

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In women, both depression and anxiety are correlated with menstrual cycle stage. Similarly, in the rodent, levels of locomotive, anxious, and depressive-like behaviours vary across the estrous cycle. In both species, a drop in plasma progesterone precedes the onset of behavioural changes. Progesterone's metabolite allopregnanolone may contribute to these behaviours, through

modulation of extrasynaptic $GABA_A$ receptors. Licking/grooming (LG) received by rat dams during the first week of life differentially programs the female hypothalamo-gonadal-pituitary axis, affecting progesterone levels. Low LG offspring show a greater difference in progesterone between proestrus and metestrus than High LG offspring. Given that low parental care is predictive of offspring affective disorders, we hypothesized that Low LG offspring would show increased anxiety, locomotion, and depressive-like behaviour, and exhibit greater variability in behaviours between estrous cycle stages relative to High LG offspring. Adult Low and High LG offspring were tested at proestrus and metestrus on the forced swim test and locomotor activity chamber. Animals were also tested on the elevated plus maze following finasteride treatment, which blocks conversion to allopregnanolone, or placebo. Brains were collected and analyzed for $GABA_A$ subunit expression, and plasma allopregnanolone levels were assayed. LG phenotype shaped cycle-related patterns in locomotor activity. Only Low LG offspring showed cycle-dependent depressive-like behaviour. Surprisingly, while High LG offspring showed decreased anxiety at metestrus, Low LG animals increased anxious behaviour at proestrus. Finasteride decreased general activity and removed all behavioural differences. Additionally, we report that Low LG animals show abnormal correlations between plasma allopregnanolone levels and $GABA_A$ receptor delta subunit expression, specifically in the limbic prefrontal cortex and dorsal hippocampus, regions associated with emotionality. Findings from this research link early life experience to estrous cycle-dependent changes in affect, and demonstrate that variations in maternal care mediate allopregnanolone-modulated behaviours, potentially through $GABA_A$ receptors.

Nicotine replacement therapy – what's the harm?
[P80]

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Cigarette smoking is a prototypal addictive disorder characterized by tolerance, withdrawal and use despite high personal cost. A cost that is especially high among pregnant women who continue to smoke regardless of the risks to their unborn child. As a result, public health organizations worldwide have been urging nicotine replacement therapy (NRT) be made available to pregnant women as a means to wean them off tobacco.

Nevertheless, nicotine remains a neuroteratogen that elicits cell damage, subsequently disrupting proper brain development. In studies of neurodegeneration and acute chemical toxicity, estradiol has been shown to elicit a neuroprotective effect through estrogen receptor (ER) activation. While estrogen involvement in neuroprotection is widely accepted, the specific underlying molecular and cellular mechanisms remain unclear. Accumulating evidence suggests that estrogen-mediated neuroprotection might be activated through glial cell interaction, mitigating CNS inflammation and protecting neurons critical for learning and memory. Though the overall

nicotine dose might be downgraded when compared to smoking, the question remains, does NRT alter the expression of these cellular targets of neuroprotection, potentially putting individuals at risk for future cognitive and behavioural impairments?

Randomly assigned nulliparous female Wistar rats were injected subcutaneously with 1 mg/kg/day of nicotine bitartrate or saline for 2 weeks before mating until weaning (PND 21). Pups (saline n=6 and nicotine, n=6) were sacrificed at 26 weeks of age and the hippocampal formation (an area synonymous with cognitive performance) was processed for immunohistochemical staining of GFAP (glial fibrillary acidic protein), Nissl (used for neuronal marking) and ERα (estrogen receptor α).

Results show that gestational exposure to low-level nicotine replacement increases the expression of GFAP and ERα in several areas of the hippocampal formation (including the CA1 and entorhinal cortex). While additional research is needed, these findings put forward the idea that NRT might indeed interfere with proper brain development, contributing to long-term adverse health effects in the offspring.

Identifying brain and gonadal gene expression patterns during natural and induced sex change in black sea bass (*Centropristis striata*)
[P57]

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Teleost fish exhibit diverse reproductive strategies, and many species are hermaphroditic. The mechanisms of sex change in protogynous species (those that undergo female-to-male change) has primarily been studied in haremic, coral reef fishes, such as gobies and wrasses. Comparatively less research, however, has focused on commercially important species with non-haremic social structures. Black sea bass (*Centropristis striata*) is a high value, protogynous teleost that supports both commercial and recreational fisheries along the U.S. Atlantic coast, and considerable research has focused on its development as an aquaculture species. Sex change in black sea bass is often accelerated in captive juveniles and adults, and remains poorly understood in both wild and captive environments. The purpose of the present studies was to investigate brain and gonadal gene expression patterns during protogynous sex change. To this end, two approaches were used: 1) wild-caught juveniles (< 1 year old) were held in captivity and sampled periodically to obtain male, female, and naturally sex changing fish, and 2) older fish (1-2 years of age) were fed an aromatase inhibitor (exemestane, 1mg/g diet) to induce sex change, and sampled after 16 days. Brains and gonadal RNAs isolated from juvenile fish (n = 3/sex) will be sequenced using the Illumina platform followed by RNA-seq to identify differentially expressed genes during sex change. Selected genes found to be differentially regulated will be further assessed using real time quantitative PCR in exemestane-fed fish. Results from these studies will identify novel genes involved in sex change processes, and may further our understanding of sex change regulation in non-haremic, teleost species.

Prolactin and cortisol direct branchial aquaporin 3 expression in euryhaline tilapia
[S13, invited]

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In teleosts, aquaporins (Aqps) are expressed within key osmoregulatory epithelia where they mediate the movements of water and selected solutes across cell membranes. We investigated endocrine control of Aqp3 in the gill of euryhaline Mozambique tilapia (*Oreochromis mossambicus*), with particular attention to prolactin (Prl), growth hormone, and cortisol. Branchial *aqp3* mRNA levels were modulated following salinity challenges, with enhanced *aqp3* expression upon transfer from seawater to fresh water (FW). Accordingly, extensive Aqp3-immunoreactivity was localized to cell membranes of branchial epithelium in FW-acclimated animals. Upon transferring hypophysectomized tilapia to FW, we identified that a pituitary factor(s) is required for the elevation in Aqp3 levels that accompanies FW acclimation. The diminished capacity of hypophysectomized animals to appropriately express Aqp3 in FW was rescued by Prl replacement, an effect blocked by co-injection with cortisol. Prl stimulated *aqp3* expression in cultured gill filaments in a concentration-related manner. Consistent with *in vivo* responses, co-incubation with cortisol blocked Prl-stimulated *aqp3* expression in cultured filaments. Our data indicate that Prl and cortisol act upon branchial epithelium to regulate salinity-dependent Aqp3 expression in tilapia. Collectively, these data provide the first evidence that Prl modulates Aqp expression in a vertebrate osmoregulatory tissue.

Unliganded thyroid hormone receptor regulates developmental timing
[S11, invited]

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Thyroid hormone (TH) receptors (TRs) play dual roles in TH-dependent development in vertebrates. TR acts to repress genes involved in developmental progression in the absence of TH, and in TH circulation, it binds TH, inducing the previously repressed genes to lead to developmental changes. TR expression occurs in the absence of or low circulating TH levels during development in all vertebrates, yet few developmental roles for unliganded TRs have been established. Unliganded TRs are expected to repress TH-response genes, increase tissue responsivity to TH, and regulate the timing of developmental events. Using TALENs (Transcription activator-like effector nucleases) technique, we disrupted TR alpha (TRα) in Xenopus tropicalis to examine the role of unliganded TRα in gene repression and development. By injecting TRα TALEN mRNA into one cell of two-cell stage embryos, half side mutant

founder animals were generated. Offspring with a wild type phenotype had zero or one disrupted TRα alleles, and tadpoles with the mutant hind limb phenotype had two disrupted TRα alleles each causing a frame-shift between the two zinc fingers followed by 40-50 mutant amino acids and then an out-of-frame stop codon. We examined expression levels of TRβ, ST3 and KLF9, and early larval development with and without exogenous TH in F1 offspring. As hypothesized, we found increased expression of three TH-response genes in mutant phenotype tadpoles in the absence of TH. Similar results after the use of methimazole to block endogenous TH synthesis showed that increased TH-response gene expression and precocious development in mutant phenotype tadpoles was not due to early production of TH. Impaired induction of the three genes was observed in mutant phenotype tadpoles after exogenous TH treatment. Morphological analysis revealed reduced hind limb and gill responsivity to exogenous TH in mutant tadpoles when compared with wild type tadpoles at same age and stage. Growth rate profiling between NF stage 46 to 64 also supports our hypotheses that the lack of TRα causes early initiation and progression of metamorphosis showing difference in body size between wild type and mutant phenotype tadpoles. These results indicate that unliganded TRα regulates developmental timing by repressing TH-response genes to allow larval growth until TH is released into circulation.

The optic tectum: An emerging target for CRF action
[S5, invited]

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The role of CRF in the downstream response to stressors, specifically regulation of the HPA axis, is well established. The potential role of this peptide in modulating the various sensory modalities that detect stressors is much less studied. My laboratory has been studying the role of CRF in modulating sensorimotor integration in the optic tectum, a key brain area for detecting visual threats and coordinating appropriate defensive behaviours. Immunohistochemical, molecular, and physiological studies in our laboratory have confirmed that CRF is produced by tectal neurons and can be released in a depolarization and calcium-dependent manner from these neurons. Significantly, these neurons are located in tectal layers 6 and 8 and thus may be involved in gating the flow of retinal information to output cells in deeper tectal layers. CRF action in the tectum takes place via CRF R1 receptors and may involve the regulation of norepinephrine release from neurons arising in the A2 nucleus or locus coeruleus and innervating deep tectal layers. Tectal CRF interneurons are regulated by glutamate, the primary neurotransmitter in retinal afferents innervating the tectum. This regulation is complex and involves both a stimulatory component via NMDA receptors and an inhibitory component through an unidentified non-ionotropic receptor. Tectal CRF interneurons may play a part in modulating how visual features of prey are detected. Food deprivation lowers tectal CRF content and CRF administered icv or peripherally inhibits prey-capture in anurans. Tectal CRF neurons respond to a reactive stressor (ether vapors) that inhibits prey capture in *Xenopus laevis* and *Bufo speciosus*. Collectively our data suggest that tectal CRF neurons may play a physiological role in modulating sensorimotor decision making in the tectum in response to systemic stressors and changes in energy balance.

Assessment of testicular histology in male Gulf killifish from Barataria Bay Louisiana one year after the Deep Water Horizon oil spill
[P72]

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The long-term goal of this research is to assess the impact of contaminants from the Deep Water Horizon oil spill and its mitigation on the health and reproductive status of the Gulf killifish (*Fundulus grandis*). We sampled eleven sites throughout Barataria Bay and areas just west of the Mississippi Delta for *F. grandis* during August of 2011. *F. grandis* was found at two impacted sites in Barataria Bay (Jimmy Bay, JB; Bayou St. Denis, BSD) and a non-impacted site along the southeast Gulf coast in Texas. Here we describe the results obtained for male fish. Although we collected several fish at BSD, we only collected one male and this site was not used in the subsequent histological analysis. There were no differences in bodyweight or Fulton condition factor between males collected at JB and the non-impacted site. Since some of the fish were less than 1 g, we estimated gonadal somatic index from histological slides through the testes in each animal. GSI in fish from the non-impacted site was double that of fish from JB ($p < 0.05$). We also evaluated two aspects of testes condition, the height of the germinal epithelium and spermatic duct area. While there were no differences in spermatic duct area between fish collected at the non-impacted site and JB, germinal epithelium thickness was 2.7 fold lower in males collected at the impacted site. This is the first report of reproductive issues in fish collected from a site that was heavily impacted by the DWH spill and the resulting cleanup efforts. These differences may not be due solely to PAH exposure, as surface water, pore water, and sediment total PAHs were not different between these sites in August 2011. We are currently examining the effects of oil/surfactant mixtures on reproduction in *F. grandis*. Supported by a grant from the Gulf of Mexico Research Initiative (GOMRI).

Effects of silencing and overexpressing GH upon chicken embryonic cerebellar cell viability
[P1]

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In vertebrates, neuroprotection is a fundamental mechanism for survival. In the central nervous system (CNS), neurons must be protected against damage caused by several insults such as hypoxia, ischemia or excitotoxicity. Previous reports have shown that growth hormone (GH) is

involved in cell survival and plays an anti-apoptotic role in several cell types, including neurons. We have previously reported that GH is locally expressed in chicken cerebellar cells, and its concentration increases when submitted to hypoxia either *in vivo* or *in vitro*. Furthermore, the addition of exogenous GH increases cell viability and decreases apoptosis of cerebellar cells in cultures treated with hypoxia (0.5% O_2) and low glucose (1g/L) conditions (HLG). To determine the role of local GH in neuroprotection, in this study we initially analyzed the effects of silencing and overexpressing local GH upon viability of cerebellar cells *in vitro*. A specific siRNA (siR-GH) and two plasmidic vectors: pMP-GH (containing only the sequence of the GH mature protein), and pSP-GH (containing the GH sequence including the signal peptide) were designed in order to silence and overexpress, respectively, the GH mRNA expression. The effectiveness of the siR-GH was assessed in the GH-producing cellular line GH3 where the GH concentration decreased from 5.6±1.6 to 2.5±0.4 ng/µg protein after transfection, while the GH mRNA expression declined 60%. In primary cultures of chick embryonic (ED15) cerebellar neurons, transfection with siR-GH produced a decrease of GH concentration from 53.09 ±14.3 to 23.7±15.6 ng/µg of protein, and cell viability declined 75% when analyzed by the MTT assay. On the other hand, transfection with overexpressing plasmids (pMP-GH and pSP-GH) produced an increase of cellular GH concentration from 35.9±18.4 to 60.51±16.51 ng/µg of protein with pMP-GH, and to 79.56±6.6 ng/µg protein with pSP-GH, respectively. Additionally, GH concentration increased in the culture media from 192.5 to 317.1 ng/ml when cells were transfected with pSP-GH. Furthermore, cell viability augmented 16% in cells transfected with pMP-GH, and 51% in those transfected with pSP-GH. These data support the notion that locally expressed GH may play a paracrine and/or autocrine effect on neuroprotection in the chicken embryonic cerebellar cells.
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The role of triiodothyronine in sex reversal and delay of hatching in medaka embryos [P41]

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Cumulative evidences show that thyroid hormones (THs) play an important role in many development process in mammalian and non-mammals species. Recent results suggest a more direct crossover between THs and androgen pathways. Initially we studied the crossover between THs and androgens with an in silico promoter analysis of several genes involved in the synthesis of THs and androgens in medaka (Oryzias latipes). We found thyroid response elements on androgen related genes; such as 5-alpha-reductases 1 (two TREs) and 11-beta hydroxysteroid dehydrogenase 2 (five TREs) genes, involved in the synthesis of 5-alpha-dihydrotestosterone and 11-Ketotestosterone, respectively. Then, to determine the biological effect of THs in the process of gonadal development have been reared medaka eggs from fertilization to hatching at different concentrations of triiodothyronine (T3; 0.05, 0.5 and 5 nM). The control without addition of T3 presented regular sex reversal of genetically males (XY) to phenotypically female (around 10%), observed by the development of ovary. The chronic exposed at low concentration of T3 increased the masculinization (development of testis) in genetically females (XX). This sex

reversal was observed at all T3 treatment, with the concomitant reduction of female percentages. On the other hand, recently was observed in fish that Type 3 Iodothyronine Deiodinase (dio3) presents an important role in the regulation of hatching. The dio3 is the primary inactivating deiodinase, which terminate the TH action. When larvae of medaka were exposed to T3 the highest dose presented a severely delay of hatching of 4 days. These observations support the involvement of THs in different development processes of fish, as gonadal fate, and regulation of hatching during the transition of embryo to larvae stage.

Can dietary 18β-glycyrrhetinic acid supplementation ameliorate the effects of an abrupt ionoregulatory challenge in a freshwater teleost fish?
[P106]

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The liquorice plant (*Glycyrrhiza sp.*) is a perennial legume and botanical that has long history of use for its medicinal benefits. As a consequence, liquorice root (LR) and liquorice root derivatives (LRDs) have received attention with respect to their anti-viral, anti-fungal, and anti-inflammatory properties as well as their interaction with the vertebrate endocrine system in a manner that is thought to curb the deleterious effect of stressors. LRDs include the saponin-glycoside glycyrrhizic acid, which in mammals is hydrolyzed into18β-glycyrrhetinic acid (18β-GA) by intestinal bacteria. 18β-GA exhibits an affinity for mammalian glucocorticoid and mineralocorticoid receptors which can be attributed to a triterpenoid steroid structure that is biochemically similar to cortisone. Therefore, despite medicinal benefits at appropriate doses, the harmful consequences of excess LR consumption can manifest as pseudohyperaldosteronism that can cause hypertension as well as salt and water imbalance in mammals. In this study it was hypothesized that the LRD 18β-GA will (at an appropriate dosage) ameliorate the effects of an abrupt ionoregulatory challenge in an aquatic vertebrate, the freshwater (FW) rainbow trout (*Oncorhynchus mykiss*). This hypothesis is built on recent observations that despite the functional absence of aldosterone in teleost fishes, dietary 18β-GA alters the abundance and activity of select ionoregulatory transport proteins in gill tissue of rainbow trout in a manner that could be considered beneficial if the fish were exposed to ionoregulatory perturbation. Yet despite this, 18β-GA did not alter systemic endpoints of salt and water balance. To test the hypothesis, rainbow trout held in FW were fed either a control diet or diets supplemented with 18β-GA (0, 5 and 50 μg 18β-GA/g diet) for two weeks and then abruptly exposed to ion-poor water (IPW). Alterations in circulating ion levels indicate that fish fed a low dose of 18β-GA cope with an abrupt exposure to IPW better than control and higher dose treated fish and data suggest that this may be linked, in part, to gill tissue responsiveness to cortisol. Therefore, it would seem that LRDs have the potential to act through the endocrine system to ameliorate ionoregulatory disturbance in fishes and possibly the deleterious effects of other stressors.

Vascular and neuroprotective actions of vasoinhibins and prolactin in the retina
[S9, invited]

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Prolactin (PRL) acquires inhibitory effects on blood vessels after undergoing specific proteolytic cleavage. The resulting PRL fragments (vasoinhibins) act directly on endothelial cells to inhibit vasopermeability, vasodilation, angiogenesis, and vascular survival, and inactivation of endothelial nitric oxide synthase is one of several mechanisms mediating their actions. Ocular vasoinhibins, derived from PRL expressed in the retina or from systemic PRL, exert an essential suppression of blood vessel growth, dilation, and remodeling under normal conditions. In diabetic retinopathy, disruption of the quiescent state of blood vessels, altered glial function, and neuronal cell death lead to visual impairment. Increasing circulating levels of PRL in diabetes can protect the eye against the deleterious effects of diabetic retinopathy. Hyperprolactinemia, the intravitreal delivery of vasoinhibins or of adeno-associated virus vectors encoding vasoinhibins lead to vasoinhibin accumulation in the retina, which reduces the diabetes-induced increase in blood retinal barrier permeability by targeting both its main inner (vascular) and outer (retinal pigment epithelium) components through nitric oxide and reactive oxygen species pathways. Moreover, by activating retinal PRL receptors, hyperprolactinemia can protect against photoreceptor apoptosis, gliosis, and neurotrophin down-regulation that lead to altered retinal function. The PRL/vasoinhibin system operates to maintain retinal physiology and offers potential treatment strategies against diabetic retinopathy and other vasoproliferative retinopathies, where both vascular- and neuronal-protective activities are required. Supported by CONACYT grant SALUD-2011-1-1611594.

Optimisation of ovulation and oviposition in the barred frog (*Mixophyes fasciolatus*) by induction with human chorionic (hCG) gonadotropin with pregnant mare serum gonadotropin (PMSG) priming
[S8, contributed]

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The collapse of amphibian biodiversity across the world in recent decades, due largely to the impacts of the global pandemic of chytridiomycosis, has focussed renewed interest on methods of assisted reproduction, including protocols for the induction of ovulation and oviposition. *Xenopus laevis* and *Silurana tropicalis* have become key model species in developmental biology because of their ready ovulation following induction with human chorionic gonadotropin (hCG) with or without priming with pregnant mare serum gonadotropin (PMSG). Relatively few other amphibian species respond well to mammalian gonadotropins such as PMSG and hCG, and for a number of species, other protocols based on GNRH and dopamine have been

developed. We tested the efficacy of PMSG priming and hCG induction in a species (*Mixophyes fasciolatus*) of the barred river frogs of eastern Australia, a genus in which 40% of species are threatened, and which might be a target for assisted reproduction. Administration by injection of hCG alone (900 to 1400 IU) induced oviposition in around 30% of females (10/27 injected females), compared to 0% in saline injected females (0/6). However, priming with two doses of PMSG (50IU, 25 IU) significantly increased the rate of oviposition ($p = 0.035$) on average to around 50% (31/62 females) and up to 67% (6/9 females) in the optimal protocol. The effect of PMSG priming increased as the interval between the first priming dose of PMSG and the first hCG injection increased from 3 to 6 days. The optimal protocol for *M. fasciolatus* involved two priming doses of PMSG (50IU and 25 IU) administered at 6 and 4 days, respectively, prior to two doses of hCG (100 IU), 24 hours apart. Viability of the induced eggs was confirmed by IVF for some females. Besides providing a viable induction protocol for *M fasciolatus*, this study, to the best of our knowledge, demonstrated for the first time in anuran amphibians that ovulate following hCG administration, PMSG priming significantly increases the rate of ovulation and oviposition.

Maternal stress-induced behavioural reprogramming in offspring
[P50]

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In most oviparous species, the environment experienced by females during the egg maturation period can alter offspring phenotypes. In fish, these maternal effects are believed to be partly mediated by the deposition of the female's proteins, RNAs, and hormones in the eggs. One of these maternal factors is cortisol, a hormone involved in the stress response. In salmonids, female plasmatic cortisol levels are reflected in the eggs. Elevated maternal plasmatic cortisol and high egg cortisol levels have been associated with offspring increased aggressiveness and decreased learning abilities respectively. Thus, maternal stress during egg formation could have adverse effects on the offspring through a rise in egg-deposited cortisol.

To understand how maternal stress shapes the phenotype of the progeny, it is essential to isolate the effects of maternally-conveyed cortisol, the proposed main mediator for stress-induced developmental reprogramming, from those of other maternal factors. We thus characterized the behavioural phenotype of maternal pharmacological and physical stress in brook trout juveniles. During 10 weeks prior to fertilisation, females were either 1) fed cortisol-sprayed food, 2) handled once a week or 3) left undisturbed as control. A fourth treatment consisted of bathing half of the eggs from the control females in a cortisol solution for 3 hours before fertilization. We aimed to stay within physiological levels of cortisol. In normal hatchery settings, juveniles are released into the wild for stocking after 6 months and must survive in a new and potentially harsh environment where food is scarce and predation risk is high. Therefore we measured essential behaviours for survival in this context (learning & memory in a maze, boldness and neophobia) at that age.

Our statistical models revealed that all fish completed the maze significantly faster on the last day of training (day 5) compared with the beginning of trials regardless of treatment, thus demonstrating training effectiveness and suggesting that offspring's learning capacities were not influenced by maternal stress. Memory, boldness and neophobia were also unaffected by treatment. These results suggest that aquaculture strains of brook trout might be stress-resistant enough that higher plasmatic and egg cortisol levels than the ones our treatments gave rise to would be necessary to reprogram the progeny's phenotype or that maternal stress does not affect these behaviours in brook trout.

Secretoneurin as a potential regulator of radial glial cell function in neurosteroidogenesis and neurogenesis
[P58]

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Teleost models offer an excellent opportunity to investigate both neuroestrogen production and adult neurogenesis because of the functions of the radial glial cell (RGC). The teleostean brain can regenerate neurons post-injury through the neurogenic activity of the RGCs as they are neuronal precursors for neurogenesis. Besides their function in neuronal regeneration, RGCs also exclusively express the estrogen synthesis enzyme, aromatase B (*cyp19a1b*). RGCs play a crucial role in neurosteroidogenesis and neurogenesis, however the permissive factors and signaling mechanisms that control these functions have yet to be elucidated. This research proposes that the granin-derived neuropeptide, secretoneurin (SN) can regulate the functions of the RGCs. Using a primary culture of highly purified goldfish RGCs, *in vitro* SNa exposure significantly ($p<0.05$) increased the relative aromatase B mRNA abundance in a dose-dependent relationship. To study this effect of SN *in vivo,* administration of SN through intracerebroventricular injection produced a significant ($p<0.05$) down-regulation of aromatase B mRNA levels in the telencephalon and hypothalamus five hours post-injection. Although inconclusive, these pilot studies suggest SN can regulate neuroestrogen production in RGCs. Moreover, *in vitro* SNa dose-dependently ($p<0.05$) downregulates transcript levels of brain- and glial- derived neurotrophic factors in RGC cultures. These findings indicate that SN may regulate RGC functions, which may have implications for neuroendocrine regulation of neurosteroidogenesis and neurogenesis in the adult brain.

Defining an ancient neuromuscular-endocrine interaction in the Metazoa: TCAP and its role in glucose metabolism in skeletal muscle
[P3]

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Encoded in the terminal exon of the four vertebrate teneurin proteins is an independent bioactive peptide termed teneurin C-terminal associated peptide (TCAP-1-4). Teneurins and TCAP are found in most metazoans studied to date. In vertebrates, TCAP-1 is involved in signaling pathways associated with metabolism, stress, and neuroprotection. Recent studies indicate that TCAP-1 enhances neuronal metabolism via upregulation of glucose transporters leading to significant glucose uptake in the brain, associated with a concomitant decrease in blood glucose. TCAP-1 is now known to bind and activate latrophilin (LPHN), a GPCR that binds teneurin with high affinity. The teneurin-latrophilin adhesion pair is the only trans-synaptic protein unit that has been conserved between invertebrates and vertebrates with respect to neuronal signaling. Both TCAP and latrophilins are expressed in rodent skeletal muscle. TCAP induces glucose transport into skeletal muscles yet inhibits the PKC-DAG-IP3 response. These studies indicate a novel interaction of the regulation of the central nervous system and skeletal muscle control.

The antagonistic roles of teneurin C-terminal associated peptide (TCAP) and corticotropin-releasing factor (CRF) in stress-related behaviours in the vase tunicate, *Ciona intestinalis* [P74]

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Teneurin C-terminal associated peptide (TCAP) is a neuropeptide expressed from the terminal exon of teneurins. TCAP and corticotropin-releasing factor (CRF) are both highly implicated in the regulation of stress-related behaviours, as established in rodent models. It is proposed that TCAP and CRF have antagonizing roles, as results suggest them to be anxiolytic and anxiogenic, respectively. In this study, we studied the roles of TCAP and CRF in the vase tunicate, *Ciona intestinalis.* As a urochordate, this species contains only one isoform of TCAP and CRF, thereby establishing *Ciona* as an excellent model organism. To analyze stress-related behaviours, different types of contractions were observed using Behaviour Stimulation Tests; contractions include buccal opening contractions, cloacal opening contractions, lateral contractions, longitudinal contractions and expulsions. Preliminary results show that TCAP significantly increases duration of lateral contractions and total number of contractions, while CRF increases number of buccal expulsions. This suggests that TCAP increases feeding response, which is associated with low-anxiety behaviour, whereas CRF induces excretion of food, which is associated with high-anxiety behaviour. Therefore, this provides strong evidence of the antagonistic roles of TCAP and CRF in urochordates, which corroborates previous results from vertebrates. Currently, *Ciona* is posing a major threat to aquaculture along the east coast of North America, and thus this research may have practical applications to control the spread of this organism.

Analyzing the activation of the melanocortin-2 receptor (MC2R): projecting a role for Transmembrane Domain 4 (TM4) and Extracellular Loop 2 (EC2) in the activation of *Xenopus tropicalis* MC2R

[S5, invited]

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There are two amino motifs in ACTH(1-39) that are required for the activation of teleost and tetrapod melanocortin-2 receptor (MC2R) orthologs: $H^{6}F^{7}R^{8}W^{9}$ and $K^{15}K^{16}R^{17}R^{18}P^{19}$. This presentation will review hypotheses to explain the sequence of events that appear to be involved in the multiple step activation of teleost and tetrapod MC2R orthologs by ACTH(1-24). In this regard, site directed mutagenesis experiments performed on the MC2R ortholog of the amphibian *Xenopus tropicalis* that indicate a role for amino acid residues in Transmembrane Domain 4 (TM4) and Extracellular Loop 2 (EC2) in the activation process will be presented. These results will be compared to parallel studies on human MC2R and rainbow trout MC2R to provide an overview of the proposed phylogeny of MC2R activation in teleosts and tetrapods.

Physiological and behavioural correlates of reproductive "decisions" in female red-sided garter snakes (*Thamnophis sirtalis parietalis*)
[P59]

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For animals with limited opportunities for reproduction and feeding, the high energy demands placed on females during reproduction means that they are likely unable to recover sufficient energy stores to be able to reproduce in consecutive years. Body condition has been used as a proxy for recent reproductive history in such species. We recently found that both hormonal responses to capture stress and receptivity to mating vary with body condition in female red-sided garter snakes, a species with limited breeding opportunities. Our results suggest that reproductive history influences both stress responsiveness and mating behaviour. However, no study has directly examined if body condition accurately reflects reproductive history. We posit that females that did not give birth during the previous summer will have a higher body condition, reduced stress responsiveness, and be more likely to mate and give birth the following year. We tested this hypothesis by comparing the influence of body condition versus two measures of reproduction (receptivity and reproductive outcome) on hormonal stress responses. We collected unmated females from a den site in Manitoba, Canada during the spring mating season, subjected them to 4 h of capture stress, and collected blood samples before (0 h), during (1 and 2 h), and after (4 h) stress treatment. After treatment, we placed each female in an arena with 15 courting males and recorded latency to copulate for up to 60 min. Females that did not mate within 60 min were given additional opportunities to mate on successive days with newly-collected males. We assigned positive or negative body condition to each female based on its residual from a regression of body mass on snout-vent length for all snakes. We found that stress responses were smaller in females that mated compared to those that did not mate, but they did not vary with body condition. These data suggest that stress responses are influenced less by body condition than by reproductive activity. In the lab, we recorded the number of offspring produced by each mated female during the summer. The stress responses of mated females

varied with whether females gave birth, but not with body condition. Body condition was, however, related to the number of offspring produced. Together, these data suggest that variation in the sensitivity of the hypothalamus-pituitary-adrenal axis is related to reproductive history and may contribute to reproductive "decision-making".

The presence and the functions of insulin-like peptides in the Chagas' disease vector *Rhodnius prolixus*
[S2, contributed]

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Insect insulin-like peptides (ILPs) are functional analogs of insulin and were first discovered in the silk moth *Bombyx mori* more than 30 years ago. Since then, ILPs have been identified in many other insect species, such as *Drosophila melanogaster*, the honeybee *Apis mellifera* and the yellow fever mosquito *Aedes aegypti*. Studies have shown that insect ILPs are not just involved in metabolism, but also in growth, development, reproduction, diapause, neuronal function and behaviour. The cell signaling components of the insulin pathways are well conserved throughout evolution; however the number of different peptides and receptors differ between species. In *Drosophila,* as well as in *A. aegypti*, there are eight known ILPs, while in *A. mellifera* there are only two peptides. On the other hand, *Drosophila* has only one insulin receptor (InR) while *A. mellifera* has two known InRs. Studies have shown the presence of insulin-like immuno-reactivity in identified neurosecretory cells in the brain of the Chagas' disease vector *Rhodnius prolixus*, and ILPs are intimately associated with the circadian rhythm and the release of ecdysteroids in this insect. *R. prolixus* is a hematophagous hemipteran that undergoes long periods of starvation between each blood meal, which can account for up to 10 times the insect's initial body weight. Many physiological events in *R. prolixus* are dependent on the blood meal or triggered by the feeding process, such as egg-laying in adult females. Considering that ILPs are known to be involved in a wide range of signaling pathways, our main objective is to investigate the role of these peptides in *R. prolixus* development and reproduction. We have identified two different ILP genes in the *R. prolixus* genome and partially cloned their cDNA sequences. *In silico* analyses of the putative peptides indicate that RpILP1 structure is very similar to other insulins, including chains A and B, and having a C domain. The putative RpILP2 seems to lack the C domain. The analyses also suggest that both prepropeptides have signal peptides and conserved cleavage sites for the assembly of the mature hormones. Quantitative PCR has been utilized to determine the expression levels and the spatial distribution of both RpILPs. Further experiments into the physiological roles and localization of the functional peptides are being conducted.

Corticotrophin-releasing hormone (CRH) regulates depressive-like behaviours and BDNF expression in the mesocorticolimbic system following global cerebral ischemia
[S5, contributed]

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The brain's reward circuitry plays an important role in mediating mood disorders, including depression. Brain-derived neurotrophic factor (BDNF) is elevated within this system following chronic exposure to stress, a phenomenon shown related to corticotropin-releasing hormone (CRH) activition. The current study investigated the effects of CRHR1 blockade on depressive-like behaviours and BDNF and TrkB expression in mesocorticolimbic structures. Adult male Wistar rats (N =70) were exposed to 10 minute global cerebral ischemia or sham operation, which was induced by four vessel occlusion (4VO). Antalarmin (2µg/µl) or a saline vehicle was injected intracerebroventricularly 30 min before 4VO. Sucrose preference, forced swim, and social interaction tests were performed to measure depressive-like behaviours. Thirty days post ischemia, levels of BDNF and TrkB receptors were assessed in the prefrontal cortex (PFC), ventral tegmental area (VTA), and nucleus accumbens (NAc) using immunohistochemistry. Western blots and RT-PCR complemented assessment of BDNF activity in the PFC and NAc. Our findings indicate beneficial effects of CRHR1 blockade on emotional and social behaviour in ischemic rats. At the biochemical level, Antalarmin treatment regulated altered expression of BDNF in the PFC, NAc and VTA post ischemia. Together, the observations demonstrate that CRH activation of type 1 receptor subtypes has effects on plasticity markers in mesocorticolimbic structures that may be involved in the development of depressive-like behaviour following global cerebral ischemia.

Integrative molecular responses of estrogens in the teleost brain
[S4, invited]

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Female and male sex steroid hormones have roles in feminizing or masculinizing brains and do so by modulating gene expression patterns involved in proliferation of neuronal cells, synaptic plasticity, among others. Distributions of soluble sex steroid hormone receptors and membrane receptors have been described in the brain of fish with each having specific functions in different parts of the brain. We investigated gene expression responses in the brain of male fathead minnows is response to two concentrations of 17 alpha ethinylestradiol (EE2) (5 ng/L or 50 ng/L aqueous exposures), the "pure" antiestrogen, ZM189,154 (ZM,100 ng/L aqueous exposure), and a mixture of ZM and EE2. Among the biological processes altered by 5 ng/L EE2, we observed changes in insulin-like growth factor receptor signaling, glucose homeostasis, apoptotic mitochondrial regulation, and innate immune response, among others. When added together with EE2, ZM reversed changes of expression for some of the transcripts but not others, suggesting that both genomic and non-genomic mechanisms are involved in estrogen signaling in the brain. Using a proteomics approach, we investigated rapid changes in the phosphorylome

following E2 treatment. Phosphorylation changes were observed in proteins related to neuronal processes, nerve regeneration, synaptic plasticity, neurite outgrowth, and mitochondrial damage, among others. In follow up experiments, we examined the effects of estrogen signaling in the telencephalon, a neuroendocrine tissue of the brain. We observed that 5 ng/L waterborne treatments of EE2 resulted in changes in proteins related to apoptosis, respiratory chain, neuron network morphology, and long term synaptic potentiation. Taken together these studies suggest that estrogen regulates brain plasticity and important processes via both soluble and membrane-bound estrogen receptors.

Glycemia in free-ranging birds: No evidence for regulation by glucocorticoids during acute stress
[P4]

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Acute stress in vertebrates, including birds, elevates the secretion of glucocorticoids such as corticosterone (CORT). This elevation may promote behavioural and physiological changes, such as mobilization of energy reserves, that help organisms cope with stress and avoid its potentially deleterious consequences. Hyperglycemia is often cited as a CORT-mediated physiological effect of acute stress. Few avian studies, however, have investigated whether acute stress actually increases plasma glucose (GLU). In addition, although the magnitude of the CORT elevation during acute stress in free-ranging birds often varies seasonally, no avian study has examined whether the plasma GLU response to acute stress parallels this variation. We measured plasma CORT and GLU in response to standard capture and restraint of free-ranging adult male Rufous-winged Sparrows, Peucaea carpalis, which we sampled at the same location and during four life history stages: early summer pre-breeding, summer breeding, autumnal post-breeding molt, and non-breeding (winter). Neither baseline (pre-stress) CORT, baseline GLU, nor the acute stress-induced increase in plasma CORT changed seasonally. By contrast, during the stress response, plasma GLU increased during pre-breeding (18 + 6 %), did not change during breeding, and decreased during molt (26 + 6 %) and the non-breeding season (13 + 6 %). This seasonal variation may reflect differential adjustments of the plasma GLU response to stress as a function of the life history stage. Alternatively, in the present study, date of capture (hence, also life history stages) was negatively correlated with minimum daily ambient temperature, suggesting that the GLU response to stress may have been temperature-modulated. Additional analyses failed to demonstrate consistent relationships between glycemia and either plasma testosterone, uric acid, body mass or fat reserves. These data show that in some conditions glycemia in free-ranging birds can vary considerably (up to 26 %) within a short period (30 min), and they do not provide evidence that plasma CORT regulates glycemia during acute stress.
Supported by NSF Award IOB-1026620 (PD).

Mineralocorticoid receptor is associated with cellular membranes and may mediate cortisol-stimulated CREB phosphorylation in rainbow trout brain

[S10, contributed]

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In vertebrates, including teleosts, corticosteroids are known to elicit rapid, nongenomic cellular effects, in addition to their classical genomic action via transcription regulation. However, the mechanisms involved are far from clear. Teleosts possess both glucocorticoid (GR) and mineralocorticoid (MR) receptors; however it remains controversial whether the teleost MR have a distinct endogenous agonist, homologous to aldosterone in mammals. To further complicate matters, the physiological role of MR in teleosts is poorly understood, despite the transcript abundance of MR gene throughout the body. We tested the hypothesis that MR is involved in the rapid nongenomic cortisol signaling in rainbow trout. To this end we characterized the membrane and subcellular association of MR in the brain and liver of trout and the rapid effects of this receptor activation on extracellular signal-regulated kinase (ERK) and cAMP response element-binding protein (CREB) phosphorylation. MR was enriched in the liver membrane fraction, and synaptosomal fraction in the midbrain and telencephalon compared to the total tissue homogenate. Membrane localization and cortisol binding of MR was further confirmed by immunoprecipitation and elution using a cortisol affinity column. The rapid phosphorylation of CREB and ERK was measured in response to mineralocorticoid ligands (cortisol, aldosterone and deoxycorticosterone) either alone or in combination with eplerenone (MR antagonist) in the brain and liver. Deoxycorticosterone (DOC) had no significant effect on protein phosphorylation in the brain and liver, while cortisol rapidly elevated phosphorylation of CREB, but not ERK, (what about ERK?) in the midbrain, telencephalon, and liver. This rapid cortisol effect was suppressed in the presence of eplerenone in the brain, but not in the liver. Together, these results support a nongenomic role for cortisol involving MR signaling in the brain.

Habitat-associated behavioural, hormonal and transcriptional evolutionary divergence in sticklebacks
[P112]

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Animals that colonize novel environments often face new ecological challenges, which result in the evolution of striking differences in morphology, physiology and behaviour. However, we understand very little about the mechanisms underlying behaviour divergence, which is central to understanding its evolution. The adrenergic, serotonergic, dopaminergic and glucocorticoid networks (composed of signalling peptides and their receptors) are associated with the hormonal and behavioural response to stress, and also with social behaviours, such as sociability and aggressiveness. After the last glacial retreat in the Northern hemisphere, marine threespine

sticklebacks repeatedly invaded freshwater habitats differing in ecological conditions from the ones faced by the marine ancestor. Our objective was to test if sticklebacks from freshwater and marine populations reared in a common environment diverge in behaviour, stress reactivity and brain molecular network expression. We found that laboratory reared F1 freshwater sticklebacks showed significantly lower sociability, as well as higher aggressiveness and activity than F1 juveniles originating from a contrasting marine habitat and reared in the same conditions. They also showed significantly lower adrenergic reactivity measured by their ventilation rates during a confinement stress. Compared to marine individuals, freshwater sticklebacks also showed lower whole brain expression of serotonergic 5HTR2B receptors and higher expression of adrenergic (ADRB2), serotonergic (5HTR2A) and dopaminergic (DRD2) receptors. We found no differences in expression of components of the glucocorticoid network (brain: CRF, CRF-R2, GR1, GR2, MR, pituitary: POMC1, POMC2), in brain monoamine levels (5HT, DA, NE), metabolite levels (5-HIAA, DOPAC, MHPG), or turnover rates. Our molecular level results are concordant with the higher aggressiveness and activity and lower sociability of these freshwater sticklebacks and with their lower reactivity to an acute stressor. Knowing how candidate molecular networks differ in activity in the brain of individuals from populations that differ in ecologically-relevant behaviours is essential to understand behaviour evolution, and our results suggest that these networks are potential evolutionary targets.

Novel function of leptin as a stimulator of glycolysis
[S1, contributed]

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Glycolysis is one of the most ancient and conserved biochemical processes for energy (ATP) production in both prokaryotic and eukaryotic organisms. Hormones are critical to regulating this foundational cellular process to meet shifting energy demands. Both glycolysis and leptin production are enhanced with hyperosmotic and hypoxic stress in order to meet the energy requirements under these conditions. However, it is unclear whether leptin might regulate glycolysis to maintain energy homeostasis in vertebrates. The purpose of these studies was to assess if the predominant form of leptin (LepA) might induce glycolysis in the tilapia (*Oreochromis mossambicus*), where the hormone increases during seawater challenge and stimulates glucose mobilization (glycogenolysis). A transcriptomic analysis of the tilapia pituitary rostral pars distalis (RPD), containing a nearly pure population of prolactin (PRL) cells, revealed leptin stimulates the expression of the glycolytic enzyme, glyceraldehyde 6-phosphate dehydrogenase (GAPDH) in a covariable manner to the regulation of other hypoxic stress response genes. Additionally, the gene expression of phosphofructokinase (PFK), the rate-limiting enzyme of glycolysis, was stimulated by leptin. Further orthogonal tests show that recombinant tilapia LepA increases GAPDH and PFK mRNA levels in a dose-dependent fashion in RPD during 6 h incubation. Likewise, leptin stimulates total glycolytic activity (lactate secretion) and PFK activity within 6 h. Glycolytic activity correlated significantly to both PFK mRNA levels and enzymatic activity. The potential signaling mechanisms for leptin action were assessed. LepA stimulates STAT3 and ERK phosphorylation in the RPD. The stimulatory effect

of leptin on glycolysis and PFK activity was suppressed by a STAT3 (Stattic) but not an ERK (PD98059) blocker, indicating the hormone stimulates glycolysis through a STAT3 mediated pathway. Leptin stimulation of ERK signaling is most likely linked to leptin action in stimulating PRL release. To assess whether LepA might broadly regulate glycolysis, we tested its effects in another cell type, namely hepatocytes, where LepA is predominantly produced and may exert local effects. In hepatocyte incubations, LepA stimulates total glycolytic activity and PFK mRNA levels, but had little effect on GAPDH gene expression. These results identify a novel action of leptin as a direct stimulator of glycolysis through a STAT3-mediated mechanism in vertebrates.

Ovarian ecdysteroid rhythms: an ovarian clock or response to rhythmic neuropeptides?
[P28]

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Endocrine rhythms distribute timing information to cells and tissues that lack other access to this information and therefore serve to synchronize cellular and physiological events in diverse tissues in an animal. In larval insects, the steroid moulting hormones (ecdysteroids) are secreted with a circadian rhythm by the prothoracic glands (PGs) and mediate temporal order throughout development. This rhythm is entrained by the action on the PGs of the neuropeptide prothoracicotropic hormone (PTTH), secreted from the brain. In adult insects, PTTH is still secreted rhythmically, but ecdysteroids are now produced in females by the ovaries. However, ecdysteroid secretion remains under circadian control. We examined the action of neuropeptides on ecdysteroid secretion by the ovaries of *Rhodnius prolixus* and whether a circadian clock is present in the ovaries. We show that both brain extracts and bombyxin (an insulin-like peptide, ILP) stimulate ecdysteroidogenesis by ovaries. Follicle cells express the clock protein PERIOD (PER) briefly and non-rhythmically during egg development. Transfer of insects from12 hr light: 12hr dark to LL (continuous light) abolishes PER expression, but treatment of LL ovaries with a 4 hr pulse of brain extract or recombinant PTTH (rPTTH) induces the reappearance of PER. We conclude that PTTH and ILPs are regulators of ovarian ecdysteroidogenesis, and induce PER expression in ovaries. However, the function of PER in ovaries is not consistent with a PER-based ovarian clock. Therefore, it is probable that the circadian rhythm of ecdysteroids in adults is driven by rhythmic neuropeptide stimulation and not by a clock in the ovaries.

Identification of California condor (*Gymnogyps californianus*) estrogen receptors 1 and 2 and their activation by endocrine disrupting chemicals
[S8, contributed]

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The primary challenge facing the continued recovery of the critically endangered California condor (*Gymnogyps californianus*) is lead poisoning resulting from scavenging carcasses containing fragments of spent lead-based ammunition. To mitigate the risk of lead exposure there is interest in establishing condor populations in coastal regions where marine mammal carcasses present abundant scavenging opportunities. However, in the only condor population currently inhabiting a coastal environment, there is evidence that exposure to endocrine disrupting chemicals (EDCs) such as dichlorordiphenyltrichloroethane and its metabolites (DDTs) and polychlorinated biphenyls (PCBs) is impairing reproduction. To address how these chemicals might disrupt condor endocrine function, we have identified and cloned condor estrogen receptors 1 and 2 (ESR1 and ESR2) and investigated their activation by EDCs. All DDTs significantly activated condor ESR1 and ESR2 at 10^{-6} -10^{-4}M. The most potent DDT agonist of ESR1 was *o,p'* –DDT followed by *p,p'* –DDT > *o,p'* –DDD > *o,p'* -DDE > *p,p'* –DDE > *p,p'* -DDD. For condor ESR2, which to our knowledge is the first ESR2 cloned from a bird of prey species,*p,p'* –DDT produced the greatest activation followed by *o,p'* –DDT > *o,p'* –DDE > *o,p'* –DDD > *p,p'* –DDD > *p,p'* –DDE. In general, condor ESR2 was more sensitive to EDCs than ESR1 with PCB52, PCB138, PCB153, bisphenol A, dieldrin, *trans*-nonachlor, *p,p'* -DDD, and *p,p'* -DDE all stimulating a higher maximal activation for ESR2 than ESR1. Although significant activation of condor ESRs by specific EDCs, such as *p,p'* –DDE, occurred at high (micromolar) concentrations, they correspond to circulating concentrations reported in coastal birds. Finally, phylogenetic analyses of ESRs of condor and 40 other avian species identified a single amino acid position in ESR2 under positive selection. Site directed mutagenesis was performed on condor ESR2 to produce variants with amino acids at this position corresponding to those found in other avian species. Receptor activation by EDCs, but not E_2, varied between the ESR2 mutants suggesting this amino acid may play an important role in EDC sensitivity. Together, these findings broaden our understanding of EDC interactions with ESRs in avian species. Specifically for condors this knowledge could be used as a tool to screen food sources at coastal sites and ultimately identify areas with low chemical exposure for condor release and relocation.

Growth hormone (GH) internalization in embryonic retinal ganglion cells: A synaptogenesis modulator?
[S9, contributed]

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Growth hormone (GH) exerts its biological effects through the GH receptor (GHR), which is present in the chicken embryo eye from ED (embryonic day) 2 of the 21-day-incubation period. In the embryo, GH gene expression also occurs in the neural retina from ED2 and retinal GH promote cell survival and induce axonal growth of immunopanned retinal ganglion cells. These local autocrine or paracrine actions are blocked following the immunoneutralization of endogenous GH or when retinal GH production is reduced by siRNA knockdown. Neuroretinal GH is therefore of functional importance before the appearance of somatotrophs (at ED12) and

the onset of pituitary GH secretion to the peripheral plasma (at ED15-17). Endocrine actions of pituitary GH in the development and function of the chicken embryo eye are, however, unknown. This possibility has therefore been investigated in ED15 embryos and using the quail neuroretinal derived cell line (QNR/D). During this research, we studied for the first time, the coexistence of exogenous (endocrine) and over-expressed GH (autocrine/paracrine) in QNR/D cells. *In ovo* systemic injections of Cy3-labeled GH (150 μg/kg, via the chorioallantonic vein) demonstrated that GH in the embryo bloodstream was translocated into the neural retina and internalized into retinal ganglion cells (RGC's). Pituitary GH may therefore be functionally involved in retinal development during late embryogenesis. Cy3-labelled GH was similarly internalized into QNR/D cells (which provide an experimental model for chick embryo RGCs) after its addition into incubation media. The uptake of exogenous GH was by a receptor-mediated mechanism and maximal after 30-60 min, and was followed by degradation of the internalized hormone. The exogenous (endocrine) and over-expressed GH (autocrine/paracrine) was biologically active, since, within the QNR/D cells it induced STAT5 phosphorylation, promoted IGF-1 expression and increased the growth associated protein 43 (GAP43) immunoreactivity (an accepted synaptogenic and axogenic marker). *Ex-ovo* intravitreal injections of Cy3-GH in ED12 embryos resulted in GH internalization and STAT5 activation. Interestingly, the labeled GH accumulated in perinuclear regions of the QNR/D cells, but was not found in the cytoplasm of neurite outgrowths, in which endogenous retinal GH is located. This suggests that exogenous (endocrine) and endogenous (autocrine/paracrine) GH are both involved in retinal function in late embryogenesis but they co-exist in separate intracellular compartments within retinal ganglion cells. This work provides evidence that GH is functionally active in the growth and differentiation of the chicken neuroretina during embryonic development.
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Exposure effects of two progestins, gestodene and levonorgestrel, on the reproductive behaviour and fitness of the fathead minnow (*Pimphales promelas*)
[P34]

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Progesterone receptor ligands are important regulators of reproduction. Synthetic progestins and endogenous progestogens have been measured in human wastewater effluent and animal agricultural runoff, and early lab studies have demonstrated profound effects of these chemicals on the reproduction of fish and amphibians. In this study, we exposed fathead minnows to environmentally relevant concentrations of two progestins to examine the exposure effects on reproductive and behavioural endpoints. We used a flow through exposure system consisting of thirty-two 15 L glass tanks (8 tanks per treatment, 6 full volume turnovers/d). Two reproductively active females and one male were placed into each tank containing a single breeding tile and were acclimated to the exposure system tanks for 7 d. Treatments began on day 8, with each tank receiving H_20 only, EtOH (vehicle control), 10 ng/L, or 100 ng/L of gestodene (GES) or levonorgestrel (LNG). Fecundity (defined as the number of eggs laid by each triad per d) and embryo viability (the resulting number of eyed embryos after 48 h) were quantified daily. Reproductive behaviour was recorded using a web-camera mounted at the front of each tank so

that the inside of the breeding tile and the surrounding outside areas were clearly visible. A 2 h recording was obtained daily from each tank, and various reproductive behaviours (lateral displays, lateral quivers, rubbing of the tile ceiling, etc.) quantified using partial interval sampling. After 8 d of exposure, all fish were euthanized and examined for the presence of nuptial tubercles, fatpad, finspot, and differences in gonadal stage of gametogenesis. While this study is currently ongoing, we expect both GES and LNG to decrease fecundity and embryo viability, masculinize females, and disrupt normal reproductive behaviours. As the effects of GES and LNG on reproductive behaviours in the fathead minnow have not yet been examined, results from this study will provide novel information regarding the impacts of these chemicals on what is considered to be a particularly sensitive endpoint, and may also help to further define relationships among any physiological, morphological, and behavioural effects.

Endocrine regulation of ion transport in marine fish: intestinal mineralization supports osmoregulation
[S13, invited]

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Seawater fish sustain an ionic disequilibrium with seawater to keep their plasma osmolality around 300-350 mOsm/kg. The gills remove excess salts from the plasma through an epithelial mechanism driven by chloride secretion and energized by the basolateral sodium pump. This process, involves (or causes) a concomitant water loss driven by the dehydrating effect of salinity that makes water replacement of the highest importance to sustain body ionic regulation. Drinking of seawater supplies the unprocessed fluid for potential water absorption. However, the fluid ingested requires processing to allow net water absorption in the intestine. The formation of intestinal carbonate aggregates, as part of the fluid processing, seems to be an important driving force to facilitate water absorption. However, two conditions are essential for intestinal mineralization: high calcium availability (and/or magnesium) as substrates and high pH to drive the precipitation. While both substrates are in high concentration in the intestinal lumen i.e. ingested seawater, the alkaline condition is driven by epithelial bicarbonate secretion. This chemical process of ion trapping promotes continuous luminal fluid processing by decreasing osmolality, which impacts on calcium homeostasis and favors water absorption in the intestine. Grounded on our work in the endocrine control of calcium regulation we initially hypothesized that intestinal alkaline secretion could be under endocrine regulation. The prediction was firstly substantiated in vitro where we established a regulatory role for calcitropic hormones in epithelial bicarbonate secretion, in the opposite to that observed for calcium movements. Furthermore, the process of aggregate production was later demonstrated to be hormone-dependent in vivo. In further studies we have confirmed regulatory actions of other factors such as the pituitary hormone prolactin, salinity and trans-membrane and soluble adenylyl cyclases. Based on the new evidences from our work, we suggest that the endocrine system is the connecting mechanism of the three most important ends of carbonate aggregate formation in the intestine of marine fish: water absorption, calcium regulation and bicarbonate secretion.

Transgenerational reproductive effects of pharmaceuticals in zebrafish (*Danio rerio*) raised from chronically exposed parents
[P35]

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In recent years, there has been increasing concern about the occurrence, fate and toxicity of pharmaceuticals in the aquatic environment. Pharmaceutical concentrations range from ng L-1 to µg L-1 in the environment and have been documented to induce alterations in behaviour, reproduction, development and overall physiological homeostasis of vertebrates. We have demonstrated that 6 week chronic, aqueous exposure of adult zebrafish to carbamazepine (CBZ) and gemfibrozil (GEM) (10 µg L^{-1}) decreased reproductive output and increased the occurrence of atretic oocytes suggesting a direct effect on embryonic development. To investigate the possibility of any transgenerational effects from parent to offspring, embryos from the exposed parents were collected after four to six weeks exposure and reared in clean water until sexual maturity. At sexual maturity, the breeding success of the first filial (F1) generation was assessed via pairwise and reciprocal mating crosses. Based on 75 pairwise breeding events, the reproductive success was significantly different with treatment. Control animals produced a clutch in 34% of pairs compared to 11% and 17% for pairs whose parents were exposed to CBZ and GEM, respectively. Reciprocal crosses with exposed females and control males had a 31% clutch success rate, similar to the control pairs. However, reproductive success was only 9% for control females paired with exposed males, from either pharmaceutical treatment, suggesting a large male effect. Behavioural analyses suggested that exposed males engaged the females less frequently for courtship. Sperm analyses showed that exposed males had altered spermatozoa morphology and swimming speed. Overall, this study suggests there may be parental impacts from chronic exposure to environmentally relevant concentrations of pharmaceuticals on the reproductive biology of fish.

The stress response suppresses innate but not adaptive immunity in the house sparrow
[P73]

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Stress-induced (SI) immunosuppression is commonly observed in wild animals, but the mechanisms that mediate this suppression are unclear. Research on these mechanisms is of especial interest in urban avian species because urban birds are exposed to a variety of anthropogenic disturbances and have high rates of pathogen transmission. To begin investigating the relationship between the stress response and immune function, we determined how the activation of the stress response by restraint influenced the adaptive and innate immune branches of the adult, male House Sparrow (*Passer domesticus*), a common urban bird. In addition, we exposed the birds either to 10 minutes or to 2 hours of restraint to discern if SI

immunosuppression was regulated by genomic or non-genomic mechanisms. The activation of the stress response did not affect the function of the adaptive branch, which was assessed by measuring the amount of swelling in response to a phytohemaglutinin injection. However, the activation of the stress response suppressed the function of the innate branch, which was assessed with both agglutination-lysis and *E. coli* bacterial killing assays. This suppression manifested as soon as after 10 minutes and persisted for 2 hours of restraint. These results suggest that the stress response may suppress the innate immune response through a non-genomic mechanism or through both a non-genomic and a genomic mechanism. Future experiments will determine if increased plasma glucocorticoids during the stress response regulate SI immunosuppression and if this response is mediated through the low-affinity glucocorticoid receptor. Supported by National Science Foundation Award 1026620 (P.D.) and by the Central Arizona Phoenix Long-Term Ecological Research project (S.G.).

A global ovary gene regulatory network derived from a large-scale analysis of chemical effects on the fathead minnow
[S10, invited]

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Complex networks underlie fundamental functions in biology, including reproduction. However, limited information is available as to how genes interact on a global scale when key organs such as the ovary are exposed to chemicals. Here, we have developed a fathead minnow (*Pimephales promelas*) ovary global gene interaction network from a compendium of different chemical exposures and examined the relationships within the network to better understand fish ovary biology by perturbing its normal functioning at different levels using chemical probes. We analyzed gene expression changes across 1,472 microarrays samples from ovaries of fathead minnow exposed to a total of 15 different chemicals representing 298 different conditions over 23 different experiments. A gene regulatory network was inferred from all 15,208 microarray probes using the algorithm Context Likelihood of Relatedness. A significance threshold estimator was applied to the inferred network resulting in 7,035 significant probes and 62,612 connections. Gene Ontology enrichment analysis found a modular structure for the network, with high connectivity genes enriched for most basic biological functions, and low connectivity genes with more specialized biological functions. A significant difference in biological processes was found between hub (highly connected) genes and non-hub genes. More than 74 percent of the connections in two of the subnetworks, and 37% of the connections in a third subnetwork were also present as known connections found in curated databases and the literature. Combining multiple microarray dataset improved our ability to identify significant gene-gene interactions, but in the process we lost the capability of identifying chemical specific interactions. Our results also suggest that combining multiple microarray datasets can be used to improve the prediction accuracy of network interactions.

A role for cortisol in altering the molecular physiology of the tight junction complex of adult rainbow trout integument
[P107]

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The barrier properties of adult teleost fish skin are well documented. However, almost nothing is known about the molecular physiology of an essential component of this boundary tissue, the tight junction (TJ) complex. To gain further insight into the adult teleost fish skin TJ complex, this study examined freshwater (FW) rainbow trout (*Oncorhyncus mykiss)* skin TJ protein transcript expression and abundance from three different perspectives: (1) spatial distribution (dorsal, lateral and ventral skin regions) (2) response to environmental change (abrupt exposure to ion poor water, IPW), and (3) response to elevated systemic cortisol levels (a hormone known to alter the paracellular permeability of vertebrate epithelia). A comparison of relative claudin (*cldn*) mRNA abundance indicated that *cldn-30* is the most abundant in the integument while regional differences in transcript abundance of *cldn-1, -3a, -5a, -5b, -6, -10c, -32a* and zonula occludens-1 (*ZO-1*) were observed. Exposure to IPW altered transcript abundance of cingulin (*cgn*) and 14 *cldns* that were suggestive of changed paracellular permeability along the dorsoventral axis of the integument. Further analysis of *cldns* (*cldn-3a, -5, -6, -8d, -10c, -30* and *32a*) that exhibited significant change in skin following IPW exposure suggested that *cldn-5b, -30* and *-32a* may also be present in vasculature of the dermis, whereas *cldn-3a, -6, -8d,* and *-10c* are found in the epidermis only. Finally, following cortisol treatment *cldn-3a, -5b, -6, -8d, -10c, -30* and *-32a* mRNA abundance variously altered in dorsal, lateral and/or ventral skin regions. Together, data indicate that the molecular components of adult fish skin TJs exhibit differences in spatial distribution and in their response to environmental change and cortisol, a hormone that plays a key role in mediating systemic change in response to an altered environment.

IGF regulation of Y-box binding protein 1 (YB-1; Ybx1) in the ovary: A potential mechanism of how the growth axis influences ovarian follicle activation
[S6, invited]

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Follicle activation is a major issue for research in ovarian physiology. Using proteomics approach, we have identified the *50-kD Y-box binding protein 1* (YB-1; Ybx1), a transcription factor and an mRNA-binding protein, as a potential gatekeeper that controls follicle activation in the zebrafish. YB-1 is the most abundant protein in the primary growth (PG) oocyte, but its level decreases dramatically during the transition from PG to vitellogenic stage. The knockout of Ybx1 gene by TALEN showed that the folliculogenesis was blocked at early stage of follicle

development. Our recent work showed that Ybx1 in the zebrafish ovary is subject to regulation by external endocrine and paracrine factors such as IGF-1. Treatment of PG follicles with IGF-I activated the PI3K-Akt pathway, which stimulated Ybx1 phosphorylation at S82. The phosphorylation of Ybx1 further induced translocation of some Ybx1 molecules to the nucleus where it functions as transcription factor, and its degradation in the cytoplasm through the ubiquitin-proteosome pathway. These results suggest that Ybx1 may serve as a critical point at which the growth axis, via IGF-I, activates the reproductive axis.

Regulation of the stress axis during chronic social stress
[Plenary]

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Many species of fish establish social hierarchies as a consequence of competition for limited resources such as feeding territories or mates. Typically, dominant fish enjoy preferential access to the limited resource and are aggressive towards more subordinate fish. Subordinate fish, by contrast, often exhibit marked behavioural inhibition including reduced activity and feeding. These behavioural differences are accompanied by distinctive physiologies, particularly with respect to regulation of the hypothalamic-pituitary-interrenal (HPI) axis. Social stress causes chronic activation of the HPI axis, leading to sustained elevation of circulating cortisol levels. The impact of this chronic activation of the HPI axis may be modulated, however, by reduced target tissue sensitivity to cortisol. For example, liver glucocorticoid receptor protein levels in subordinate rainbow trout were significantly lower than control values. Subordinate trout also exhibit a lowered cortisol response to an acute netting stressor, and an attenuated cortisol response to serotonin administration, probably because cortisol synthesis in response to ACTH is reduced in subordinate fish. Thus, chronic social stress impacts regulation of the HPI axis. In female fish, these changes may in turn impact development of the stress axis and stress responsiveness in their offspring. For example, offspring of subordinate female zebrafish exhibited lower cortisol levels in response to an acute stressor than did offspring of control mothers. The mechanisms underlying such effects pose an exciting challenge for future research.

Effect of LPS on dopamine and Parkinsonian-like behaviours
[P99]

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In mice, exposure to stress during puberty can cause long lasting effects on behaviour and brain functioning. Exposure to the bacterial endotoxin lipopolysaccharide (LPS), during puberty, can lead to enduring alterations in both reproductive and non-reproductive behaviours. In the present study, we examined whether pubertal LPS treatment altered dopamine expression and dopamine-sensitive behaviours in male and female mice. Two separate cohorts of mice were used. The first

cohort was treated with saline or LPS at 6 (puberty) or 10 (adulthood) weeks of age. Four weeks later, mice were euthanized and brains were perfused, extracted and stained to examine the expression of tyrosine hydroxylase (TH), an important enzyme for dopamine production. The second cohort was also treated with saline or LPS at 6 or 10 weeks of age. Four weeks later, mice were exposed to a series of behaviour tests (like the Rotorod, the activity monitoring, the forepaw stride length during walking, the gri! d test and the olfactory test) that are sensitive to dopamine levels in the brain to examine Parkinsonian-like behaviours. Our results showed that, while exposure to LPS during adulthood increases TH expression, LPS treatment failed to reproduce these effects when administered in pubertal mice. This research gives a better understanding of the effect of LPS on the production of dopamine and on the long-term consequences of altered dopamine production.

Impact of the feminizing Wolbachia wVulC strain on the androgenic hormone gene expression which is involved in male differentiation of the terrestrial isopod *Armadillidium vulgare*

[S2, invited]

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In crustaceans, the androgenic gland (AG), thanks to the synthesis of the androgenic gland hormone (AGH), controls the differentiation of the primary and secondary male sexual characters. This gland first discovered in Amphipods by Charniaux-Coton in 1954 was also described in decapods and isopods. The sequence of the AGH was deciphered in the terrestrial isopod *Armadillidium vulgare* more than 40 years after the discovery of the AG. We first sequenced 12 new AGH cDNA and investigated the molecular evolutionary patterns of this protein at a large scale. The phylogenetic relationships of AGH sequences allowed to distinguish two main clades corresponding to members of the Armadillidiidae and the Porcellionidae families which is congruent with the narrow specificity of AG heterospecific grafting. We then examined AGH gene expression during post embryonic *A. vulgare* development and showed that this gene is expressed before AG differentiation. This hormone might also be the target of virulence factors of the feminizing Alphaproteobacteria Wolbachia which infect numerous terrestrial isopod species. Experimental injection of Wolbachia into *A. vulgare* males revealed unexpected increase of AGH gene expression in gonads. This huge effect is not even inhibited by siRNA compared to uninfected controls. In parallel, we developed a novel targeted-genome enrichment procedure that allows specific isolation of endosymbiotic DNA and successfully sequenced using Next Generation Sequencing technologies 7 strains of Wolbachia that induce either host feminization or cytoplasmic incompatibility in terrestrial isopods. Comparative genomics were then performed to identify bacterial effectors involved in Wolbachia symbiosis. A conserved-motif recognition analysis using HMMER3 also allowed us to identify eukaryote-like proteins such as ankyrin or tetratricopeptide repeats containing proteins that are known to be involved in host-symbiont interactions, including the virulence of the bacteria.

Naphthenic acids from petroleum extraction disrupt larval development and metabolism in *Silurana (Xenopus) tropicalis*
[S4, contributed]

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Naphthenic acids (NAs) are defined as the total carboxylic acids present in crude oil. These NAs are used commercially as emulsifiers and wood preservatives. Some NAs that remain in sediment after oil spills and in oil sands process affected water (OSPW) from oil extraction activities in Alberta, Canada have been found to be toxic. NA molecules exhibit hundreds of different chemical structures and based on their structure, some could have endocrine disrupting properties. The toxicity NAs and its effects on development are largely unknown. To address this knowledge gap, we exposed *Silurana (Xenopus) tropicalis (S. tropicalis)* to two different Sigma Aldrich commercial extracts of NAs (SA1 and SA2). Frog embryos (4 h post-fertilization) were exposed to extracts SA1 and SA2 and after the 72 h of exposure, 10 larvae/treatment were pooled together for subsequent total RNA extraction and cDNA synthesis. A 4 x 44,000 gene probe custom Agilent microarray developed for *S. tropicalis* was used to study the effects of NAs on the frog transcriptome. The expression of 631 and 665 genes were significantly altered by the exposure to SA1 and SA2, respectively ($p < 0.05$). Gene ontology (GO) analysis (GeneSpring 13.0 GX) showed that a subset of molecular functions, biological processes, and cellular components were significantly altered by NAs. For example, monooxygenase activity and the xenobiotic metabolic process were affected by both SA1 and SA2. As determined by GO, numerous transcripts were classified as having novel P450 activities, including some with aromatase-like activity. Further expression and activity analyses are required to confirm the GO predictions. Exposure to NAs have also induced malformations and reduced length of the frog embryos. Research on the relationship between the chemical composition of NA mixtures and *S. tropicalis'* survival, development, and growth is ongoing and imperative to understand the environmental impacts of NA exposure. This project is supported by CONACYT (Mexico), NSERC (Canada), and University of Ottawa Research Chair Program.

5*α*-dihydrotestosterone in female sablefish (*Anoplopoma fimbria*)
[S7, contributed]

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5α-dihydrotestosterone (DHT) is a potent androgen in mammals with multiple roles. However, the physiological actions of DHT in fishes are not well known. In a recent study we demonstrated that previtellogenic ovarian follicles of sablefish produce DHT in vitro when testosterone (T) is given as a substrate, raising the possibility that DHT plays a role during early ovarian development. The present study aimed to better understand the role of DHT during oogenesis in this species. For this, we characterized the reproductive cycle in adult females, including seasonal changes of plasma and ovarian extract sex steroid levels by li*quid chromatography tandem-mass spectrometry*. In parallel, we evaluated potential effects of DHT on the reproductive endocrine axis of prepubertal cultured specimens.
Adult female sablefish (≥ 5 years old) were caught in coastal Washington over 12 months. Transcript levels of key pituitary gene transcripts (gonadotropin –Gth- subunits, estrogen and androgen receptors, and *kisspeptin 2- kiss2*), and plasma and ovarian extract sex steroids (12 steroids total*) were analyzed in relation to ovarian development*. No significant differences were found in pituitary mRNAs or sex steroid levels during the transition to the secondary oocyte growth, while transcripts for Gth subunits, androgen receptor 1 and 2, and *kiss2*, as well as levels of androstenedione, T, and 17β-estradiol (E2) displayed distinctive patterns during vitellogenesis and final maturation. Surprisingly, DHT was undetectable in both plasma and ovarian extracts throughout the entire reproductive cycle.
Prepubertal cultured female sablefish were treated with sustained-release cholesterol pellets containing DHT (0, 0.125, 0.5 and 2.5 mg) for 4 weeks. Effects of DHT on key pituitary and ovarian (Gth receptors and sex-steroid related genes) transcripts were analyzed in relation of plasma sex steroids and gonadal development. Mid- and high- doses of DHT increased transcript levels for ovarian aromatase (*cyp19a1a*), but did not induce significant changes in plasma E2, or pituitary or ovarian transcripts.
In conclusion, juvenile sablefish produce DHT in the presence of exogenous T, and treatments with DHT have a stimulatory effect on ovarian *cyp19a1a* gene expression. In contrast, adult fish do not show detectable levels of this androgen at any reproductive stage (from cortical alveolus to post-spawning). Future studies during the onset of puberty should be addressed to elucidate the role of this steroid in sablefish.

In silico **pathway analysis linking perturbed steroidogenesis with gonad growth in fathead minnows (*Pimephales promelas*) exposed to 17α-ethynylestradiol**

[P81]

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It is expected that the mathematical analysis of biochemical networks will lend to functional interpretations of organismal fitness or susceptibility when perturbed. We applied the optimization framework of *constraints-based flux balance analysis* (FBA) to assess effects on

steroidogenesis (or steroid hormone production) and gonad growth in adult male and female fathead minnows exposed to the synthetic estrogen, 17α-ethynylestradiol (or EE2). We augmented a previously developed stoichiometric model of piscine steroidogenesis with a biomass reaction constraining the contributions of various (and functionally relevant) steroid hormones to biomass production (or gonad growth). *In silico* analysis successfully predicted effects of EE2 exposure on fathead minnow gonad growth (%gonadosomatic index or %GSI) and perturbed steroid hormone production. Specifically, FBA accurately predicted no effects of exposure on male %GSI and a significant reduction for female %GSI. Furthermore, *in silico* simulations accurately identified disrupted reaction fluxes catalyzing productions of androgens (in male fish) and progestogens (in female fish), an observation which agreed with *in vivo* experimentation. The approach presented may be an important step towards incorporating *in silico* predictions with toxicological risk assessments.

Fluoxetine bioaccumulation and concentration in *Daphnia magna* – studying toxicity in planktonic food webs
[P42]

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Pharmaceuticals are found at increasing levels in wastewater treatment plant effluents, and drinking water around the world. After being ingested by humans, these compounds are excreted into wastewater systems unchanged, or as active metabolites. Fluoxetine hydrochloride (Flx) is one of the most prescribed antidepressants in the world and has a relatively high persistence in aquatic environments. Fluoxetine is known to have toxic effects on various aquatic species, and levels of Flx ranging from 50 ng/L to 99 ng/L have been detected in the Great Lakes Region. Zooplankton can get exposed to Flx via two potential routes, direct exposure from water or indirectly from ingested phytoplankton. *Daphnia* species are key components of freshwater zooplankton and while direct exposure to Flx has been previously tested, the effects of indirect exposure remain unclear. The purpose of this study is to investigate the effects of fluoxetine on the freshwater crustacean *Daphnia magna* via direct and indirect routes. Specifically we are assessing the consequences of Flx on *Daphnia* fitness by measuring mortality and reproduction. Furthermore, we developed novel assays to investigate swimming behaviour and embryonic development. Preliminary data shows that Flx affects *Daphnia* development, reproduction, and swimming behaviour. If Flx has an impact on *D. magna*, there is the potential of bioaccumulation through the food chain, which could lead to unknown consequences for humans.

Isolation and characterization of the corazonin receptor in the kissing bug, *Rhodnius prolixus*
[P29]

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Neuropeptides control many physiological and endocrinological processes in animals, acting as neuroactive chemicals within the central and peripheral nervous systems. Corazonin (CRZ) is one such neuropeptide found within insects and other arthropods. CRZ has a variety of physiological roles associated with control of heartbeat, cuticle colouration, and ecdysis behaviour initiation. These physiological effects are mediated by the CRZ receptor (CRZR) which is a G protein-coupled receptor (GPCR). In order to understand the role of the CRZ signalling pathway in *Rhodnius prolixus*, the cDNA sequence encoding the RhoprCRZR has been isolated and cloned. It was found to have seven transmembrane domains which is a characteristic of GPCRs. The spatial expression pattern of the RhoprCRZR transcript was examined using quantitative PCR, and the RhoprCRZR transcript was found to be expressed in the central nervous system and several peripheral tissues, including the dorsal vessel, abdominal dorsal epidermis, and prothoracic glands. This data gives clues for possible functions of the CRZ signalling pathway in *R. prolixus.* To examine these functions, the receptor is being knocked down using RNA interference, and the effect on heart rate, growth, development, and cuticle colouration will also be monitored.

Starfish gonadotropin, relaxin-like gonad-stimulating peptide: the gene and its expression [P30]

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Relaxin-like gonad-stimulating peptide (RGP) in starfish is the only known invertebrate peptide hormone responsible for final gamete maturation, rendering it functionally analogous to gonadotropins in the vertebrates. Previous studies have shown that the coding DNA sequence (CDS) of RGP in starfish Asterina pectinifera consists of 351 base pairs (bp) with an open reading frame (ORF) encoding a peptide of 116 amino acids (aa), including a signal peptide (29 aa), B-chain (19 aa), C-peptide (44 aa) and A-chain (24 aa). Though A. pectinifera is an endemic Japanese species and widely inhabits on the rocky shores from northern to southern Japanese waters, RGP ORFs are exactly the same in local populations. This suggests that RGP is a highly conserved peptide in A. pectinifera. However, a genomic DNA sequence of RGP was unidentified yet. Thus, we examined the RGP gene prepared from testis of A. pectinifera. A functioning gene of the RGP consisted of 3,896 bp comprising two exons (exson I: 208 bp, exson II: 2,277 bp) and one intron (1,411 bp). Promoter genes, the CCAAT and the TATA boxes, were present in the 5'-upstream of RGP gene. It is interesting that a polyadenylation signal (AATAAA) was found over 2 kbp far from stop codon. Whereas the ORF length is 351 bases, the transcript consisted of 2,485 bases. This shows that only 14% of RGP mRNA is translated into the peptide. Furthermore, the transcription activities of RGP gene were measured in various organs of A. pectinifera using real-time quantitative PCR with specific primers for RGP. mRNA was expressed in high level in the radial nerves. The expression was also observed in the cardiac stomachs and pyloric caeca, although in low amounts, but it was a trace level in the gonads and tube feet. This suggests that the RGP gene is transcribed mainly in the radial nerves. Then, RGP

is synthesized as a prepro-RGP translated from its mRNA in radial nerves. After the formation of three disulfide cross-linkages between the A- and B-chains and within the A-chain, mature RGP is produced by elimination of the signal and C-peptides.

A gene expression screen for genes synergistically regulated by thyroid hormone and glucocorticoid in mouse hippocampal neurons
[P92]

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Thyroid hormone (T_3) deficiency during neonatal development results in morphological and functional changes in the hippocampus. Glucocorticoids (e.g., corticosterone - CORT) both facilitate and inhibit hippocampal development and function, which depends on their level and duration of elevation. Few studies have looked at whether these hormones interact to regulate similar or different sets of target genes in the hippocampus, or if they may cooperate to regulate gene expression. Here we investigated gene regulation by microarray analysis following treatment of the mouse hippocampal-derived cell line HT-22 with T_3 or CORT alone, or the two hormones for 4 hr. We identified 425 genes that were regulated by CORT, 293 induced and 132 repressed. There were 203 genes regulated by T_3, 116 induced and 87 repressed. We found 69 genes synergistically up-regulated, and 80 genes synergistically down-regulated by T_3 plus CORT treatment. We validated a subset of genes from several regulatory categories by RTqPCR. Among the most strongly synergistically induced genes was Kruppel-like Factor 9 (*Klf9*), a transcription factor, and Cytochrome b561 (Cyb561), a transmembrane protein whose physiological functions involve posttranslational processing of neuropeptides. We found that induction of *Klf9* and *Cyb561* persisted in the presence of cyclohexamide, supporting that they are direct nuclear receptor target genes. Earlier we identified an ancient, ultraconserved nuclear hormone enhancer element to explain synergistic regulation of *Klf9* (*Klf9* synergy module). Here we focused on Cyb561 and discovered two putative Cyb561 synergy modules (CSMs) located in upstream and intronic regions of the gene. Chromatin-immunoprecipitation (ChIP) assay for thyroid hormone receptor (TR) showed strong association with both CSMs, supporting that Cyb561 is a direct TR target gene. Forced expression of a dominant negative TR strongly inhibited T_3, CORT and synergistic induction of Cyb561 mRNA. Our findings support that, like *Klf9*, the TR functions as a 'gatekeeper' for GR entry to the Cyb561 locus, which explains in part the molecular basis for hormone synergy. (Supported by NINDS 1 R01 NS046690 and NSF IOS 0922583 to RJD, and NIH 1T32HD079342-01 and MCubed funding to JRK).

Sex steroid regulation of pituitary gonadotropins during primary oocyte growth in coho salmon
[P60]

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In salmonids, the gonadotropins (GTHs) are differentially expressed throughout development, with plasma levels of follicle-stimulating hormone (FSH) being prominent during early to mid gametogenesis and plasma luteinizing hormone (LH) remaining low until final gamete maturation. Considerable research has been done to investigate the differential regulation of GTHs by hypothalamic and gonadal factors including sex steroids, but many questions remain: 1) the factors stimulating an initial increase in FSH synthesis and release at the onset of puberty are not well defined; 2) the role of androgens in regulating GTHs in females is largely unexplored; and 3) the effects of estrogens on GTH synthesis and release are unclear due to species- and stage- specific differences. To clarify the role of estrogen and androgen on GTH expression, we implanted prepubertal coho salmon with slow-release cholesterol pellets containing either vehicle, estradiol (E2), or a non-aromatizable androgen, 11-ketotestosterone (11-KT), at two stages: late perinucleolar stage (LPN) or early cortical alveolar stage (ECA). Plasma steroid levels in steroid-treated fish were approximately 10 ng/mL over the experiment. Fish were sampled at 1, 3, 7, and 21 days after treatment and pituitary glands were collected. Pituitary RNA was isolated for analysis of transcript levels by quantitative PCR. At both stages, mRNA levels of LH beta subunit (lhb) and gonadotropin-releasing hormone receptor 1 (gnrhr1) were strongly and rapidly upregulated by E2; approximately 70- and 4-fold by 1 day and 500- and 3-fold by 21 days, respectively. Glycoprotein alpha 2 (cga2) mRNA levels were also upregulated by E2 at 7 and 21 days post treatment in LPN stage fish. In contrast, 11-KT had no consistent effects on cga2, lhb or gnrhr1 mRNA levels over the 21-day experiment. Transcript levels of FSH beta subunit (fshb) were not regulated by E2 or 11-KT in LPN stage fish over the course of the study. However, in ECA stage fish, E2 treatment significantly decreased fshb mRNA levels at 7 and 21 days post injection. These results suggest that negative feedback of E2 on fshb develops during late primary oocyte growth in coho salmon, while E2 positively regulates lhb, gnrhr1 and cga2. 11-KT does not appear to regulate GTH mRNAs at this stage, but effects of 11-KT on pituitary protein content or plasma levels cannot be ruled out. Supported in part by National Science Foundation grants OISE-0914009 and IOS-0949765 to GY and PS.

Pituitary gonadotropins are targets of endocrine disruption in salmon and trout
[P43]

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There is evidence that chemicals in the environment are capable of disrupting reproduction and gonad development in humans, wildlife, and fish. In vertebrates, the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are involved in all aspects of gonad development and function. Despite their central role in regulating reproduction, only

limited data are available on impacts of endocrine disrupting chemicals (EDCs) on the gonadotropins, or pituitary function generally. Using high-throughput sequencing and RNA-Seq, we previously demonstrated that waterborne exposure of previtellogenic coho salmon to 12 ng/L 17α-ethynylestradiol (EE2) for up to 6 weeks had widespread effects on the pituitary transcriptome. At 6 weeks, LH beta subunit (lhb) was upregulated 395-fold and was the most significantly altered transcript, while FSH beta subunit (fshb) was downregulated -3.5 fold. Based on the strong response of lhb and fshb to EE2, we investigated the direct effects of estradiol (E2) and EE2 on gonadotropin mRNAs and secretion in a 3-day in vitro assay using rainbow trout primary pituitary cells. Exposure of pituitary cells from previtellogenic trout to 0.1 to 100 ng/mL E2 resulted in 8- to 20- fold upregulation of lhb and a 3- to 5-fold increase in the gonadotropin releasing hormone (GnRH)-stimulated secretion of LH. EE2 exposure resulted in similar levels of lhb induction. In contrast, when pituitary cells from vitellogenic trout were exposed to E2, lhb was upregulated 1.5 fold at all concentrations, indicating that vitellogenic animals are less responsive to estrogen-induction of lhb. In contrast to our previous in vivo results, no differences in fshb mRNA levels were observed in response to E2 or EE2 exposure in vitro at either stage, suggesting that estrogen does not have a direct effect on fshb gene expression. These results indicate that environmental estrogens can alter gonadotropin production in sexually immature salmonid fish, with potential implications for gonad growth and development. Further, we have shown that pituitary cell culture can be useful for screening chemicals with potential endocrine disrupting activity. This project was supported in part by Washington SeaGrant project RB49 and EPA-Star project R835167.

Ocular growth hormone: a comparative perspective
[S9, invited]

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The eye is an extrapituitary site of growth hormone (GH) production. GH mRNA and GH proteins are, for instance, present in the neuroretina of reptiles (iguana), birds (chickens) and mammals (rat, mice and humans). Within the neural retina GH is produced in retinal ganglion cells (RGCs), the pigmented epithelium and it is expressed in the inner and outer nuclear layers and is present in the photoreceptor layers. Within the eye, GH is also present in the choroid, lens, and cornea and it is secreted into vitreous humor, in which, it is bound to opticin, a proteoglycan binding protein, unique to the eye. The production and secretion of GH in the eye is likely to be similar to that in the pituitary gland, as it is similarly induced by the same hypothalamic releasing factors (eg. GH-releasing hormone and thyrotrophin-releasing hormone), that are also expressed locally within the eye. The eye is also an endocrine, autocrine or paracrine site of GH action, as GH receptors (GHRs) are widely expressed in ocular tissues and GH actions have been demonstrated in the visual systems of fish, amphibian, birds and mammals. These actions include the growth, proliferation and survival of ocular cells and the growth and synaptogenesis of neurons. These actions are of physiological significance, as they are impaired by the immunoneutralization of ocular GH and by its siRNA knockdown. These actions are also of

pathophysiological relevance, as visual defects accompany pituitary GH excess or deficiency and the death of RGCs in glaucoma directly correlates with a loss of GH within the visual system. Pituitary and ocular GH are thus of functional importance in eye development and vision in vertebrates.
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Interactions of nesfatin-1-GnRH-kisspeptin in murine hypothalamic cells *in vitro*
[P61]

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In recent years, it has been conclusively demonstrated that various endocrine factors such as hormones with key roles in energy homeostasis are also involved in the regulation of reproduction. Metabolism and reproduction are tightly interlinked. However, the hormonal mechanisms underlying interactions between metabolism and reproduction are not fully understood. Nesfatin-1 is an 82 amino acid metabolic regulatory peptide derived from nucleobindin-2 (NUCB2). NUCB2 mRNA and protein significantly increases in the hypothalamus of both female and male rats during puberty-to-adult transition. Fasting at puberty causes a suppression of nesfatin-1 and a decrease in circulating luteinizing hormone (LH) levels in rats. Administration of nesfatin-1 to both males and females increases circulating LH and testosterone (T) levels in males. These results point to a significant role for nesfatin-1 in the neuroendocrine regulation of the hypothalamus-pituitary-gonadal axis. This research aimed to study whether/how nesfatin-1 affects hypothalamic reproductive hormones, including kisspeptin-1 (Kiss-1) and gonadotropin releasing hormone (GnRH). We found the expression of mRNAs encoding Kiss-1, Kiss-1 receptor GPR54 (Kisspeptin receptor), GnRH and NUCB2 in a mouse hypothalamic cell line (GT1-7 cells). GT1-7 cells are immunopositive for NUCB2/nesfatin-1, GnRH and Kiss-1. Synthetic nesfatin-1 (100 nM) increased Kiss1-R mRNA expression in GT1-7 cells *in vitro* at 1 hour post-incubation. Both NUCB2 and GnRH mRNAs were increased in GT1-7 cells after 2 hours of incubation with 1000 nM kisspeptin-1. GnRH peptide at 100 nM decreased NUCB2 and KiSS-1 mRNA expression in GT1-7 cells *in vitro* at 1 hour post-incubation. These results demonstrate that nesfatin-1 likely induces LH synthesis/secretion through the stimulation of GnRH/Kiss-1 systems. In addition, NUCB2/nesfatin-1 appears to be a novel target of kiss-1 action, which requires additional confirmatory studies.

Mechanisms of sex determination in South American silversides: from TSD to GSD
[S6, invited]

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Sex differentiation in silversides can be easily modulated by the water temperature experienced during embryogenesis or early larval development (TSD, temperature-dependent sex determination). In general, low temperatures produce female-biased whereas high temperatures produce male-biased populations, with proportions that vary according to species or populations. However, since the discovery of the sex-determining gene *amhy* (Y-chromosome-linked, anti-Müllerian hormone) in *Odontesthes hatcheri*, it is becoming clearer that genotypic factors (GSD, genotypic sex determination) are also present in this group of fish. In this study we examined the presence of *amhy* gene in several *Odontesthes* species and analyzed its role in the process of sex determination of *Odontesthes bonariensis*.
The homologue of *amhy* gene was found in 11 species of *Odontesthes* genus. A field screening analysis revealed the presence of sex reversal in about half of the species. Interestingly, species showing no sex reversal inhabit marine or estuarine environments whereas those with sex reversal inhabit inland waters. A more detailed analysis of *amhy* in *Odontesthes bonariensis* revealed that it is functional, especially at intermediate, mixed-sex producing temperatures. Furthermore, our expression analysis showed that the autosomal *loci amha* may also be crucial for testis differentiation in this species. Overall, this study showed that genotypic and environmental sex determinants can coexist in *Odontesthes* species.

Expression and localization of two glycoprotein hormone receptors in the ovary and thyroid implies that hypothalamic-pituitary-ovary & -thyroid axes overlap in the sea lamprey, *Petromyzon marinus*
[S7, contributed]

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In gnathostomes, the pituitary hormones LH and FSH regulate gonadal activity, and TSH regulates thyroid activity/metabolism. In contrast, there is only one major glycoprotein hormone (GpH) identified in the sea lamprey, *Petromyzon marinus*, that may be involved in both reproduction and metabolism. Two glycoprotein hormone receptors (GpH-Rs) have been identified in lamprey that are both expressed in ovary and thyroid tissues. It is not known if lamprey GpH stimulates one or both receptors at the gonad or thyroid. Therefore, this research was to determine the expression of these two GpH-Rs in response to GnRH, as well as to determine the localization of GpH-Rs in the ovary and thyroid of sexually mature, adult lamprey. In our *in vivo* experiment, injection with lamprey gonadotropin releasing hormones (GnRH-I, -II, -III) resulted in increased RNA expression of both lamprey GpH-Rs in ovary and thyroid compared to controls. All GnRH treated fish had decreased concentrations of thyroxine and elevated estradiol concentrations compared to controls. These results suggest that both receptors at the thyroid and gonad are responsive to lamprey GpH. Support: NSF IOS-1257476, AES NH00624, AES NH00571.

Sniffing out endocrine disruption from complex mixtures with a new genome, metagenomics, and systems approaches
[S10, invited]

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Linking molecular changes to adverse outcome pathways can be quite challenging, particularly when systems approaches are hampered by access to bioinformatics resources. In fact, the vast majority of sentinel species have limited to no genetic information. An important animal group is the Ranids; the largest frog family. Multiple Ranid species have been instrumental in identifying endocrine disruption as they have an obligate transition from an aquatic tadpole to a terrestrial frog that is dependent upon thyroid hormone (TH) action. This presentation focuses on recent progress made in the application of systems approaches toward identifying adverse outcomes associated with olfaction in the North American bullfrog tadpole. We developed *de novo* RNA-seq and quantitative real time polymerase chain reaction (qPCR) pipelines to investigate the impact of exposure to an endocrine disrupting chemical (EDC) mixture from common pharmaceuticals and personal care products found in municipal wastewater. Linked with electro-olfactography, behavioural tests, and bacterial metagenomics analyses in the rostral region, we are able to better define the consequences of EDC exposure and relate molecular changes in gene expression to adverse outcome pathways. With our sequencing and assembly of the first "true frog" genome, further large scale analyses are possible in identifying important pathways involved in TH signaling. The methods and approaches described in this study are applicable to other species for which genomic information is lacking.

Follicle stimulate hormone and vascular endothelial growth factor increase the production of sphingosine-1-phosfate in cultured of bovine granulosa cells
[S6, contributed]

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In several cell types, sphingosine-1-phospate (S1P) promotes cellular proliferation and survival. Previous results from our laboratory show that bovine granulosa cells from large healthy follicles had a higher concentration of S1P than cells from atretic follicles. This suggests that S1P sustains follicular growth and dominance. However the mechanisms that regulate S1P production in follicular cells are not clear. The synthesis of S1P depends on the phosphorylation of Sphingosine-Kinase-1 (SK1) by protein kinase C (PKC). However, whether FSH via PKA and VEGF via tyrosin kinase could phosphorylate SK-I has not been investigated. To test this hypothesis, bovine ovaries were collected from the local abattoir. Granulosa cells were harvested and 75 X 103 viable cells seeded in a 96 well plate in McCoy's 5a medium containing 10 ng/mL of insulin and 1 ng/mL of LR-IGF-I and cultured for 48 h in a humidified atmosphere with 5% CO2 at 370C. Cells were treated with FSH (0, 0.1, 1 or 10 ng/mL) and VEGF (0, 0.01, 0.1, 1, 10 or 100) in a 4X6 factorial design. Concentrations of S1P were quantified by HPLC and corrected by cell number. FSH at 1ng/ml increased ($P < 0.05$) S1P concentrations in culture media. Similarly, the addition of 0.01 ng/mL of VEGF to the culture medium increased ($P <0.05$) S1P production. However, in both cases higher concentrations of FSH or VEGF produced a decline in SIP production to levels similar to the non-treated cells. Based in these results we evaluated the effect of 1 ng/mL of FSH, 0.01 ng/mL of VEGF and their combination on the concentration of phosphorylated-SK1 by Western blot. Both FSH and VEGF treatment increased SK-1 phosphorylation ($p<0.05$), However, the treatment combination of FSH and VEGF did not show an effect on the phosphorylated enzyme. In conclusion, our results show that FSH (1 ng/mL) and VEGF (0.01 ng/mL) increase SK1 phosphorylation in bovine granulosa cells in vitro, resulting in an increase in enzyme activity and the production of sphingosine-1-phospate. These results reinforce the hypothesis that SIP is produced by the follicle and acts in an autocrine fashion to support follicle development.

Jab1 is a coregulator of the thyroid hormone receptor beta 1
[P82]

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Thyroid hormones (THs) exert multiple biological effects in vertebrates mainly induced by genomic pathways, which involve their binding to thyroid hormone receptors (TRs). TRs interact with the TH-response elements located at the promoter regions of TH-dependent genes and recruit coregulators to induce or inhibit their expression. In teleosts, the TH 3,5-diiodothyronine (3,5-T2 or T2) regulates the expression of TH-dependent genes through the binding and activation of a specific isoform of the TRβ1, the long or L-TRβ1, that contains a 9 aminoacid insert in the ligand binding domain (LBD), different from that activated by T3, the short or S-TRβ1, which lacks the insert. Furthermore, T2 seem to promote the recruitment of a coregulator population to the ligated L-TRβ1 isoform different from that recruited by T3. We initially looked for coregulators that interacted with the T2+L-TRβ1 complex using a yeast 2-hybrid screen. Jab1 was identified as a binding partner of the full L-TRβ1+T2 complex. To study the interaction mechanisms and the transcriptional effects of Jab1 bound to the TRβ1 isoforms, we performed: 1) EMSAs with L-TRβ1 and Jab1, in the presence of T2; and 2) transactivation

assays with L-TRβ1, S-TRβ1 or delta NTD L-TRβ1 plus increasing concentrations of Jab1, in the presence or absence of T2 or T3. Our results showed that Jab1 enhances: 1) L-TRβ1+T2 complex formation and 2) T2- and T3-dependent L-TRβ1 mediated transcription; furthermore the presence of the NTD is necessary for T2 but not T3-dependent transcription, even in the presence of Jab1. Unexpectedly, Jab1 repressed T3-dependent S-TRβ1 mediated transcription, suggesting an opposite role as a corepresor when interacting with this TRβ1 isoform. These results show that Jab1 is a specific coactivator of the L-TRβ1+T2 complex and a corepressor of the S-TRβ1+T3 complex, suggesting that TH divergent biological functions could be mediated by isoform- and ligand-specific TRβ1 partner protein interactions that result in opposite functional outcomes (Supported by PAPIIT: IN201614-25).

The regulation of metamorphic development in a marine environment revisited – Exogenous cues and endogenous modulators
[S14, invited]

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Metamorphosis is characterized by the loss of larval tissues and structures and an irreversible transition to a juvenile morphology. In various marine invertebrate groups it punctuates a transition between disparate developmental states resulting from divergent selective pressures in the larval versus the juvenile habitat. In sea urchins, environmental cues induce the process of settlement once larvae are metamorphically competent and this transition is accompanied by drastic morphological change. Hormones have been previously implicated in the regulation of larval development and juvenile morphogenesis in this group, yet the regulatory machinery underlying the process remain largely unknown. Here we present an expanded model of sea urchin metamorphosis and provide empirical evidence for histamine signaling in the regulation of metamorphic competence. Specifically, our data provide insight into an extensive histaminergic nervous system in larvae of the purple sea urchin Strongylocentrotus purpuratus. We provide evidence that histamine directly regulates metamorphic competence and arm retraction, two essential components of the metamorphic transition in sea urchins. We further hypothesize that histamine interacts with nitric oxide, another critical regulator of metamorphosis in sea urchins. Technical advances including morpholino injections into late larval stages allow to directly manipulate metamorphic stages and further explore the regulatory machinery underlying this fascinating life history transition.

Hypothalamic gene expression and gonadal steroid hormone levels during transitions in reproductive status in naked mole-rats
[S12, invited]

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Naked mole-rats are a eusocial mammalian species; they live in large colonies with a single breeding female, called the queen, and 1-3 breeding males. Breeders are socially dominant though non-reproductive subordinates can transition to dominant breeding status if they are removed from their colony and housed with an opposite sex conspecific. Interestingly, adult subordinates exhibit little to no sex differences in their overall body size, behavioural profile, external genitalia, or circulating steroid hormones. However, traditional sex differences in gonadal hormones and reproductive behaviours are seen in breeders. To determine how sex differences in neuroendocrine function develop in breeders, we measured circulating levels of gonadal steroid hormones in plasma and the expression of steroid hormone receptor mRNA in the brains of male and female animals as they transitioned in reproductive status. We removed male and female subordinates from their colony and paired them with an opposite sex animal for either 1 day, 1 week, 1 month, or until they became breeders (i.e., produced a litter). We compared all groups to in-colony subordinates. Hypothalamic tissue was collected and species-specific primers were used with qPCR to measure mRNA of androgen receptor (AR), estrogen receptor (ER) alpha, progesterone receptor (PR), and aromatase. No differences were seen in the 1 day and 1 month groups. At 1 week post-removal, males had reduced ER alpha mRNA and females had increased aromatase mRNA. Striking effects were seen in breeders where females had a 3-fold increase in aromatase and 5-fold increase in ER alpha and PR whereas breeding males had reduced PR and increased AR. Circulating gonadal steroid hormones (E, P, and T) varied by sex and group. In females, E and P were higher in the 1 month and breeding groups while T showed no group differences. For males, T was increased at 1 week, 1 month, and in breeders while E and P did not change. These data confirm that sex specific changes in neuroendocrine function are associated with the transition to reproductive status: sex differences in circulating gonadal steroids and in hypothalamic gene expression only emerge weeks to months after animals are removed from reproductive suppression in their home colony.

Secretogranin IIb plays an important role in zebrafish cerebral artery development
[P93]

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Secretogranin II (SgII)-derived neuropeptide secretoneurin (SN) is an important factor in nervous, endocrine and vascular systems, but putative functions in embryonic development remain unknown. Here, we have established both sgIIa and sgIIb knockout zebrafish lines by TALENs (transcription activator-like effector nucleases). Moreover, sgIIa/b double mutant line was obtained by crossing sgIIa knockout zebrafish with sgIIb knockout zebrafish. Intriguingly, both homozygous sgIIb and sgIIa/b mutant embryos are defective in hindbrain central arteries (CtAs) development, while CtAs development in homozygous sgIIa mutant embryos is not affected. The CtAs defective phenotype in sgIIb mutant embryos can be partly rescued with injected SNb mRNA. Western blot analysis showed that both phosphorylation of extracellular signal-regulated kinase (p-ERK) and protein kinase B (p-AKT) were significantly decreased in sgIIb mutant embryos. The decreased p-ERK was also detected in sgIIb mutant embryos using

whole mount inmmunofluorescence stained with anti p-ERK. The up-regulated expression of Notch1b mRNA was found in both sgIIb and sgIIa/b double mutant embryos. This in vivo evidence in the present study uncovers important role for sgIIb in cerebral artery development.

Teneurin C-terminal associated peptide (TCAP): An independently transcribed peptide involved in stress and cellular metabolism and a possible ancestor to the ligands of the secretin GPCR family
[P5]

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The teneurin transmembrane protein and its receptor, latrophilin, comprise the only trans-synaptic adhesion pair that is conserved between invertebrates and vertebrates. Both protein genes are thought to have originated from a lateral gene transfer event that introduced the prokaryote genes into a single-celled metazoan ancestor. At the extracellular carboxy-terminus of each teneurin protein is a 40-41 residue sequence we have called the teneurin C-terminal associated peptide (TCAP). Recently, we have established by 5'-RACE-PCR and northern blot, that TCAP-1 is independently transcribed. Further evidence suggests that the ancestral TCAP-like sequence found on the original prokaryote protein acted as a toxin within a bacterial defense system. Comparison of the TCAP primary sequence indicates a structural similarity with the Secretin GPCR family of ligands. Moreover, its putative receptor, latrophilin, possesses a hormone binding domain similar to that found in the Secretin family of GPCRs. Additional evidence indicates that the Secretin GPCR family evolved directly from the Adhesion family of GPCRs, which latrophilin is a member of. Recent studies have established that extant TCAP-1 is a potent regulator of glucose transport in neurons and muscle cells. Interestingly, TCAP-like sequences can be found in prokaryote glucose regulating peptides and proteins. Taken together, we hypothesize that the original function of the ancestral TCAP was to affect glucose metabolism in cells and the peptide has been exploited for use in cellular metabolism in metazoans.

The orexigenic peptide ghrelin and its relationship with metabolic challenges mammals face during reproduction
[P6]

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Ghrelin, a 28 amino acid peptide, is secreted from the stomach and binds to receptors in brain, pituitary and other peripheral tissues, including the ovaries, and plays a major role in the regulation of energy balance by increasing food intake. In these studies we investigated ghrelin's contribution to metabolic changes occurring during pregnancy and lactation. Thus, rats with a

point mutation in the gene encoding the ghrelin receptor (GHSR-KO), rendering them insensitive to ghrelin and WT rats were mated with males of the same strain. In study 1, these groups were compared on weight gain during pregnancy, litter size and weight at birth, as well as maternal and litter weight change across lactation, maternal food intake and length of lactational anovulation. No strain differences on any of the pregnancy measures were observed, but as expected, GHSR-KO dams ate significantly less than WT rats in the first 15 days postpartum. Further, GHSR-KO dams had a longer period of lactational infertility and their litters gained weight at a slower rate than those of WT mothers. In study 2, a cross fostering design was used to investigate the relative contribution of strain of mother and strain of pup to these effects. Maternal but not pup strain influenced both food intake in the first 15 days postpartum and pup weight gain from day 16 to 25. Length of lactational anovulation was affected by both dam and pup strain and was longest in GHSR-KO mothers nursing GHSR-KO pups and shortest in WT mothers nursing WT pups. Together, these studies suggest that in postpartum rats, ghrelin makes a significant contribution to the adaptation of energy balance pathways to the energetic demands of milk production. The mechanisms through which ghrelin has these effects are currently being investigated by comparing mother-litter interactions, circulating hormone levels, as well as differences in hypothalamic gene expression of neuropeptides related to food intake and reproductive function between GHSR-KO and WT rats on different days across lactation.

Regulation of *Drosophila* food intake and lifespan
[S1, invited]

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Energy acquisition is fundamental to all animals. Neuronal and genetic mechanisms regulate all aspects of feeding behaviour, including food-seeking, initiation of feeding, and meal termination. Numerous studies have demonstrated that these mechanisms are highly conserved across species ranging from humans to flies. However, in mammals—especially humans—complex cognitive processes (i.e. hedonic feeding) interfere with the basic mechanisms of energy homeostasis that direct feeding behaviour in simpler organisms. *Drosophila* provides a genetically tractable, simple model system for dissecting the basic rules that govern central aspects of energy homeostasis. To study these systems, we have developed high-resolution methods for measuring total food intake in adult *Drosophila*. Flies are able to modify food intake volume to compensate almost perfectly for changes in nutrient concentration, suggesting that altered consumption reflects true changes in energy demands and/or metabolism. Using this as a model, we characterize two paradigms—caloric restriction (CR) and cold-/hypothermic-induced longevity (CHIL)—to show how total food intake relates to feeding preferences and longevity. Although our work has focused on assessing energy acquisition, future development of methods for accurately measuring nutrient assimilation and energy usage will provide a complete accounting of energy balance in this important model organism.

Investigating how developmental exposure to bisphenol-A (BPA) and ethinyl estradiol (EE2) affect F1 parental care and F2 pup parameters

[S4, contributed]

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The nature and extent of care received by an infant can affect social, emotional and cognitive development, features that endure into adulthood. Here, we employed the monogamous, California mouse (*Peromyscus californicus*), a species, like the human, where both parents invest in offspring care, to determine whether early exposure to endocrine disrupting chemicals (EDC: bisphenol A, BPA; ethinyl estradiol, EE) of one or both F1 parents altered their behaviours towards their F2 pups. F1 adult (~90 days old) males and females from each group were paired with either controls or another F1 partner developmentally exposed to the same EDC which resulted in 7 treatment combinations (n = 4 to 7 pairs/combination). Parental behaviours were recorded with an infrared video camera in the dark and light cycles from post-natal days (PND) 0 to 5. F2 pup weight and temperature were determined, as we have done previously. We are currently measuring the pup ultrasonic vocalizations (USV) with Avisoft-UltraSoundGate 116Hb, microphones. Data to date show that females exposed to either compound spent less time nursing, grooming and being associated with their pups than controls, although there was little consequence on their weight gain. Care of pups by males was less affected by exposure to BPA and EE, but control, non-exposed females appeared able to "sense" a male partner previously exposed to either compound and, as a consequence, reduced their own parental investment in offspring from such pairings. In general, F2 pups derived from parents who were both exposed to BPA or EE was less than that of controls during the early PNDs. F2 pup communication and analysis of USVs are ongoing and will be presented at the meeting. The data emphasize the potential vulnerability of pups born to parents that had been exposed during their own early development to EDC, and that effects on the male, although subtle, also have consequences on overall parental care due to lack of full acceptance of the male by the female partner. A reduced amount of time spent suckling did not translate into reduced growth of the pups, except in the cases where the pups were hyper-groomed. F2 pups also demonstrated a significantly lower body temperature than the other groups, possibly because of reduced parental huddling. Besides examining how reduced parental care effects F2 pup communications, we are also currently investigating the transcriptomic profile within hypothalamuses of F1 parents exposed to BPA and EE.

Neuropeptides in vertebrate reproduction: many players at the table
[Plenary]

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Since the discovery of GnRH back in the early 70s, the number of neuropeptides shown to modulate gonadotrophic activity in vertebrates has increased over the years. Among other findings, the discovery of GnIH in birds in 2000 and that of kisspeptins in mammals in 2003 were milestones in the progress of our knowledge. While these peptides have obviously key functions in the fine regulation of the GnRH neurons of birds and mammals respectively, their precise functions in other vertebrates are still a matter of debate. More recently, neurokinin B and dynorphin stepped into the picture at least in mammals in which KNDY neurons mediate the negative feedback of estradiol and are key regulators of LH pulsatile release. However, the roles of these different new comers in the central regulation of reproduction in other vertebrate groups is still not well defined and some time controversial. This lecture intends to summarize the most recent data obtained in different vertebrates, notably in fish regarding the potential involvement of these neuropeptides and in particular kisspeptins, in the regulation of gonadotrophin release.

Is the combined contraceptive pill as oestrogenic to fish as we think?
[S3, contributed]

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Assessing the risks posed by complex mixtures of environmental contaminants is a major challenge for aquatic toxicology. As *in vivo* testing of all possible mixtures is clearly impractical, computational modelling will be required in future. Chemicals integrate their effects within the underlying molecular networks governing organism physiology, resulting in complex and often unpredictable outcomes. Systems toxicology offers a powerful approach to develop the evidence base to understand chemical mixture interactions and identify their mechanisms. Three-spined stickleback (*Gasterosteus aculeatus*) were exposed to ten individual priority and emerging pollutants (B(a)P, $CdCl_2$, dibutyl-phthalate, ethinyl-oestradiol, fluoxetine, gemfibrozil, ibuprofen, levonorgestrel, PCB-118 and triclosan) in flow-through systems for 4 days. In addition, sticklebacks were exposed to twenty-six different mixtures of these compounds. Hepatic transcriptomics and MS-metabolomics were employed to characterize the molecular responses of the fish to these acute sub-toxic exposures. We found that although many molecular alterations can be explained by additive response models, some genes were activated in chemical mixtures alone whilst others were silenced by the presence of certain chemicals in the mixture. The latter was particularly evident when two specific chemicals, namely ethinyl-oestradiol (EE_2) and levonorgestrel (LV), a widely used synthetic progestin were present in the mixtures. LV antagonised the action of EE_2 in terms of key genes (vitellogenin, choriogenin) that lead to oestogenisation; ironically EE_2 and a progestin are always used together to make up the active ingredients of the contraceptive pill, suggesting that the overall oestrogenising potential of the pill is somehow overestimated when EE_2 is used on its own in fish exposures. Preliminary analysis suggests that the mechanism of this antagonistic behaviour between the two chemicals appears to be simple; LV appears to down regulate the gene expression of oestrogen receptor ($ER\alpha$), preventing the downstream effects that the ligant oestrogen (EE_2) could have initiated on

its own. Subsequent chronic exposure studies confirmed a strong anti-oestrogenic/androgenic effect of the synthetic progestin, challenging the long-held assumption that the contraceptive pill is highly oestrogenic to fish. To our knowledge this is the first report of this antagonism between two key components of the contraceptive pill. Our data are discussed not only in the context of novel findings but also in terms of monitoring data from UK rivers and estuaries looking at endocrine disruption biomarkers over the past 20 years.

Characterization of the gonadotropin-releasing hormone receptor in *Aplysia californica*
[P83]

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Gonadotropin-releasing hormone (GnRH) is the most upstream neuroendocrine activator of reproduction in vertebrates. A GnRH-like molecule was previously identified in the mollusk, *Aplysia californica,* and interestingly, this GnRH (ap-GnRH) does not appear to have a reproductive role. In an earlier study, we cloned the full-length cDNA of a Type II putative ap-GnRH receptor (ap-GnRHR). This receptor contains two potential translation start sites, each accompanied by a Kozak sequence, suggesting the translation of both a long and a short form of the receptor is possible. The putative ap-GnRHR maintains the conserved structural features and motifs of other known Type II GnRH receptors and shares high sequence identity with the octopus GnRHR. The expression of both long and short forms of the putative ap-GnRHR is confined to the central nervous system. The goal of this study is to examine, through a series of functional characterizations, if these two receptor isoforms are authentic ap-GnRHR. The cDNA encoding the long or the short receptor was subcloned into the pAWG vector and transfected into a protostomian cell line, the Drosophila S2 line. Transfected cells were subject to a radioreceptor assay using ^{125}I-labeled ap-GnRH as a radioligand. Further, they were treated with various concentrations of ap-GnRH or a related peptide, *Aplysia* adipokinetic hormone (ap-AKH), and measured for the accumulation of cAMP and inositol phosphate (IP). Radioreceptor assay revealed that only the long form of the receptor selectively bound to the radioligand, with cold ap-GnRH displacing the bound radioligand at EC_{50} of 3.54×10^{-8} M. Cells transfected with either form of the receptor did not respond to ap-GnRH or ap-AKH treatment with cAMP accumulation. However, cells transfected with the long receptor increased intracellular IP in a dose-dependent manner when treated with ap-GnRH, leading to a 3- to 6-fold increase in IP accumulation over controls. The results from our studies show that the long form of the putative ap-GnRH is indeed a bona fide ap-GnRHR that binds to ap-GnRH with high affinity to activate a signaling pathway commonly utilized by other GnRHRs.
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Adrenergic regulation of hepatic glycogenolysis in the European eel *Anguilla anguilla* at different silvering stages
[P8]

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The European eel (*Anguilla anguilla*) has a complex life cycle that includes migrations to and from seawater at different life stages. Immature specimens (yellow eels) spend up to 20 years in fresh or brackish water then metamorphose into silver eels, through the so called "silvering process", and start a long migration to the Sargasso Sea for reproduction. Strong changes occur during the silvering process: bodies become silver, eyes enlarge, gonads develop and the atrophy of the alimentary tract takes place. Profound modifications in hormone levels occur and sensitivities to these hormones are also expected to change. Although catecholamines (CA) may not be involved in silvering induction, their role in energy metabolism makes them a key to understanding the regulation of fuel availability in fish initiating a long reproductive migration. This study's aim is to investigate the adrenergic control of glucose metabolism in liver of eels collected from the Comacchio lagoon (North Adriatic Sea, Italy) at different silvering stages during different seasons. Eels were captured in Oct-Nov and Dec. Morphometric parameters were measured and animals were classified as silver eels at the 3rd and 4th stage, respectively. For comparison, yellow eels at the 2nd stage were collected during autumn and spring. Hepatocytes were isolated through collagenase perfusion and glycogen levels and glucose release were evaluated. As expected, in all animals epinephrine stimulated glucose release in a time and dose-dependent manner through cAMP elevation. However, hepatocytes sensitivity to the hormone slightly appeared to vary from stage to stage. To evaluate whether epinephrine sensitivity is related to a differential expression of adrenergic receptors in hepatocytes, expression of α1- and β2- AR mRNAs was evaluated, revealing a higher AR mRNA expression in silver than in yellow eels.The increased expression of AR mRNAs in silver eels may indicate a different modulation of CA on liver metabolism, which may concern glucose metabolism and also lipid metabolism (not addressed in this study). This is the first investigation on adrenergic regulation of glucose metabolism in the liver of European eel at the silver stage.

Environmental gestagens disrupt endocrine systems associated with reproduction and metamorphosis in amphibians
[S3, invited]

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Endocrine disruption by various compounds has become an emerging concern especially for aquatic wildlife because surface waters are the main sink of endocrine disruptors (ED). In the past research focused on (anti)estrogenic, (anti)androgenic, and (anti)thyroidal substances affecting reproduction and development in vertebrates but further endocrine systems might be also targets for ED. More recently environmental gestagens, including the natural progestogens (e.g. progesterone (P4)) and synthetic progestins, have been identified as potential ED. Gestagens have been supposed to affect vertebrate reproduction via progesterone receptors

especially by progestins being the major compound of the "mini pill" for contraception of humans. Amphibians are suitable models to assess endocrine disruption and also targets of gestagens. Exposure to progestogens seems to be less effective in comparison to progestins (e.g. levonorgestrel (LEVO)) because LEVO affected male mating calling of *Xenopus laevis* whereas P4 had no effect at all. However, this might be due to the particular androgenic mode of action of LEVO on androgen receptors. During larval exposure LEVO disrupts sexual development in *X. laevis* by affecting gene expression of pituitary gonadotropins and gonadal steroidogenic enzymes. Surprisingly, in parallel LEVO also impairs metamorphosis by disruption of the thyroid system. However, the underlying molecular mechanisms need still to become elucidated. In the study by Kvarnryd et al. (2011) larval exposure to LEVO had long lasting effects on *X. tropicalis* adults affecting especially females lacking oviducts and having histopathological patterns of the ovaries. Recently, in order to get a better insight concerning the mechanisms how gestagens might affect endocrine organs we shift from in vivo experiments to in vitro organ cultures to reveal potential direct effects of gestagens on target organs and the recent findings will be presented.

Krüppel-like factor 9 is a circadian transcription factor that regulates clock output genes and participates in corticosteroid modulation of peripheral circadian oscillators
[S5, contributed]

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In mammals cellular circadian rhythms are maintained in part by a core transcription-translation feedback loop that oscillates with an approximately 24 hour periodicity. This loop is necessary to maintain free-running circadian activity rhythms in the absence of external cues. While this loop runs independently of endocrine influences in the central neural pacemaker (the suprachiasmatic nucleus of the hypothalamus), in many other neural tissues and peripheral organs it is exquisitely sensitive to modulation by glucocorticoids (CORT). The core clock genes *Per1* and *Per2* are directly and rapidly upregulated by CORT bound to the glucocorticoid receptor (GR). This strong upregulation results in resetting of circadian rhythms in GR-expressing cells and has been hypothesized to be an important mechanism for synchronizing circadian oscillations in peripheral tissues. However, other transcription factors may participate in circadian transcriptional regulation. The zinc-finger transcription factor Krüppel-like factor 9 (Klf9), an evolutionarily conserved immediate-early CORT/GR target gene, has previously been shown to have circadian oscillations in expression in mouse liver and human keratinocytes. We conducted chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) in a mouse hippocampal cell line (HT22) to identify Klf9 genomic targets, and found that Klf9 associated with several circadian genes, including *Per1-3, Dbp, Tef,* and *Nr1d1*. For *Per1*, the regions bound by Klf9 overlapped with known GR binding sites. We therefore tested the hypothesis that Klf9 acts as a feed-forward repressor of circadian clock gene expression. Forced expression of Klf9 in HT22 cells delayed CORT induction of *Per1*, suggesting that Klf9 may act as a feedforward brake on *Per1* induction, which we hypothesize is necessary for proper resetting of the clock under conditions of elevated CORT. Forced Klf9 expression also repressed expression

of the clock output gene *Dbp*. ChIP assays on HT22 cells synchronized with CORT showed association of Klf9 with a *Dbp* intronic enhancer necessary for circadian expression at a time point that coincided with high *Klf9* mRNA and low *Dbp* mRNA, consistent with rhythmic repression of this locus by Klf9. Our data suggest that Klf9 may be a novel link between the hypothalamo-pituitary-adrenal axis and peripheral circadian clocks (Supported by NINDS 1 R01 NS046690 and NSF IOS 0922583 to RJD, and NIH 1T32HD079342-01 and MCubed funding to JRK).

The impact of pubertal immune stress on learning and memory between the sexes
[S12, contributed]

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Research into puberty as a critical developmental period suggests that early activation of the innate immune response influences various aspects of brain function and cognition, including learning and memory. However, the enduring effects of a pubertal immune challenge on spatial memory and its relation to the sexes remain to be investigated. The objective of the current study is to examine sex differences in short- and long-term hippocampal-dependent visuo-spatial memory and cognitive flexibility following a single pubertal exposure to the bacterial endotoxin lipopolysaccharide (LPS). A sample of 40 male and 40 female CD-1 mice was shipped at three weeks of age. At six weeks of age (i.e. pubertal), male and female mice were injected intraperitoneally with LPS (1.5 mg/kg) or 0.9% sterile saline control (1.5 mg/kg). To examine the effects of gonadal hormones on learning and memory, mice underwent either gonadectomy or sham-operation upon at nine weeks of age. Following one week of recovery, at ten weeks of age (i.e. in adulthood), the mice were tested in the Barnes Maze and Morris Water Maze during their dark cycle. An open field test was used to assess differences in locomotor activity. Based on previous research, it is expected that (1) LPS-injected mice outperform their saline counterparts and (2) males outperform females regardless of treatment, and (3) that gonadectomized females will perform better than sham-operated females on visuo-spatial memory tasks. Taken together, these findings suggest important interactions between pubertal immune stress and gonadal hormones on cognitive function.

Claudin-8 tight junction protein isoforms and cortisol-mediated alterations of teleost fish gill epithelium paracellular permeability
[S13, contributed]

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Because the internal fluids of freshwater (FW) teleost fishes are hyperosmotic relative to their surroundings, these organisms experience obligatory ion loss across epithelia that directly interface with water (e.g. the gill epithelium). The tight junction (TJ) complex is broadly acknowledged to limit paracellular ion loss across the FW fish gill epithelium as it occludes the

paracellular cleft and therefore acts as a barrier to impede paracellular ion movement. The vertebrate TJ complex is composed of a large number of TJ proteins, and the largest of these, the claudin (Cldn) superfamily of TJ proteins, have recently been implicated in establishing the barrier properties of the FW fish gill epithelium. Cortisol is an important ionoregulatory hormone in teleost fishes and its role in mediating mechanisms of transcellular ion transport across ionoregulatory epithelia is well established. It has also recently been established that cortisol can significantly reduce paracellular permeability of the FW teleost fish gill epithelium, and this has been linked to the actions of cortisol on select TJ proteins. Nevertheless, a clear picture of how this is achieved has yet to emerge, due in part to the complexity of the system. Cldn-8 is (generally speaking) a barrier forming TJ protein that due to genome duplication or tandem gene duplication events typically exhibits four isoforms in teleost fishes (Cldn-8a, -8b, -8c and -8d). In the gill epithelium of teleosts, *cldn-8d* appears to be the most abundant *cldn-8* isoform and *cldn-8d* abundance is reported to increase dramatically under circumstances that coincide with the *in vitro* establishment of gill epithelium confluence and the development of gill epithelium electroresistive properties. In addition, *cldn-8d* abundance also increases in association with cortiol-mediated reductions in gill epithelium paracellular permeability. This study sought to examine the broader role of Cldn-8 isoform contribution to corticosteroid regulation of gill epithelium permeability by examining the cortisol responsiveness of all Cldn-8 isoforms and by using transcriptional knockdown of cortisol sensitive Cldn-8 isoforms as a tool to experimentally dissect the specific role of these TJ proteins in the endocrine–mediated regulation of gill epithelium permeability.

Do environmental conditions matter? Gonadal androgens have neither direct nor indirect effect on male growth in a captive gecko
[P75]

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Changes in the effect of gonadal androgens (GA) on male growth are considered to be the proximate mechanism of the shifts in sexual size dimorphism (SSD) among reptiles. Positive effect of GA on male growth was reported in male-larger species of squamates, while negative in female-larger species. In contrast to this generally accepted hypothesis, in our previous study we documented in geckos that GA do not affect male growth under constant thermal conditions. This discrepancy could be attributed to the effect of GA on thermoregulation and therefore on body temperature, which might affect metabolic rate, general activity and hence indirectly growth. We tested this possibility by monitoring growth of control (placebo treatment) and castrated males in the male-larger Madagascar ground gecko (*Paroedura picta*) kept in social isolation in thermal gradient for more than a year. Low testosterone levels in castrated males were verified by the liquid chromatography-mass spectrometry. We did not find any differences between treatment groups in growth rate, final body size or amount of adipose tissue. Castration also affects neither metabolic rate measured as oxygen consumption nor foraging activity of experimental geckos in open field. In castrates, we found reduction in tissues directly connected with reproduction (hemipenes and spermaducts associated with kidneys). Enlarged livers in

castrates, the only other significant difference between treatments, are probably connected with their larger energy supplies. In conclusion, GA seems to have neither direct nor indirect effect on the male growth and hence ontogeny of SSD in geckos and the evolutionary changes in SSD found in this group cannot be attributed to changes in GA metabolism.

Involvement of parathyroid hormone in calcium regulation and gill cartilage development in zebrafish
[108]

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The present study investigated the role of parathyroid hormone (PTH) in Ca^{2+} homeostasis and cartilage formation in developing zebrafish (Danio rerio). We demonstrated that translational gene knockdown of PTH1 decreased Ca^{2+} uptake at 4 days post fertilization (dpf). Additionally, the numbers of epithelial Ca^{2+} channel-expressing cells were reduced in PTH1-deficient fish. Knockdown of PTH1 caused a shortening of the jaw and impeded the development of gill cartilage. Disorganization of chondrocytes in craniofacial cartilage also was observed in fish experiencing PTH1 knockdown. The results of real-time PCR demonstrated that PTH1 morphants failed to express the transcription factor glial cell missing 2 (gcm2). Co-injection of PTH1 morpholino with gcm2 cRNA rescued the phenotypes observed in the PTH1 morphants, suggesting that the defects in PTH1-deficient fish were caused, at least in part, by the suppression of gcm2. Taken together, the results of the present study reveal critical roles for PTH1 in promoting Ca^{2+} uptake and gill cartilage formation during development.

Brain and plasma oxytocin levels under basal and mating conditions in the female rat
[P62]

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Sexual activity is known to affect oxytocin (OT) neuron activation in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). However the specific response of each nucleus to the different components of mating is unclear. Furthermore, there has been no consensus on what changes occur in plasma OT levels following mating in rats. Therefore, we hypothesized that changes in brain and plasma OT levels are dependent upon the quality of the sexual experience, and the physical stimulations received. To verify this hypothesis we designed three studies. In Experiment 1, proestrus and metestrus female rats were used to investigate baseline plasma and brain OT levels. In Experiment 2, ovariectomized sexually-experienced animals were used to study the effect of vaginal cervical stimulation (VCS) and paced mating on OT levels. Females were either vaginally masked or unmasked, and were either paced or non-paced during a 30 min trial. In these two studies, brain OT was extracted and both brain and plasma levels were measured with an ELISA. Finally, in Experiment 3 we examined the peripheral projections of OT neurons from the PVN and SON using retrograde tracing with fluorogold (FG) and dual

labeling in the PVN and SON in cycling females. Our results show that OT levels in the PVN and the SON were similar at baseline, not correlated with plasma OT levels, and unaffected by the estrous cycle. For Experiment 2, VCS increased OT levels in the PVN but did not affect the SON, or OT levels in plasma. Paced mating had no effect on brain or plasma OT levels. Finally, results from Experiment 3 showed that the PVN and SON both projected equally to the periphery in this species at both proestrus and metestrus. These findings indicate that VCS is important for the activation of OT in the PVN during mating. However, the lack of correlation with changes in plasma levels during mating suggests that OT is not being released from the PVN into the periphery. Additionally, both the SON and PVN project equally to the periphery, and thus can equally contribute to OT peripheral release. Plasma and brain OT levels are not affected by the estrous cycle. Interestingly, the role of the SON appears to be unrelated to sexual behaviour in the sexually-experienced female rat. In conclusion, our results suggest that the OT PVN neuron population may be particularly important for the contribution of OT to mating in the female rat, and that VCS is required for OT release in this nuclei.

Identifying molecular mechanisms underlying crosstalk between the thyroid hormone and androgen axes in the frog *Silurana tropicalis*
[P63]

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The objective of this study was to examine molecular markers of crosstalk between the thyroid hormone (TH) and androgen axes in the frog *Silurana tropicalis*. Transcriptional changes within a target tissue are not the result of a single linear signaling pathway, but rather reflect pathway integration upon multiple regulatory levels. Identifying molecular mechanisms underlying cross-regulation *in vivo* is consequently difficult. Adult brain, liver, testicular, and ovarian tissues were exposed *ex vivo* to triiodothyronine (T3; 50 nM), iopanoic acid (IOP; 10 μM – a TH disruptor), testosterone (T; 5 μM), and to both 5α- and 5β-dihydrotestosterones (DHTα; DHTβ; 5 μM) for 6 h. Enzyme-linked immunosorbent assays were used to measure hormone levels in media following the exposures. Real-time RT-PCR analysis confirmed significant increases in TH-related transcripts in the brain, liver, and gonads following T3 exposure (TH receptors: *trα* and *trβ*; deiodinases: *dio2* and *dio3*), thereby highlighting regulation by THs. Local synthesis and metabolism of THs was confirmed by decreases in *trβ* in the ovary tissue following treatment with IOP; however adult tissues appeared overall unaffected by direct exposure to the deiodinase inhibitor, IOP. Direct crosstalk between the TH and androgen axes was not observed in adult male tissues; however cross-regulation was observed in adult females. The 5α-reductase type 2 mRNA levels decreased with both T3 and IOP treatments. Significant increases in the concentration of estradiol in media following T3 and IOP treatments corroborates with this observed decrease. Changes in circulating DHT levels were not observed between the T3 and IOP treatments. Interestingly, androgen treatments confirmed reciprocal crosstalk between the TH and androgen axes. All three androgens induced a significant increase in *trβ* expression levels in the testis tissue. Liver *trβ* and *dio3* transcript levels were also increased significantly with T treatment. Overall, these findings reveal tissue- and sex-specific molecular mechanisms

of crosstalk, as well as the maintenance of cross-regulation between the TH and androgen systems at sexual maturity in frogs.

Developmental expression of *TRα* and *TRβ* in the limb of the direct-developing frog *Eleutherodactylus coqui*
[P94]

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Direct development is a life-history strategy that has evolved independently in at least a dozen Anuran lineages. Direct-developing frogs, including the Puerto Rican coquí, *Eleutherodactylus coqui*, hatch from terrestrial eggs as miniature adults. Their embryonic development is characterized by precocious formation of adult features, such as limbs, and the absence of tadpole-specific features, such as jaw cartilages. In metamorphosing frogs, limb growth is a metamorphic change mediated by thyroid hormone (TH). Because TH has widespread and diverse effects throughout metamorphosis, changes in TH signaling could underlie the evolution of direct development. Specifically, changes in temporal or spatial expression of the nuclear thyroid receptor α (TRα) or thyroid receptor β (TRβ) in the target tissue could facilitate the early development of limbs. Primers were designed for quantitative RT-PCR in primer3 for *TRα* and *TRβ*. Total RNA was isolated from limbs at each developmental stage. qRT-PCR was used to examine *TRα* and *TRβ* expression in the developing *E. coqui* limb. *TRα* expression is significantly higher than *TRβ* expression throughout most of limb development and peaks at stage 10. *TRβ* expression is low during early development and rises significantly throughout limb development. Prior studies suggest limb development in direct developing frogs is independent of TH. However, these data are approximately similar to *TRα* and *TRβ* expression patterns observed in the developing limb of the metamorphosing frog *Xenopus laevis*, where limb development is dependent on TH. These data suggest that the *E. coqui* limb is at least TH competent and thyroid mediated development may begin earlier than previously thought. This work is an important first step in describing the mechanism of direct development and will serve as a comparison to examine the developmental basis of this life history strategy in other amphibian groups.

Age and sex differences in the effect of chronic partial sleep disruption on the corticosterone response to a novel stressor in mice
[P100]

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Puberty is marked by dramatic changes in the neuroendocrine system. Exposure to stressors during this period may result in altered future hormonal responses. Exposure to chronic sleep disruption has been shown to alter the responsiveness of the hypothalamic-pituitary-adrenal

(HPA) axis to future novel stressors. Since there are important age and sex differences in the responsiveness of the HPA axis to stressors, the objective of the current study was to examine the age and sex differences in the responsiveness of the HPA axis to a novel stressor after chronic sleep disruption. Based on previously published work, we hypothesized that chronic partial sleep disruption will induce a sensitization of the HPA axis in pubertal male and female mice and in adult female mice, but not in adult male mice. We predicted that in response to a novel stressor, there will be (1) an increased corticosterone response in sleep deprived (SD) pubertal male and female mice and in adult female mice, with (2) a higher corticosterone response in SD pubertal females than in adult females, and (3) no change in the corticosterone response in SD adult male mice, compared to non-sleep deprived (NSD) mice. To test these hypotheses, mice were sleep deprived for four hours per day for seven continuous days, and on the last day, they were subjected to a restraint stress, immediately followed by euthanasia and blood collection. Females across all groups showed higher corticosterone levels than males. The corticosterone response to a novel stressor was significantly increased in adult SD females compared to NSD adult females. This increased corticosterone response was present but not significant between the SD and NSD pubertal females. There was no difference in the corticosterone response in SD and NSD pubertal and adult males. This study provides evidence that there are age and sex differences in the effect of chronic partial sleep disruption on the responsiveness of the HPA axis to a novel stressor.

Characterization of novel neuropeptides modulating fish reproduction
[S7, invited]

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The HPG axis plays a critical role in the control of fish reproduction. This axis is tightly controlled by GnRH promoting LH and FSH synthesis and release from pituitary gonadotrops. However, the mechanisms whereby puberty and function of the reproductive axis begun to be deciphered only recently, and apparently involve a plethora of neuropeptide hormones, which impinge and integrate at the hypothalamic centers governing reproduction. These novel neuropeptide will be the focus of the current lecture. 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, as a model fish, in terms of receptor transactivation, distribution in the brain and pituitary and finally in in vivo experiments. Two h after ip injection of tiNKB, the plasma levels of both FSH and LH were increased, whereas tiNKF was more effective in increasing LH levels. However, tiNKB was more effective than tiNKF in increasing both GTHs from tilapia pituitary dispersed cells. Using in situ hybridization and fluorescent immunohistochemistry, we have shown that LH cells possess NKB and its receptors mRNAs, whereas FSH cells possess mainly NKB receptors. LPXRFa peptides have been characterized for their ability to inhibit GTH release in birds and stimulate GH release in frogs. Signal-transduction analysis of the tilapia LPXRF-R showed stimulation of the PKA pathway, whereas the huNPFFR1 showed suppression of forskolin-induced activity in this system. Administration of the tilapia LPXRFa-2 peptide to primary cell culture of pituitaries, or to reproductive female by ip injection, positively regulated both LH and

FSH release. Using double-labeled fluorescent ISH and immunofluorescence, βLH cells were found to coexpress both lpxrf and lpxrf-r mRNA, whereas some of the βFSH cells coexpressed only lpxrf-r mRNA. No coexpression of lpxrf-r was identified in GH-positive cells. However, endocrine factors that has a negative control on the HPG axis are less characterized in teleost fish. We further characterized tilapia spexin and its receptors. An ip injection of tilapia spexin significantly inhibited both LH and FSH release, with some differences between genders and reproductive stages. Our next step will be to study the organization and interrelationship of neuropeptides in the fish brain and pituitary leading to successful reproduction.

Structural and transcriptomics analysis of IGF ligand, receptor, and binding proteins in a short-lived fish
[P95]

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Considerable progress has been made in recent years in discovering genes that are involved in regulating lifespan. Many of these genes are members of the Insulin/Insulin-like growth factor Signaling (IIS) pathway. Signaling through this conserved pathway was found to promote aging in *C. elegans* and *Drosophila*. In vertebrates, this signaling pathway has diverged into the insulin pathway and the IGF signaling pathway. While there is some evidence to suggest that the IGF pathway has retained the pro-aging effects of the ancestral IIS pathway, contradictory evidence has also been reported. Whether the vertebrate IGF signaling pathway has pro- and/or anti-aging effects is still under debate. Furthermore, the vertebrate IGF signaling system contains another crucial component, the IGF binding proteins (IGFBPs). These proteins bind IGF with high affinity and are able to inhibit or enhance IGF signaling by regulating the IGF ligand and receptor interaction. The roles of these IGFBPs in aging remain largely unexplored. This is because the currently used vertebrate model organisms, i.e. rodents and zebrafish, have a long lifespan of 3-5 years. Recently, the short-lived teleost fish *Nothobranchius furzeri*, has emerged as a promising vertebrate model for aging biology. While some strains of this species have lifespans of up to 9 months in the lab, an inbred strain (GRZ) has a lifespan of only 3 months, making it comparable to that of *C. elegans* and *Drosophila*. As an initial step to study the role(s) of IGF signaling in aging, we have cloned and characterized major components of *Nothobranchius* IGF signaling pathway, including IGF-I, IGF-II, IGF1 receptor, and six IGFBPs. Structural and phylogenetic analyses suggest that the major components of IGF system are highly conserved in this teleost fish. As in the case of many other teleost species, some of these genes are duplicated and retained in *Nothobranchius furzeri.* Transcriptome analysis was performed and the spatial expression of these IGF related genes have been determined throughout the entire life span. These data will be reported and discussed.

Fusion of testis is beneficial to male reproduction in *Spodoptera litura* (Lepidoptera)
[P31]

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Insects usually have two bilateral symmetry testes. In some species of Lepidoptera, two testes of larva fuse together during pupating metamorphosis and one single testis is formed at pupal stage. The fusion of testis was recorded in many agricultural lepidopteran pests which have strong fertility. Howerver, in economic lepidopteran insect silkworm, two testes do not fuse during metamorphosis development and keep separation in all developmental stages. The influence of testes fusion on sperm development and reproduction, its biological significance and molecular regulation mechansim are not clarified yet.

Using lepidopterous pest *Spodoptera litura* as experimental animal which testes fuses together during testicular development, the influence of testes fusion on sperm development and its biological significance was investigated. The two testes of *S. litura* larvae at day 4 of 6th instar (L6D4) are separated and they are move against each other, attach closely and fuse together during pupation at day 6 of 6th instar (L6D6). The testes completely fuse together at day 1 after pupation (PD1). A barrier was placed in the middle of two testes of *S. litura* at larval stage L6D4 by our developed microsurgery method. As a kind of control, unilateral testis was removed using same method. The wound was healed 2 days after surgery and *S. litura* showed normal pupation, eclosion and copulation. The mortality, pupation rate, percentage of adult emergence and mating success after microsurgery were no difference compared with control or slam worms. Unfusion testes were successfully obtained at both pupal and adult stage by microsurgery method. When testis was blocked to fuse at pupal stage, the number of sperm bundles decreased significantly compared with control groups. And the fertility (percentage of egg hatch) of female adult mating with male adult which had unfusion testes declined significantly in comparision with control groups The results indicated that unfusion of testis led to decrease of sperm bundle and impairment of male reproductive fertility in *S. litura* and fusion of testes in *S. litura* may benefit the male reproduction.

To gain gene expression profiles and identify the genes that may be involved in the testis fusion, gene expression patterns in the testes at L6D4 (pre-fusion), L6D4 (fusing), PD1 (post-fusion) stages were analyzed by using RNA-seq method. A total of 63,292 unigenes were generated, of which 28,909 unigenes were annotated. Compared to pre-fusion and post-fusion testes, 2,846 and 6,318 DEGs (differentially expressed genes) were found in the fusing testes, respectively. Out of these DEGs, 2457 and 4,616 were up-regulated at the fusing stage, respectively, and 1,329 were specifically up-regulated at the fusing testes as compared both pre-fusion and post-fusion testes. Genes that are involved in cuticular proteins, tubulin, serine proteases, glutamine synthetase, lysocardiolipin acyltransferase, lectin and chitin deacetylase were significant differentially expressed during the process of testes fusion.
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miRNA regulation of endocrine dependent cancer
[Presidential symposium]

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miRNAs are a class of non-coding RNAs that modulate the protein expression of target mRNAs by promoting mRNA degradation or inhibiting mRNA translation. miRNAs have been identified to possess both oncogenic or tumor suppressor roles in cancer development and progression. Similar to hormonal function, miRNAs possess the capacity to regulate large, yet specific patterns of gene expression to exert their cellular effects. It is therefore not surprising that hormones involved in cancer progression exert their co-ordinated yet distinct cellular activities, at least partially, through regulation of miRNA expression. I will present examples, identified in our laboratories, of endocrine regulated miRNAs that possess specific roles to either promote breast cancer cell proliferation (miR 26) or dissemination (let-7g). We have demonstrated that estrogen decreases the expression of miR 26a and 26b, dependent on c-MYC, which promotes estrogen stimulated cell proliferation *in vitro* and *in vivo*. Cross-screening of genes predicted to miR 26 regulated and also demonstrated to be estrogen regulated, with functional and clinical analyses of these genes, identified two genes (CHD1 and GREB1) critical for estrogen stimulated breast cancer cell proliferation. Estrogen also decreases the expression of another miR, let-7g, dependent on p44/42 MAP kinase, which promotes estrogen stimulated cell invasion and dissemination. Decreased let-7g expression in breast cancer is an independent predictor of lymph node metastases and poor survival outcome. Let-7g was determined to regulate two clinically relevant genes in involved in metastasis, GAB2 and FN1. Hence, estrogen regulates distinct miRNAs in breast cancer cells to co-ordinate specific cellular processes involved in breast cancer progression.

Applied comparative endocrinology and novel hormone entities: Finding a pathway to commercialization
[S8, invited]

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Investigation of endocrine systems amongst the over 3 million species of animals is a rich source of discovery of novel hormones, receptors and associated mechanisms. Indeed, most of the hormones developed for therapeutic uses in humans were initially found in non-human species. However, the route from discovery to the first clinical trials of a new drug entity and its eventual commercialization has become increasingly more complex. Moreover, the prohibitive cost of such an endeavour makes independent development out of practical reach by academic scientists. Despite these obstacles, there are a number of advantages for engaging in a path to commercialization. These may include increased funding opportunities, a closer relationship with industry and increased opportunities for graduate students and trainees. Protagenic Therapeutics, Inc., (PTI) is an American-Canadian biotechnology company founded on an algorithm to identify

novel peptides in the genome. This led to the discovery of the teneurin C-terminal associated peptides (TCAP), a novel family of highly conserved peptides found in most metazoans. Variants of these peptides are currently being developed for FDA applications in the area of neurology and metabolism. However, because of the uniqueness of these peptides, the pathway to development was far from clear. Thus, PTI has developed considerable experience in the development of novel peptides for commercialization. A practical approach for peptide development will be presented.

Seasonal life-history transitions between reproduction, migration, and foraging in red-sided garter snakes: The role of neuropeptide Y and arginine vasotocin
[P64]

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Many animals exhibit seasonal changes in life-history stages, and these seasonal transitions are often accompanied by dramatic switches in behaviour. For example, some migrating animals undergo a transition from reproductive behaviour to feeding behaviour. While the neuroendocrine mechanisms that regulate such behavioural transitions are poorly understood, neuropeptide Y (NPY) and arginine vasotocin (AVT) are excellent candidates: NPY regulates feeding behaviour in all vertebrates studied to date, while brain AVT regulates reproductive behaviour in many organisms. We previously showed that feeding male red-sided garter snakes (*Thamnophis sirtalis parietalis*) have more neuropeptide Y (NPY) immunoreactive (-ir) cells in the brain compared to non-feeding males, but no difference in arginine vasotocin (AVT) cell number. In this study, we asked if seasonal changes in NPY and/or AVT are concomitant with spring migration away from the breeding grounds, as snakes are transitioning from reproductive to foraging behaviour during this time. We collected male and female snakes in different migratory stages during the spring and fall. Brains were processed for NPY and AVT immunohistochemistry and the total number of labeled cells quantified for each individual. Surprisingly, females did not exhibit seasonal changes in either NPY or AVT. In contrast, males had significantly more NPY-ir cells during the fall compared to the spring in the cortex and posterior hypothalamus, likely reflecting increased feeding behaviour during the summer foraging period. As predicted, males had significantly more AVT-ir cells in the preoptic area and bed nucleus of the stria terminalis during the spring mating season compared to the fall. Neither NPY- nor AVT-ir cell number varied significantly with migratory status, indicating that seasonal changes in these neuropeptides are not directly related to migration. We then asked if the observed seasonal changes in NPY and AVT in males are related to the transition in behaviour from courting to non-courting. Compared to courting males, non-courting males had significantly more NPY-ir cells in the cortex and more AVT-ir cells in the supraoptic nucleus. Collectively, our results suggest that NPY and AVT play a role in regulating seasonal transitions in male reproductive behaviour, rather than regulating migration per se. Further, these data indicate that both NPY and AVT may be involved in mediating sex differences in the timing of life-history transitions.

Anti-apoptotic effects of growth hormone (GH) and IGF-I in chicken cerebellar cell cultures during the hypoxia injury
[P84]

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It is known that growth hormone (GH) and IGF-I, and their corresponding mRNAs, are locally expressed in the Central Nervous System (CNS), suggesting that these hormones may be involved in some functional roles mediated by autocrine/paracrine mechanisms in the brain, such as neuro-protection in the CNS after a severe insult, for instance damage by hypoxia. Previous results showed that GH and IGF-I concentrations increased 1.7- and 1.4-fold, respectively, in primary cerebellar neuron cultures exposed to hypoxia. Both GH and IGF-I may act as neuroprotective factors and have been implicated in cell survival, inhibiting apoptosis in several cell types. We studied the possible neuroprotective role of GH and IGF-I in a model of ischemic neuronal injury (hypoxia and low glucose, HLG) using primary chicken cerebellar neuron cultures. The viability of cerebellar neurons exposed to HLG decreased 2.5 times, compared to the control. However, treatment with recombinant chicken GH (rcGH, 1 nM) or IGF-I (40nM) significantly recovered viability (to 1.7 and 2 times, respectively) of the cell cultures. Likewise, the addition of rcGH decreased the number of apoptotic cells marked by TUNEL (15.3±2.2%) when compared with those exposed only to HLG (68.1±12.1%). Similarly, the activity of caspase-3 in cerebellar neurons exposed to HLG (7.5±1.3 Units) was significantly reduced by rcGH (1.5 times) and IGF-I (1.2 times) treatments (4.8±0.9 and 6.0±0.53 U, respectively). On the other hand, addition of rcGH and IGF-I together only inhibited caspase-3 activity 1.5-fold when compared with HLG, showing no synergistic effects. Immunoneutralization of GH by co-incubation with a specific anti-GH antibody blocked the effect upon the activity of caspase-3, whereas the addition of anti-IGF-1 did not modified the GH protective effect. These results suggest that the neuroprotective effect of GH could be direct, and not mediated by IGF-I, in the embryonic chicken cerebellum where the locally expressed GH may act as an autocrine/paracrine survival factor that preserves cellular viability and inhibits apoptotic cell death.
Supported by PAPIIT-DGAPA, UNAM 208812, 206813, 206115; CONACYT 118353.
Technical support by Courtois, G. is acknowledged.

Antiapoptotic effects of growth hormone are mediated by PI3K/Akt pathway in the chicken bursa of Fabricius
[P96]

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Growth hormone (GH) is expressed in several extra-pituitary tissues, including the primary and secondary lymphatic organs of the immune system. In birds, GH mRNA and protein expression show a specific developmental distribution pattern in the bursa of Fabricius (BF), particularly in epithelial and B cells. Changes in the bursal concentration and distribution of locally produced GH during ontogeny suggest it is involved in B cell differentiation and maturation, as well as in a functional survival role in this organ which may be mediated by paracrine/autocrine mechanisms. Here, we analyzed the anti-apoptotic effects of GH in BF and the intracellular signaling pathways involved in this activity. Also, we studied if this was a direct effect of GH or mediated by IGF-I. Bursal cell cultures showed an important loss of their viability after 4 h of incubation and a significant increase in apoptosis. However, treatment with 10 nM GH or 40 nM IGF-I significantly increased B cell viability (16.7±0.67 and 13.4±1.12%, respectively) when compared with the untreated controls. In addition, the presence of apoptotic bodies (TUNEL) dramatically decreased (5.5-fold) after GH and IGF-I treatments, whereas co-incubation with anti-GH or anti-IGF-I, respectively, blocked their anti-apoptotic effect. Likewise, both GH and IGF-I significantly inhibited caspase-3 activity (by 40±2.0%) in these cultures. The addition of 100 nM wortmaninn (a PI3K/Akt inhibitor) blocked the GH protective effects. Also, GH stimulated (3-fold) the phosphorylation of Akt in bursal cells, and adding wortmannin or an anti-GH antibody inhibited this effect. Furthermore, GH was capable to stimulate (7-fold) the expression of Bcl-2. Taken together, these results indicate that the anti-apoptotic activity of GH observed in the chicken bursal B cell cultures might be mediated through the PI3K/Akt pathway. Supported by PAPIIT-DGAPA-UNAM IN208812, IN206813, IN206115; CONACYT 178335. JLLA received a PhD fellowship from CONACYT (200220)

Sex or candy? Neuroendocrine regulation of seasonal life-history transitions
[Gorbman-Bern New Investigator Lecture]

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Examples of life-history transitions are readily apparent in nature and include seasonal changes in reproductive behaviour, migratory activity, foraging and hibernation. Intriguingly, both sex and phylogeographic differences in the timing of life-history events occur in many species, yet the neuroendocrine factors underlying these differences remain enigmatic. Using common garter snakes (*Thamnophis sirtalis*) as a model system, research in my lab focuses on the neuroendocrine mechanisms that mediate the seasonal transition from winter dormancy to spring mating behaviour and from spring mating to migration and summer foraging. We use an exceptionally well-studied population of red-sided garter snakes as an "anchor" in our comparative approach, with the goal of more quickly identifying the factors that govern reproductive timing. Although the red-sided garter snake is infamous for its temporally dissociated reproductive pattern (i.e., mating does not coincide with peak gonadal activity), we recently found exciting evidence that the neuroendocrine gonadotropin-releasing hormone (GnRH) system is strongly modulated by environmental temperature. Moreover, temperature-induced increases in GnRH are sexually dimorphic. These results suggest that the hypothalamus-pituitary-gonad axis is indeed critical to reproductive regulation in this dissociated breeder, and provide compelling support for a neuroendocrine mechanism underlying sex differences in

reproductive timing. To better understand the phenomenon of seasonal reproduction, we also investigate the factors that inactivate reproductive behaviour at the end of the mating season. Thus far, our research indicates that adrenal glucocorticoid hormones are critical to this life-history transition. Specifically, decreased glucocorticoids directly activate feeding behaviour and likely induce migration from the breeding grounds. This transition to feeding behaviour is concomitant with increased neuropeptide Y cells in the brain, a potent regulator of appetite and feeding in all vertebrates. Together, these data advance the unconventional hypothesis that glucocorticoids are *necessary* for the expression of energetically costly sex behaviour. As we continue to investigate the neuroendocrine factors that regulate life-history transitions, comparative analyses among different populations, species, and environments will reveal critical insight into how organisms successfully orchestrate seasonal rhythms within a changing environment.

Early-life bisphenol-A (BPA) exposure impairs adult leptin sensitivity and predisposes to obesity
[P44]

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Bisphenol-A (BPA) is a component of polycarbonate and many other types of plastics. BPA has been characterized as an endocrine disruptor, most famously due to observations of its estrogenic activity in various experimental models. Because BPA is a common constituent of food and drink containers, and because it can leach out under certain conditions, human exposure is nearly ubiquitous.

Emerging evidence from our lab has shown that CD-1 mice exposed to low, environmentally relevant doses of BPA early in life exhibit an adult phenotype characterized by sex-specific metabolic disruptions. Specifically, male mice exhibit impaired glucose tolerance, and females a propensity toward diet-induced obesity. Given the sensitivity of the hypothalamus to the organizational effects of sex steroids, we explored the possibility that early-life BPA exposure adversely affects the development of hypothalamic feeding circuitry to bring about these effects. To test this hypothesis, we used male and female CD-1 mice exposed pre- and post-natally to either a control diet, a diet containing BPA (appx. 13.5 µg/kg/day), or the estrogenic diethylstilbestrol (DES) as a positive control (appx. 3 µg/kg/day). Serum from pups was collected on PND2, 8, 10, 12, 16, and 21 for analysis of circulating leptin. Results from this study show that BPA and DES exposed pups have respectively delayed and blunted postnatal leptin surges – a state of affairs that points to a role for leptin in the organizational effects of early-life xenoestrogen exposure. qRT-PCR analysis of leptin mRNA expression in white adipose tissue collected at the same time suggests that BPA and DES act at the transcriptional level to bring about these effects. Both male and female BPA-exposed mice showed a reduced density of POMC projections into the PVN. This phenotype was rescued in female BPA-exposed animals given daily injections of supplemental leptin (5 µg/g/day). Adult offspring from this experiment were resistant to leptin-induced suppression of food intake, body weight loss, and hypothalamic POMC upregulation. Taken together, these data suggest that BPA, a

known obesogen, may exert its effects through developmental programming of the hypothalamic melanocortin circuitry.

In vitro regulation of hepatic leptin A synthesis and secretion by glucose and stress hormones in a teleost fish, the tilapia (_Oreochromis mossambicus_)
[P10]

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Leptin is a 16 kDa cytokine critical for regulating energy expenditure in vertebrates, yet little is known about how the hormone interacts with the endocrine stress axis, particularly in fishes and other ectotherms. Studies in tilapia and other fishes have shown that leptin A (LepA) is the dominant form of leptin and that its mRNA levels in the liver acutely rise with systemic glucose during seawater challenge, as well as under hypoxic conditions. In tilapia, the hormone increases plasma glucose and decreases liver glycogen, indicating potent glycogenolytic actions. These data suggest the hormone may be involved in the adaptive stress response by mobilizing energy reserves, namely carbohydrates. Currently the regulatory interactions between the classical stress hormones (e.g. cortisol, epinephrine), metabolites (e.g. glucose), and leptin remain undescribed. We evaluated the actions of cortisol, epinephrine, and glucose in regulating LepA in the liver, the major site of hormone production in the tilapia (*Oreochromis mossambicus*). Using primary hepatocyte incubations and a homologous LepA ELISA, we show that LepA synthesis and secretion declines as ambient glucose levels increase (10-25 mM). These data suggest a negative feedback inhibition whereby leptin stimulates glucose release (glycogenolysis) during the initial stress response and glucose subsequently acts to inhibit leptin synthesis and secretion. Cortisol at physiological concentrations stimulated hepatic lepa mRNA and LepA secretion within 6 hours. Epinephrine, a major adrenergic stress hormone, stimulates LepA secretion in a dose-dependent fashion within 15 minutes, but had little effect on lepa mRNA expression in hepatocytes. The response was accompanied by increases in glucose release likely indicating a classical glycogenolytic effect of the adrenergic hormone. These data suggest hepatic LepA is sensitive to ambient glucose and is stimulated by both catecholamines and glucocorticoids. The results indicate that leptin plays an integral role in the vertebrate stress response to promote energy mobilization in conjunction with the classical stress hormones.

Does developmental exposure to the endocrine disrupting chemicals, bisphenol A (BPA) and ethinyl estradiol (EE2) affect growth and learning memory in painted turtles (_Chrysemys picta_)?
[P45]

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Many environmental estrogens adversely impact wildlife species through feminization or demasculinization. One such chemical is bisphenol A (BPA), which is present in many common household products. Current estimates indicate global production of BPA at 15 billion pounds annually. Bisphenol A has been identified in almost all aquatic habitats tested to date; thus, raising concern for widespread and continued exposure of humans and wildlife. Scant information is available on the sensitivity of certain taxa, including turtles, to BPA and potential effects on growth, learning, and memory. We hypothesized turtles exposed *in ovo* to BPA and ethinyl estradiol (EE2, an estrogen present in birth control pills) would exhibit altered growth patterns and compromised spatial learning and memory, as suggested in rodent models. To test this hypothesis, we exposed painted turtles (*Chrysemys picta)* to one of five treatments: no treatment control (n = 12), vehicle control (EtOH, n = 12), BPA low dose (0.01 µg/ml, n = 12), BPA high dose (100 µg /mL (n = 12), and EE2 (0.2 µg/mL, n = 12). Every 5 weeks, growth was measured by taking mass, carapace length, carapace width and plastron length of each turtle. After 5 months, no significant effects of treatment on carapace length were noted (RANOVA p = 0.33; control=28.2 mm ± 4.3 mm, EtOH=28.9 mm ± 4.3 mm, EE2=27.8 mm ± 3.7 mm, BPA low dose = 28.7 mm ± 3.9 mm, BPA high dose= 27.5 mm ± 4.4 mm). Spatial navigation abilities were tested at 5 months of age in an aquatic maze fitted with an HD video recorder. Spatial learning was evaluated using four containers with associated visual cues (circle, square, triangle, and star) placed on each side of the maze. At the outset, each turtle was randomly assigned one of four food containers for the 14 day experimental period. Brine shrimp was rubbed across each container to control for olfactory cues. Turtles were habituated to the room 30 min prior to maze testing and the assigned container was baited. Each turtle was placed in the middle of the arena for 10 min or until the correct food container was located. Behavioural studies will be completed by March 2015, and results will be presented at the meeting. We predict significant learning and memory deficits to occur from developmental exposure to these concentrations of BPA or EE2. Our findings will provide an understanding of the sensitivity of this aquatic model species for risk evaluations of wildlife populations exposed to these chemicals.

A proto-pluritropic cell type and its expression of novel glycoprotein hormones in the pituitary of the basal vertebrate, the sea lamprey
[P65]

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In gnathostomes, the classical pituitary glycoprotein hormones are follicle stimulating hormone (FSH), luteinizing hormone (LH), and thyroid stimulating hormone (TSH). In any one species, these consist of the common α subunit (GpA1) and specific β subunits (FSHβ, LHβ, and TSHβ). In contrast to jawed vertebrates, only one pituitary glycoprotein hormone (GpH) has been

identified in lampreys and consists of unique α and β subunits, GpA2 and GpHβ, which form a functional heterodimer (Sower et al., 2015, in revision). Additionally, there is evidence for a putative thyrostimulin in lampreys, consisting of GpA2 and GpB5. The objectives of this study were to determine the localization and co-expression of the lamprey GpH and thyrostimulin subunits in the pituitary of sea lampreys (*Petromyzon marinus*) at three different life stages (larval, parasitic and adult). We used histology, transmission electron microscopy, *in-situ* hybridization (ISH), and immunohistochemistry. lGpA2 and lGpB5 transcript were shown to be expressed in all regions of the anterior pituitary (rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI)) in parasitic and adult lampreys. However, lGpHβ transcript and protein were shown to be localized predominantly in the ventral half of the PPD, while lGpA2 protein was found primarily in the PPD and PI. Dual-label fluorescent ISH showed that the α and β subunits were co-localized, suggesting they are synthesized in the same pituitary cells. Furthermore, we have identified two classical tropic cell types in the lamprey anterior pituitary (corticotropes and somatotropes); and we discovered a novel proto-pluritropic cell that may differentially produce lGpH and thyrostimulin. The ISH studies showed that these proto-pluritropic cells expressed thyrostimulin throughout the anterior pituitary, while lGpH was expressed more specifically in the ventral PPD. In summary, our studies show that there are differences in expression of the lamprey GpH and thyrostimulin subunits during different life stages and that they are co-expressed. These data provide supporting evidence along with our other studies that lampreys only have two heterodimeric pituitary glycoprotein hormones.
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Neuroprotective effect of growth hormone against glutamate/BSO-induced cell death in QNR/D cells
[S9, contributed]

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Retinal ganglion cells (RGCs) in the chick embryonic neural retina are sites of growth hormone (GH) synthesis, release and action. Retinal GH has been demonstrated to exert actions in RGCs survival, since its blockade by interference RNA and immunoneutralization resulted in the cell death of immunopanned RGCs and quail neuro retinal derived cells (QNR/Ds). The GH anti-apoptotic properties have been extensively demonstrated during chicken embryo development and but it is not known if retinal GH is neuroprotective against neurotoxicity. The cell death of RGCs is a cause of blindness and is associated with common eye diseases such as, glaucoma and diabetic retinopathies. The QNR/D cell line is a suitable model to study cell death induced by glutamate/buthionine sulfoximine (BSO). In QNR/D cells, the neurotoxic effect of glutamate is dependent of the inhibition of the glutathione peroxidase by BSO. The capacity to induce excitotoxicity in QNR/D cells was demonstrated by MTT and LDH assays. TUNEL-labeling was used to determine apoptosis induction. Results showed a dose-dependent (from 0.5 to 4 mM)

glutamate/BSO (0.5 mM)-induced cell death in QNR/D (determined by MTT and LDH survival assays). Incubation with glutamate (1 and 4 mM) + BSO (0.5 mM) and exogenous GH (100nM) resulted in a significant increase ($P<0.05$) in survival rate (27.7 and 118.2% respectively) compared with the non-GH treated cultures. Results of TUNEL staining on QNR/D's treated with GH (100 nM), BSO (0.5 mM), and glutamate (1 mM) show a significant decrease ($P<0.05$) in the number of positive apoptotic cells, 8.6 ± 0.8 per field, compared to control (13.2 ± 0.9 per field). Over-expression of QNR/D-secreted GH also resulted in neuroprotection against glutamate/BSO-induced excitoxicity. These results demonstrate a neuroprotective effect of GH against glutamate-induced excitotoxicity in RGC's. Exogenous GH therapy or increasing retinal GH expression might therefore provide therapeutic approaches for the treatment of glaucoma and similar ocular problems.
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Sub-network enrichment analysis as a tool to characterize hormone signaling pathways in the teleostean gonad
[P85]

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Sub-network enrichment analysis (SNEA) is a new bioinformatics approach that identifies key regulators or molecular targets of hormones and endocrine disruptors. SNEA uses known relationships among genes that are reported in the literature (e.g. co-expression patterns, binding, or common pathways) to build interaction networks. The database employs ~2.4 million full-text articles (as of September 22, 2014). Thus, it is a powerful approach for describing molecular endocrine networks. We have used this approach to learn more about ovary and testis maturation during reproduction in largemouth bass (*Micropterus salmoides*) and rainbow darter (*Etheostoma caeruleum*). The development of eggs and sperm, although different processes, share common changes in gene expression during gametogenesis. For example, pathways that are active in females as well as males in the early stages of gamete development are genes related to the immune system and cell division. Thus, some cell processes appear to be conserved during maturation in teleost fishes. In addition, SNEA has provided insight into the signaling pathways regulated by sex steroids in the gonad. For example, in vitro experiments in the ovary of fathead minnows (*Pimephales promelas*) treated with the potent androgen dihydrotestosterone (DHT) has revealed that cell processes related to blood (e.g. vasodilation, clotting), lipids (e.g. lipid storage, cholesterol metabolism) and reproduction (e.g. steroid metabolism) are regulated by this hormone in the mature ovary. A number of these gene networks have also been identified as responsive to DHT in the fish testis; male mummichog (*Fundulus heteroclitus*) showed up-regulation of triacylglycerol biosynthesis and down-regulation of lipid export, in addition to other pathways such as immunity and xenobiotic clearance. Progesterone signaling has also been explored in the testis of fathead minnow and SNEA has verified roles of signaling pathways such as follicle-stimulating hormone secretion in this tissue, as well as identified potential new

avenues of research, suggesting that networks related to vasopressin signaling may be of importance in this tissue. As increasing amounts of omics datasets are collected in different fish species, it will become increasingly important to synthesise the data in a meaningful way to understand the biological events that underscore hormone action in the gonad.

Transcript variability for genes in the fathead minnow ovary: Implications for molecular reproductive studies
[P86]

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Fundamental studies characterizing transcript variability in tissues, and how this variation relates to reproductive physiology, are needed if steady state mRNA levels are to be useful endpoints for studying endocrine disruption. The objectives of this study were to assess the transcript variability for steroidogenic pathway enzymes and sex-steroid receptors in the fathead minnow (FHM; Pimephales promelas) ovary. *Estrogen receptor 2b*, and *membrane progestin receptors* (beta and gamma) and *5α-reducase a3* (*srd5a3*) showed high variability in the ovary (CV = ~1.2-3.0) while *progesterone receptor* (*pgr*), *ar*, *srd5a1*, and *esr2a* showed comparatively low variability (CV = ~0.5-0.7). Using these estimates, a power analysis revealed that sample sizes for real-time PCR experiments would be >20 in order to detect a two-fold change with power of 0.8 for 7 of the 18 transcripts; thus many molecular studies conducted in the fish ovary may have insufficient power to detect small effects. A second objective of this study was to determine how gene expression patterns related to higher level reproductive endpoints (e.g. gonadosomatic index, oocytes distribution, and steroid production (testosterone, T and 17β-estradiol, E2)). E2 production was positively correlated to *cyp19a* levels, while *ar* levels were negatively correlated to T. Cyp19a levels were positively correlated to both *ar* and *pgr* (r = 0.6) and star was positively correlated to *esr1*, *esr2b*, and *ar*, suggesting that androgens play a role in regulating the transcription of key rate-limiting steps in steroidogenesis. Molecular approaches in fish are increasingly used to assess biological impacts of endocrine disruptors and environmental stressors; however studies are needed that determine how gene variability relates to higher level biological responses.

Examining the functional roles of gonadotropin-releasing hormone II (GnRH2) in the zebrafish (*Danio rerio*)
[P66]

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Gonadotropin-releasing hormone II (GnRH2) is an evolutionarily conserved neuropeptide and the most prevalent GnRH variant in vertebrates, suggesting an important evolutionary role for this hormone. Although the forebrain GnRH1 and hypothalamic-preoptic GnRH3 (specific to teleost fish) hormonal populations have been extensively studied, much less is known about the functional roles of GnRH2. While primates possess both GnRH1 and GnRH2, rodents lack GnRH2, eliminating a potentially useful model for studying the behavioural roles of this peptide. Another model organism for studying GnRH2 has been found in zebrafish (*Danio rerio*), as this animal possesses the conserved GnRH2 gene as well as the hypophysiotropic GnRH3 gene, similar to the dual GnRH system in primates.GnRH2 is mainly expressed in the mid-brain tegmentum region, but recent mappings of the neuronal projections in *GnRH2:eGFP* transgenic zebrafish in our lab show widespread GnRH2 projections extending into the olfactory bulbs, the hypothalamic region, and down through the medulla oblongata to the spinal cord, suggesting this gene may have widespread roles in a number of different physiological and behavioural processes. We have achieved targeted, heritable mutations in the GnRH2 gene, using the TALEN technology, and have developed a knockout line of these mutants, which we have verified by immunocytochemistry. These *gnrh2-/-* fish are useful tools for examining how GnRH2 is involved in reproduction, behaviour, feeding, and other physiological processes. Preliminary experiments have shown a possible difference in larval mobility/behaviour of *gnrh2-/-* fish compared to wild-type siblings. We are also examining feeding behaviour differences in these fish, as studies on other organisms have found GnRH2 injections to induce an anorexigenic response. These results and others will be presented. Although GnRH1/3 have been found to be primarily responsible in controlling reproduction and development, we have preliminary evidence that GnRH2 may also have some role in controlling reproductive morphology, as *gnrh2-/-* fish exhibit differences in sperm motility, which we are currently quantifying. Due to a lack of suitable models, deciphering the functional roles of GnRH2 has been quite elusive in the past. However, our current results with the GnRH2:eGFP and gnrh2 -/- zebrafish models allow us to have more comprehensive understanding of the neuroendocrine role of GnRH2.

Novel substrates for hormone analysis in wildlife
[S8, invited]

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In wildlife species, reproductive and stress hormones are routinely assessed in blood and other less invasive substrates, including saliva, urine, and feces. These sample types provide a picture of circulating hormone levels within the past few minutes, hours, and days. In recent years, investigation of the effects of chronic stress on reproductive output and health of captive and free-ranging populations prompted the development and application of novel approaches for hormone evaluation. Keratin-based substrates incorporate hormones over the growth period of the sample representing longer periods of time, from weeks to months. Studies in our laboratory have shown that extraction of keratin samples, including hair, feather, snake sheds, and claws, released steroid hormones in measurable quantities by enzyme immunoassay that correlated to pharmacological and biological events. Numerous challenges exist with keratin sample analysis; however, the information gained from keratin-based hormone levels has the potential to provide

valuable insight into long-term effects on population dynamics in wildlife. Studies in mammalian, avian and reptilian species will be discussed.

DNA methylation patterning in the promoter region of the V1a/oxytocin-type vasotocin receptor in the sea lamprey (*Petromyzon marinus*)
[P51]

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The sea lamprey (*Petromyzon marinus*) is an ancient jawless vertebrate at an evolutionary nexus between invertebrates and jawed vertebrates. We previously identified and characterized a lamprey receptor gene that diverged into the separate vasopressin 1a receptor and oxytocin receptor genes in higher vertebrates. Mammalian expression and tissue localization of OXT receptor mRNA is regulated by the methylation of cytosine-guanine dinucleotides (CpG) in promoter region DNA, while microsatellites in the AVPR1A gene promoter regulate V1a receptor expression. Both CpG islands and microsatellites are intermingled in the lamprey V1a/OXT receptor gene (Pm807) promoter. Although the genome-wide methylation distribution in lampreys is similar to that in higher vertebrates, the regulation of individual genes by CpG methylation has not been investigated. Lamprey Pm807 mRNA is highly expressed in the adult heart but not expressed in the liver. Our hypothesis is that differential DNA methylation in these lamprey tissues is responsible for the difference in mRNA expression. Using High Resolution Melt (HRM) PCR on bisulfite converted DNA to screen for differences in promoter CpG methylation, we compared temperature at melt completion or touchdown (T_{td}), melt start or takeoff (T_{to}), and melt temperature at which half the molecules are denatured (T_m). Higher melt temperatures indicate higher levels of methylation. HRM enabled us to pinpoint a region with tissue-specific differences in DNA melt characteristics. Sequencing revealed a specific methylation pattern that was similar for both tissue types and did not vary among individuals, with a partial level of methylation at each CpG that was consistently higher in the heart. This was contrary to the slightly higher liver T_m but consistent with the higher T_{td} in the heart. Levels of methylation could differ among cell types within a tissue, and may have no association with gene expression. However, the stable pattern in this gene promoter could indicate the binding of repressors in the less-methylated liver which is prevented in the more-methylated heart tissue. Our study offers the first evidence of DNA methylation patterning in the promoter CpG island of an individual lamprey gene, potentially demonstrating the evolutionary conservation of gene regulatory mechanisms, and promoting an understanding of how the DNA methylation regulatory switches have come to operate in development and diseases such as autism and cancer.

Hypothalamic-pituitary-adrenal (HPA) responses to stressors in pre- and post-pubertal adolescent rats compared with adult rats
[S12, invited]

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Psychosocial stressors lead to long-lasting negative consequences for cognitive, emotional, and social behaviour when experienced in adolescence, but are less likely to so when experienced in adulthood. The elevation of plasma concentrations of glucocorticoid hormone (corticosterone in rats) in response to stressors is known to influence brain development and plasticity. Thus, the age-specificity of the effects of stressors may involve age differences in HPA function. Nevertheless, little is known about HPA function in adolescence, particularly in the early post-pubertal period. In adults, testosterone dampens HPA responses to stressors, but providing testosterone to pre-pubertal rats did not eliminate the difference in HPA responses compared with adults (Eiland & Romeo, 2013). We add to these findings to show that removal of testosterone from adults (gonadectomy) did not eliminate stress-induced differences in corticosterone concentrations between pre-pubertal adolescent and adult rats, but gonadectomy eliminated the difference between post-pubertal adolescent and adult rats. Whereas testosterone administration did not affect pre-pubertal corticosterone concentrations in response to a stressor, such administration to post-pubertal adolescents increased corticosterone concentrations, rather than decrease as in adulthood. In gonadally intact rats, all three ages groups showed a significant increase in progesterone concentrations after a stress exposure, but concentrations were higher in both pre- and post-pubertal rats than in adult rats after the stress exposure. In contrast, adults showed a significant increase in testosterone concentrations in response to a stress that was not found in post-pubertal adolescents (concentrations too low to be measured in pre-pubertal). Both pre-pubertal and post-pubertal adolescents had more prolonged activation of the paraventricular and arcuate nuclei compared with adults after a stressor, as indicated zif-268 immunoreactive cell counts. These results highlight the marked shifts in endocrine and neural responses to stressors that occur within the brief pre- to post-pubertal period in rats, and the maturational changes that must occur between the early post-pubertal period and adulthood, particularly involving gonadal–adrenal axes interactions. These shifts are noteworthy considering that investigations of chronic or repeated stress exposures in adolescence involve the pre- and post-pubertal period.

Differential stimulation of the hypothalamus-pituitary-interrenal axis during smolt development of landlocked and anadromous Atlantic salmon
[P109]

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Cortisol has an important role in development of seawater tolerance of teleost fish, including preparatory adaptations for seawater entry that occur during smolt development of anadromous salmon. Landlocked Atlantic salmon migrate from streams to lakes, resulting in relaxed selection on traits associated with salinity tolerance but not on other life history changes such as downstream migration and imprinting. In this study anadromous and landlocked strains of

Atlantic salmon were reared under identical conditions after fertilization and examined for differences in seawater performance and its underlying endocrine control during smolt development. Salinity tolerance (as judged by plasma chloride 24 h after direct transfer to 35 ppt), survival and growth in the first two weeks of seawater exposure were greater in the anadromous strain. Gill Na+/K+-ATPase (NKA) activity and the abundance of the seawater isoform of gill NKA (α1b) increased in spring in both strains but were greater in the anadromous strain. Similarly, plasma cortisol levels increased in spring in both strains but were 5-fold higher in the anadromous strain in April. Pituitary POMCA1, A2 and B mRNA levels increased slightly in spring but there were only slightly different between strains. Hypothalamic mRNA levels of Corticotrophin Releasing Factor (CRF) and Urotensin I (UI) increased in spring and were higher in the anadromous strain. CRF and UI mRNA levels in the preoptic area changed seasonally but were not correlated with circulating cortisol in either strain. The results provide evidence that hypothalamic CRF and UI are involved in increases in endogenous cortisol and that relaxed selection on salinity tolerance in landlocked salmon has resulted in decreased activity of the HPI axis during smolt development.

Comparing rapid screening bioassays to assess the impacts of progestins on reproduction in the zebrafish
[P36]

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Progesterone and its derivatives are important reproductive hormones in teleost fish that are involved in oocyte maturation and ovulation; however they are also present in waterways around the world and may act as endocrine disrupting chemicals (EDCs). Progesterone levels in the environment have a range from about 2 ng/L to 50 ng/L downstream of waste water treatment plants. In this study, the effects of progesterone exposure at environmentally relevant levels were examined in a short-term spawning bioassay using zebrafish in which fish were exposed to 2 ng/L or 50 ng/L of progesterone for 7 days. Both treatments led to significant reductions in the number of eggs that were spawned over the 7 day period. Subsequent analysis failed to clearly show the mechanism of action of progesterone as there were no effects on the expression of mPr, nPr and IGF3; additional gene expression analysis is pending. Other studies evaluated the effects of progesterone using a rapid ovulation bioassay. This involved inducing ovulation in 4 hours via waterborne exposure to the progesterone derivative, and major maturation inducing hormone in teleosts, 17α, 20β-dihydroxy-4-pregnen-3-one (17,20βP). Exposure to 10 nM progesterone for 24 hours prior to 4 hours of exposure to 10 nM 17,20βP shows evidence of inhibiting ovulation induced by 17,20βP alone. The use of these two reproductive bioassays provides support that environmental exposures to progesterone could be acting as an EDC. Further studies are examining the adverse outcome pathways that mediate the effect of progesterone on ovulation.

Timing of stress, not cortisol magnitude, is an important egg viability determinant in female rainbow trout (*Oncorhynchus mykiss*)
[P76]

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Stress and cortisol have been linked to negative effects on reproduction in fishes, but the relationship between how these two factors operate is not known. The objective of this study was to determine if the timing of elevated cortisol levels in adult female rainbow trout had an effect on egg viability. Highly fertile female rainbow trout, based upon an embryo viability assessment, were selected after their maiden spawn. Cortisol was delivered via silastic implants placed in the body cavity of half the fish, which were divided into three treatment groups: 0-4, 4-8, or 8-12 months after spawning. The remaining fish comprised the respective control groups and were implanted with empty silastic tubing during the same treatment periods. Fish were monitored throughout the year, spawned approximately one year later, and eggs fertilized to assess embryo viability again (i.e., after their second consecutive spawn). Cortisol-implanted fish experienced a significant increase in circulating levels of plasma cortisol compared to sham-implanted fish and plasma cortisol remained elevated relative to controls during the four month treatment periods in each group. There were no significant differences in embryo viability, egg diameters, or plasma estradiol-17β levels between the cortisol- and sham-implanted treatments in any of the groups. However, there was a significant difference in the number of females assessed as being subfertile (<80% embryo viability) when the three treatments were compared. The majority of the females (75%) implanted immediately post-spawn (0-4 months) produced subfertile eggs, which was different compared to those treated 4-8 (33%) or 8-12 (17%) months post-spawn. These results imply that the stress induced by the presence of an implant in the body cavity early in the reproductive cycle, but not elevated plasma cortisol levels, can affect oocyte development leading to a reduction in embryo viability.

Programming effects in the adult immune response following a pubertal immune challenge
[S12, contributed]

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Puberty is a critical period of development marked by physiological and hormonal changes as well as brain remodelling and reorganization. Exposure to stressors during this period can lead to enduring alterations in brain and behaviour. Specifically, pubertal exposure to the bacterial endotoxin, lipopolysaccharide (LPS), leads to decreased sexual receptivity in adulthood even after appropriate hormonal treatment. This effect is not limited to reproductive behaviour and also extends to numerous non-reproductive behaviours, where pubertal LPS treatment alters the responsiveness of depression-like, anxiety-like and cognitive function to gonadal hormones. Moreover, exposure to immune stress during critical periods of development can program or alter the response to a second immune challenge later in life, as shown neonatal studies (Ellis, Mouihate, & Pittman, 2006). The aim of the present study was to examine potential programming effects of the adult immune system in both male and female mice as a result of

pubertal exposure to an immune challenge (LPS). We hypothesize that adult mice treated with LPS during puberty will respond differently to an immune challenge compared to adult mice who were not treated with LPS during puberty. Male and female CD-1 mice were injected with either saline or LPS at six weeks (during puberty) or at ten weeks (during adulthood) of age. Four weeks following, all mice were injected with LPS and euthanized 10 hours later. Brains were extracted and tissue was analyzed using RT-qPCR to determine pro-inflammatory cytokine mRNA expression (TNFα, IL-1β, and IL-6). We predict that cytokine mRNA expression levels will be attenuated in adult mice who received a pubertal immune challenge compared to adults who did not receive a challenge during puberty. Programming effects resulting from pubertal stress exposure will provide a step towards understanding the link between early life stress and a subsequent stress response as well as stress-related psychopathologies.

Endocrine crosstalk with microRNAs in the control of energy metabolism: insights from the rainbow trout
[Presidential symposium]

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Rainbow trout are an established research model for the investigation of endocrine regulation of metabolism. microRNAs (miRNAs) have emerged as important regulators of mammalian energy metabolism, but inspite of their high evolutionary conservation, their comparative role in rainbow trout, and teleost fish in general, are currently not well understood. We here provide evidence that specific hepatic miRNA abundance acutely increases in short-term fasted rainbow trout 4h after administration of a single meal. Subsequent studies of the postprandially upregulated miRNA-122, a liver-specific and highly abundant miRNA, revealed that both endocrine and nutritional factors play a role in the acute regulation of hepatic miRNA-122 abundance. Specifically, intraperitoneal insulin injection in fasted fish, as well as insulin stimulation of primary hepatocytes from fasted fish, mimicked the postprandial increase in miRNA-122 after 4h, providing evidence for cross-talk between the endocrine system and hepatic miRNA-122. Additionally, administration of a single meal with a high fat diet (20%) acutely attenuated postprandial miRNA-122 abundance compared to a single meal with a low fat diet (3%), suggesting rapid regulatory effects of macronutrients on hepatic miRNAs following the ingestion of a single meal. Functional characterization of miRNA-122 in rainbow trout using a specific miRNA-122 LNA inhibitor *in vivo* showed a moderate stimulatory role for miRNA-122 in postprandial hepatic glucose utilization, which, at the molecular level is linked to stimulatory role of miRNA-122 on FAS protein abundance, the rate limiting enzyme in hepatic lipogenesis. These metabolic actions are similar to findings from mammalian model systems, indicating a conservation of miRNA-122 function in vertebrates. Together, these results show the utility of rainbow trout as a comparative model organism to study the (endocrine) regulation and function of miRNA in energy metabolism in both physiological and evolutionary contexts.

Serum lipid levels and oxidative stress parameters in streptozotocin-induced diabetic rats administered aqueous preparation of *Kalanchoe pinnata* leaves
[P11]

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Diabetes mellitus is a chronic metabolic disease that according to the World Health Organization affects more than 347 million people. The increased prevalence of diabetes mellitus coupled with the lack of an effective treatment has led many researchers to investigate the potential use of medicinal plants as a viable alternative treatment. Diabetic dyslipidemia involves elevation of triglycerides, low HDL cholesterol levels and small dense LDL particles. This pattern in diabetic individuals contributes to microvascular and macrovascular complications that are the major causes of morbidity and mortality. Oxidative stress also plays a major role in the development of secondary complications. Reduced antioxidant enzymes and increased plasma glucose glycation have been reported to contribute to the production of advanced glycation products that interact with receptors to trigger oxidative stress and proinflammatory pathways. In this study, we evaluated serum lipids and antioxidant levels in streptozotocin-induced diabetic Sprague Dawley rats administered aqueous preparations of *Kalanchoe pinnata* (3 mature leaves ~ 9.96 g / 70 kg body weight or 0.14 g/kg body weight). We noted that the diabetic treated group lost weight and consumed less food. There was a decreasing trend in serum glucose in the treated diabetic group compared to the other groups. We noted a significant ($p < 0.05$) decrease in serum triglyceride levels and a non-significant decrease in serum total cholesterol in the diabetic treated group compared to the diabetic control group. Serum antioxidant levels were reduced in diabetic control group compared to the other groups. Catalase levels were significantly ($p < 0.05$) increased in the serum of the diabetic treated group compared to the other groups. Serum thiobarbituric acid reactive substances were increased in the diabetic control group compared to the other groups. Our data suggest that the administration of aqueous *K. pinnata* preparation may be effective in reducing serum lipid levels and oxidative stress indices in diabetes.

Phylogeny of the corticotropin-releasing factor (CRF) family of peptides: The structure of urocortins 2 and 3 as a window into early evolution of the ancestral CRF gene
[P52]

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In vertebrates, the CRF family of peptides includes four distinct paralogous genes: CRF, urotensin-1, urocortin-2 and urocortin-3. CRF is found in all vertebrates. Urotensin-I is likewise found in all vertebrates but is termed ‘urocortin’ in amniotes (reptiles, birds, mammals), sauvagine in amphibians and urotensin-I in all fishes. CRF and urotensin-I are direct paralogues resulting from a gene duplication early in chordate ancestry. Urocortins-2 and-3 are also direct paralogues of each other. However, only a single form of a CRF-like peptide is found in the genomes of the vase tunicates, *Ciona intestinalis*, and *C. savignii*. The primary structure of these

peptides possesses motif similarities to the insect diuretic hormones and to all four of the CRF paralogues in vertebrates. Using the 2R hypothesis as a model, this suggests that there were two separate gene duplications leading initially to a CRF-urotensin-I-like ancestral gene and separately to urocortin-2 and 3-like ancestral genes. However, the lack of a clear urocortin-2 gene in lampreys and holocephalans indicates that the functional separation of these peptide genes occurred after the separation of the CRF-urotensin-I split. The urocortin-2 and-3 sequences also possess a significant sequence similarity into the insect calcitonin-like peptide, DH31. We hypothesize that before the protostome-deuterostome bifurcation that led to insects and chordates, respectively, an ancestral CRF-calcitonin-like peptide existed. However, because both peptides have similar functions with respect to ion regulation and similar amino acid motifs, it is likely that this ancestral peptide evolved from an earlier functional peptide.

Structural and functional disruptions of the suprachiasmatic nucleus in fibroblast growth factor-deficient mice
[P87]

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Fibroblast growth factor (Fgf) 8 and its cognate receptor, Fgfr1, are essential for the development of multiple brain regions. Previous studies from our laboratory showed that reduced Fgf8 signaling led to the malformation of neuroendocrine nuclei that originated within the diencephalon, including the oxytocin system in both the paraventricular (PVN) and supraoptic (SON) nuclei. To further understand the role of Fgf8 in the development of other hypothalamic nuclei, we examined if Fgf8 and Fgfr1 deficiencies also impact the integrity of the suprachiasmatic nuclei (SCN). The SCN are principal regulators of the organism's circadian rhythm and consist of neurons that produce vasoactive intestinal peptide (VIP) as the main input neurons. The objectives of this study are (1) to examine the number of VIP neurons in the SCN of postnatal day (PN) 0 mice hypomorphic for Fgf8, Fgfr1, or both, and (2) to quantify SCN neuronal activation by cFos immunostaining in adult mice deficient in Fgf8 alone or Fgf8 combined with Fgfr1. Brains were fixed in 4% paraformaldehyde, sectioned in a cryostat, and processed for VIP and cFos immunohistochemistry. The numbers of VIP- and cFos-immunoreactive (ir) neurons were then quantified in the SCN. In general, neonatal mice harboring homozygous deficiencies in Fgf8, Fgfr1, or both combined exhibited the most severe malformation of SCN and very few SCN VIP-ir neurons. Neonatal mice harboring heterozygous deficiencies in Fgf8 alone or Fgf8 combined with Fgfr1 (called DH mice) showed less severe, albeit still significant, reductions in VIP-ir neurons. To determine if these seemingly less severe changes could still disrupt SCN function, adult wildtype, Fgf8 heterozygous and DH mice were examined for SCN cFos activation at three time points: 1 (morning), 6 (afternoon), and 11 (evening) hours after light onset. Although the SCN of WT mice stayed consistently activated at all three time points, a significant change in cFos activation was observed in Fgf8 heterozygous mice between morning and afternoon time points. These data suggest an inherent defect in the morning activation of SCN in heterozygous Fgf8-deficient mice. Overall, our studies provide strong evidence that deficiencies in Fgf8 and Fgfr1 not only impact the structural integrity of the

SCN, the former also impacts the function of the SCN by compromising neuronal activation immediately after the onset of light.

Zebrafish *lh*β and *fsh*β transgenic lines for the analysis of hormone-regulated pituitary development
[P97]

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Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are released by gonadotropes of the anterior pituitary. These hormones are key players in the endocrine control of vertebrate puberty and reproduction. The regulation of their release involves a complex interaction between a variety of stimulatory and inhibitory factors. While the multifactorial control of LH release in adult teleosts by more than 20 neurotransmitter and neuropeptides is well-studied, little is known about FSH and the neurohormonal regulation of gonadotrope development. Using trangenesis, we are establishing two transgenic zebrafish (ZF) lines that will allow us to track early development and study the localization and regulation of both LH and FSH in the developing pituitary. Two DNA constructs have been designed with the ZF *lh*β and *fsh*β promoters (~3 kB) driving a fluorescent protein. The coding sequences for a red (mCherry) and a green fluorescent protein (eGFP) are in frame with the first exon of *lh*β and *fsh*β, respectively. Both constructs harbour either eGFP or mCherry in frame with a *cmlc2* heart marker promoter. Currently we have zebrafish (150; F0) that are positive for strong eGFP fluorescence in the heart that are ~ 1-4 months of age and will be screened using PCR for the incorporation of the construct into their genome. Positive ZF will be bred to establish the *tg*:*lh*β line. Once both stable transgenic lines have been established, *tg*:*lh*β and *tg:fsh*β fish can be crossed to follow the dynamic interaction of the 2 cell types in relation to somatotropes and lactotropes. Moreover, pharmacological and genetic manipulations will be used to test the hypothesis that early gonadotrope development is driven by local intrapituitary paracrine control by traditional hormones (e.g., LH, GH, PRL) and novel peptides (e.g., secretoneurin) rather than extrinsic hypothalamic neuropeptides (e.g., GnRH, kisspeptins). Funded by NSERC and the University of Ottawa (URC and IRAP).

Regulation and function of endogenous nesfatin-1/NUCB2 in mice
[P12]

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Nesfatin-1 is an endogenous, circulating, meal-responsive anorexigenic peptide. Prohormone convertases (PCs) cleave the first 82 amino acids of the precursor protein nucleobindin-2 (NUCB2) to produce nesfatin-1. Nesfatin-1 is expressed in several central and peripheral tissues, and is secreted in a meal responsive manner. Although identified as a multifunctional peptide, the regulation of nesfatin-1/NUCB2 is poorly understood. In addition, whether endogenous nesfatin-1 is critical for energy homeostasis is also unknown. We hypothesized that carbohydrate, fat and protein differentially regulate tissue specific expression of nesfatin-1, and that the absence of endogenous nesfatin-1 leads to abnormalities in energy homeostasis. Quantitative PCR and nesfatin-1 immunoassays were used to determine nesfatin-1 mRNA expression and circulating nesfatin-1 in mice acutely or chronically fed on a high fat, protein or carbohydrate diet. NUCB2 mRNA expression was significantly lower in the liver of mice fed a high protein diet compared to mice fed other diets. Chronic intake of high fat diet caused a significant reduction in NUCB2 mRNA in the stomach, while high protein and high fat diet caused similar suppression of NUCB2 mRNA in the large intestine. High carbohydrate diet fed mice showed significantly elevated nesfatin-1 levels at 1 p.m. Serum nesfatin-1 was significantly lower in mice fed high fat, protein or carbohydrate compared to the controls at 7 p.m, just prior to the dark phase. We characterized mouse stomach ghrelinoma (MGN3-1) as a nesfatin-1 producing cell line. NUCB2 and PCs were detected in MGN3-1 cells. In vitro studies employing MGN3-1 cells determined the role of glucose, fatty acids and amino acids on NUCB2 mRNA expression and nesfatin-1 release. Glucose and L-tryptophan stimulated NUCB2 mRNA expression, while oleic acid inhibited NUCB2 mRNA expression in cells. Our results for the first time indicate that nesfatin-1 is modulated by nutrients in vitro and in vivo. Second, we wanted to determine what happens if NUCB2 production is genetically ablated in mice. It was found that deletion of NUCB2 affects body weight, food intake, insulin secretion, glucose metabolism, and whole body energy homeostasis resulting in a sexually dimorphic manner. Our results indicate that endogenous nesfatin-1/NUCB2 is critical for maintaining energy balance, and that nesfatin-1 synthesis and secretion are differentially regulated by diet components in mice.

Uncovering novel mechanisms of sex-steroid induced previtellogenic ovarian follicle growth in coho salmon (*Oncorhynchus kisutch*) using high-throughput sequencing and pathway analysis
[P37]

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Contrary to the classical view of androgens serving simply as substrates for estradiol synthesis, recent studies have revealed major roles for them in ovarian follicle growth. While many of these studies indicate that androgens have growth promoting effects, the potential of their aromatization into bioactive estrogenic steroids leaves the question of specific, direct androgen effects unanswered. Recently, we have shown that 11-ketotestosterone (11-KT), a non-aromatizable androgen, promotes growth of previtellogenic (primary and early secondary) follicles of coho salmon, both in vitro and in vivo. In order to understand the mechanisms underlying these effects, ovarian samples from fish that were implanted with pellets containing

11-KT were assessed for alterations in their transcriptome using Illumina® high-throughput sequencing and Ingenuity Pathway Analysis software. After one and three days of treatment, 68 and 811 contiguous sequences (contigs) respectively, mapping to a total of 551 annotated genes, were differentially expressed relative to controls. Some of the androgen-regulated genes include those encoding proteins implicated in hormonal signaling pathways, those involved in cell and ovarian development, and genes containing putative androgen response elements. Several highly regulated contigs could not be annotated. These results, along with analysis of which biological pathways and networks have been altered in response to 11-KT, gives insights into the fundamental mechanism driving previtellogenic ovarian development as well as identifying new androgen-sensitive targets for further study.
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Enteric hormone regulation of NUCB2/nesfatin-1 expression in mice
[P13]

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Nesfatin-1 is an anorexigenic and insulinotropic peptide encoded in the precursor nucleobindin-2 (NUCB2). It is found abundant in the stomach, pancreas, and brain. Exogenous administration of nesfatin-1 decreases food intake and stimulates insulin secretion. Incretins, glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), secreted from intestinal enteroendocrine cells also regulate insulin secretion and metabolism. We hypothesized that the intestine and liver are sources of nesfatin-NUCB2, and that GLP-1 and GIP regulate nesfatin-1 secretion *in vitro*. To test this hypothesis, we used mice tissues, and a mouse gastric cell line (MGN-3) that produces nesfatin-1. The presence of nesfatin-1 in intestinal tissues was determined using RT-PCR, and NUCB2 mRNA expression level was measured using RT-qPCR. MGN3-1 cells were treated with GLP-1 and GIP *in vitro* to determine the effects of incretins on nesfatin-1. NUCB2 mRNA expression was detected in the liver, and small and large intestines of mice. In comparison to stomach (100%), liver (~8%), small intestine (~15%) and large intestine (~ 5%) had relatively less abundant expression of NUCB2 mRNA. GLP-1 (0.001, 0.01, and 100nM) stimulates NUCB2 mRNA expression in MGN3-1 at 1 hour post-incubation. In contrast, GIP (1, 10, and 100nM) treatment after 1 hour incubation inhibits NUCB2 mRNA expression. Our results indicate the presence of nesfatin-1 in the intestine of mice. GLP-1 has a stimulatory role, while GIP has an inhibitory effect on nesfatin-1 mRNA expression *in vitro.* Future studies will focus to unravel the *in vivo* actions of GLP-1 and GIP on nesfatin-1, and the mechanisms by which GLP-1 and GIP regulates nesfatin-1.

Estrogen receptor sequence from the brain and gonads of *Chirostoma humboldtianum* (Atheriniformes: Atherinopsidae)
[P67]

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Estrogen is involved in reproduction process such as the regulation of oogenesis, vitellogenesis, gonadotropin, testicular development among others. Estrogen action is mediated for specific receptors (ER) which play a crucial role in their targets. In this work males and females of *Chirostoma humboldtianum* from Zacapu lagoon, Michoacan, Mexico were collected and the brain and gonads were removed and kept in RNAlater. Posteriorly, the total RNA was extracted and cDNA was made with SuperScript II kit. Specific oligos for alpha subtype reported for *O. bonariensis* were used in PCR. The partial sequence of 236 bp (73 aa) for three tissues was obtained and it corresponds to ligand binding domain (LBD) of ER alpha subtype. This sequence is 91% similarity with *Odontesthes bonariensis* (EU284021.1), 87% *Melanotaenia fluviatilis* (GU319956.1) and Chelon *labrosus* (DQ011293.1) and 86% with *Pseudolabrus japonicus* (DQ298133.1). Regarding the distribution of the receptor in a particular tissue, we determined that the partial sequence of the ER alpha subtype in *C. humboldtianum* is expressed in the brain, ovary and testis during the month of May.

Day length, reproduction, and immune function
[Plenary]

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Life represents energetic trade-offs between survival and investment in offspring. At higher latitudes the combined challenge of food shortage and low temperatures makes winter a particularly difficult time to reproduce and survive, and the trade-off is shifted towards investment in survival. Physiological and behavioural adaptations have evolved among nontropical animals to cope with this winter energetic bottleneck. Individuals use short days to determine time of year to shift investment to immune function, a proxy for survival. Field studies indicate that immune function is compromised and prevalence of many diseases is elevated during winter. Individuals should enjoy a survival advantage if seasonally-recurring stressors could be anticipated and countered by shunting energy reserves to bolster immune function. The primary environmental cue that permits physiological anticipation of season is daily photoperiod, a cue that is mediated by melatonin. This talk will review laboratory studies that consistently report enhanced immune function and reduced sickness behaviours, in short day lengths. Prolonged melatonin treatment mimics short days, and also enhances immune function in rodents both in vitro and in vivo. Melatonin appears to be part of an integrative system that coordinates reproductive, immunological, and other physiological processes to cope successfully with energetic stressors during winter. In addition to adult photoperiod, early photoperiodic conditions can organize (i.e., program) important physiological and behavioural survival responses later in adulthood. Our studies use day length, a simple and precise environmental factor, to probe gene expression to understand development of phenotypes. Differences in early day length exposure program adult neuroimmune responses; both peripheral and central

inflammation are affected by photoperiod. The influence of photoperiodic influences on inflammatory responses and their effects on disease processes will be reviewed.

Exploring the genetic and neuroendocrine mechanisms underlying behaviour and reproductive success in a species with male alternative reproductive tactics
[S14, invited]

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Studying the proximate mechanisms that give rise to variation within and across species is critical to understanding the origins of diversity in wild animal populations. The male ocellated wrasse (*Symphodus ocellatus*) is the ideal model system in which to study the biological underpinnings of diversity because it is one of the numerous organisms that displays multiple reproductive tactics and variation in paternal behaviour in the wild. Although genetically monomorphic, juvenile males show differences in early growth rate which determine their adult social status. Three distinct phenotypes are observed in S. ocellatus males: 1) "nesting males" build and defend nests, court females and provide paternal care, 2) parasitic "sneaker males" covertly slip into nests to spawn, but take no part in female courtship or paternal rearing, 3) "satellite males" assist nesting males in nest defense and courtship, but secretly sneak matings when the nesting male is distracted. The reproductive tactics of the ocellated wrasse have been extensively studied in the field, yet the biological underpinnings of their marked physical and behavioural differences were previously unknown. Transcriptomics and quantification of circulating hormones revealed differences in gonadal and adrenal hormones across male morphs, which create differences in gene expression in brain regions critical for social and sexual behaviours. To determine if these differences in neural gene expression and hormones underlie disparities in behaviour and influence reproductive success, we performed hormone manipulations and gene knock-down experiments in the field, followed by paternity analysis at individual nests. We found drastic shifts in neuroendocrine signaling and neural gene expression patterns following these manipulations and found that altering the neuroendocrine signaling of a single nesting male had cascading effects on the social and reproductive behaviours of all fish at the nest. We believe that these mechanistic approaches, which enable manipulations of natural variation in wild populations, will allow for the discovery of direct links between genes, behaviour, and reproductive success.

T3 and 3,5-T2 regulate expression of different gene sets in the thalamus-pituitary of tilapia (*O. niloticus*)
[P88]

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Thyroid hormones (THs) act mainly 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*. Furthermore, liver expression of L-TRβ1 is 10^6-fold higher than that of S-TRβ1, reflecting the functional relevance of this signaling pathway. These studies however have only been performed in tilapia liver and no information regarding any other tissue is available. In this regard, it is well known that THs mediate cellular proliferation and neural differentiation in the nervous system of vertebrates. With the aim of starting to explore the functional role of the two TRβ1 isoforms in tilapia brain, we first determined that the exposure in the water culture with 25 nM of T2 or T3 for 12 h is an appropriate treatment to observe clear effects in gene expression in tilapia juveniles. Among brain structures, we chose to study thalamus-pituitary due to S- and L-TRβ1 expression abundance, and performed a transciptome analysis focusing in differential expression via RNA-seq Illumina genome analyzer GIIx. Our results show that gene expression is differetially regulated by T3 and T2. While T3 regulates the expression of 111 transcripts, T2 regulates 54 transcripts; of which 95 and 38 are T3 and T2 especific respectively. Of those genes, 16 are regulated by both tyronines. These results suggest that T3 and T2 exert divergent roles in tilapia thalamus-pituitary homeostasis.

Environmental gestagens: Sources, concentrations, and exposure effects from receptor activation to reproductive behaviour
[S3, invited]

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Gestagens include native progestogens, such as progesterone and 17α,20β-dihydroxypregnenone, which bind progesterone receptors and have critically important roles in vertebrate physiology, especially reproduction. Gestagens also include synthetic progestins, such as norethinedrone, levonorgestrel, and gestodene, which are components of contraceptive pharmaceuticals. Gestagens enter the aquatic environment through wastewater treatment plant effluent, papermill effluent, and agricultural runoff. A number of gestagens have been shown to negatively affect reproduction, development, and behaviour of exposed fish and other aquatic wildlife at ng/L concentrations, and these compounds have been measured in the environment at single to low hundreds of ng/L. Given the importance of endogenous progestogens in the regulation of

gametogenesis, secondary sex characteristics, and reproductive behaviour in vertebrates and the documented exposure effects of pharmaceutical progestins and progesterone, environmental gestagens are an emerging class of contaminants that deserve increased attention from researchers and regulators alike. The potential for environmental gestagens to affect the reproductive health of aquatic vertebrates seems evident, but there are a number of important questions for researchers to address in this nascent field and these include identifying biomarkers of gestagen exposure, testing the effects of environmentally relevant mixtures, and determining what other physiological endpoints and taxa might be affected by exposure to environmental gestagens. This talk will provide an overview of the field of environmental gestagens, receptor activation characteristics, and the ability of gestagens to alter reproductive behaviour in fathead minnows, *Pimephales promelas*. (Funding by MAF- D14ZO-010 and USGS/NIWR- 2014MD321G.)

Gut melatonin response to pathogenic stress: A study in relation to the activity of digestive enzymes and antioxidant defense system in carp *Catla catla*
[P77]

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We searched hitherto unknown relationship between the concentrations of melatonin, activity of different digestive enzymes and oxidative status in the gut of a carp under pathological stress to focus possible role of gut melatonin in response mechanism to microbial infections in any fish. Accordingly, carp (*Catla catla*) were intubated with *Aeromonas hydrophila* for 3 or 6 days and the response was evaluated by measuring the titer of melatonin, relative abundance of arylalkylamine-*N*-acetyl transferase (AANAT) protein (the key enzyme in the regulation of melatonin biosynthesis), levels/activities of different enzymatic and non-enzymatic antioxidants, as well as the activity of different digestive enzymes in the gut following specific analytical techniques. The organization of gut tissues in different fish groups was also studied at microscopic level. Microbial infection resulted in a marked crypt fusion in the gut-wall and an enormous structural damage in the tunica mucosa and lamina propia leading to significant reduction in its width. With the progress of treatment, a gradual increase in the level of melatonin, band intensity of AANAT protein and the activity of different antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), was accompanied by a decrease in the level of reduced glutathione (GSH) as well as the activity of different digestive enzymes in the gut. Simple correlation coefficient analysis of the data revealed that gut melatonin levels have a positive correlation with the density of AANAT protein, the activity of different antioxidant enzymes but a negative correlation with the level of GSH and the activity of studied digestive enzymes. Taken together, our study presented first information on pathogenic response of endogenous melatonin and thereby underlined possible protective role of gut melatonin against microbial infection in any fish species. Possibly, gut melatonin ameliorates pathological stress through up-regulation of anti-oxidative enzymes, though a direct role of melatonin as the scavenger of free radicals generated during the stress in infected fish may not be ruled out before further investigation.

The sexually dimorphic vasotocin system as target for neuroendocrine disruption in birds
[S4, invited]

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In our laboratory, we investigated the effects of some xenoestrogens on an avian species: the Japanese quail. In this species, exposure to low levels of estrogens during the embryonic critical period induces a total loss of male copulatory behaviour and a female-like VT-parvocellular system phenotype, therefore these two features can be considered as good endpoints to test xenoestrogens' activity.

All the tested compounds may affect male copulatory behaviour. However, the effects are variable and they depend on the considered compound and on the dose. For example EB and DES, which are the compounds with the highest estrogenic activity, completely abolish male sexual behaviour, whereas EE2 causes a complete loss of mounting behaviour and a partial loss of the other components of the copulatory behaviour. Finally, genistein and DDE (a metabolite of DDT) slightly affect the behaviour, in a proportional dose-dependent way. The only compound that does not influence the copulatory behaviour is the selective ERα agonist PPT, thus suggesting a main role of ERβ in the demasculinization of copulatory behaviour.
Also the VT-ir system is affected in different way, not always going in parallel with copulatory behaviour. For example, whereas both high and low doses of EB completely abolish the male copulatory behaviour, VT immunoreactivity is reduced by 60% with exposure to low EB and by 90% with high EB. DES has similar effects, whereas the slight decrease of behaviour caused by the highest dose of genistein is paralleled by a demasculinization of VT system similar to that obtained in DES treated animals. The most unrelated results are observable after EE2 treatment: a demasculinized copulatory behaviour, whereas the VT system is not affected by the treatment. These observations suggest that the selective ERα activation is not sufficient to induce the demasculinization of both behaviour and VT system. This is supported by the medium-high effect of genistein on VT system: this molecule has indeed a higher affinity for ERβ than for ERα.

Taken together all these data assert the importance of avoiding alterations of the hormonal milieu during the embryonic period. VT system can be one of the most useful markers to study the effects of estrogen-like compounds thanks to its extreme sensitiveness to steroids and in particular to estrogens, even if we cannot assume it as a general endpoint to test all the types of xenosteroids.

Neurogenesis and sexual behaviour
[Plenary]

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Sexual behaviour requires the expression and processing of olfactory cues by the main (MOB) and the accessory olfactory bulb (AOB). The olfactory bulb (OB) is one of the regions in the adult brain that receives and integrates new neurons that arrive from the subventricular zone of the lateral ventricles trough the rostral migratory stream. Previous studies from our group, demonstrated that the ability to control or pace the sexual interaction is crucial in male and female rats to develop a positive affective (reward) state. We have also shown that only when females pace the sexual interaction a higher number of new cells and neurons is observed in the AOB. When the females repeatedly mate pacing the sexual interaction a higher number of new cells is also observed in the MOB. The higher number of neurons is still present 45 days after the females control the sexual interaction suggesting that mating can induce permanent changes in the number of neurons that reach the AOB. The increase in the number of neurons after paced mating appears to be mediated by opioids because the injection of naloxone before mating blocks this effect.
Studies in male rats have also shown that only males allowed to control the rate of sexual interaction show an increase in the number of neurons in the AOB after mating. When males are allowed to ejaculate 1 or 3 times they show a significantly higher number of cells than males exposed to females. As well, males that mated with females that paced the sexual interaction showed a decrease in the number of BrdU+ cells compared with the control group and with those that mated freely. Together, these results indicate that the quality of stimulation that subjects receive when controlling the rate of the sexual interaction induces a higher number of neurons that reach the OB in rats.
Preliminary results in mice indicate, contrary to what we have observed in rats, that mating in males dose not induce a higher number of neurons in the OB after mating suggesting that the neurogenesis associated with mating could be species specific.
Acknowledgments: Francisco Camacho. Research supported by CONACYT 167101 and DGAPA IN200512; IN210215

Chronic social defeat paradigm effects on GHSR and NR3C1 mRNA expression in the PFC, HIPP and VTA of C57/BL6 male mice
[P89]

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Ghrelin, a gut-derived peptide hormone involved in the regulation of energy balance also plays a role in the stress response. Receptors for ghrelin (the growth hormone secretagogue receptor or GHSR) are found in regions like the prefrontal cortex(PFC), hippocampus (HIPP) and ventral tagmental area (VTA). These regions are commonly damaged in depressed patients. Recent studies show that ghrelin is secreted during the stress response, has anti-depressant effects and protects areas like the HIPP and PFC. Furthermore, chronic stress impacts the ability of ghrelin to have these protective effects, presumably through a down-regulation of ghrelin receptors (GHSR) in these regions. To determine if this is the case, we examined the expression of glucocorticoid receptor (NR3C1) and GHSR mRNA in the PFC, HIPP, and VTA of control and mice that were placed in a chronic social defeat paradigm for three weeks, and sacrificed at the onset of the light or dark cycles. Our results show that stressed mice had lower levels of GHSR

mRNA expression in the PFC ($p. < 0.05$) and lower levels of NR3C1 mRNA expression in the HIPP ($p. < 0.05$). Both NR3C1 and GHSR mRNA expressions were elevated in the VTA ($p. < 0.05$), an area associated with reward seeking behaviours. These data support the idea that chronic stress decreases the ability of ghrelin to protect the HIPP and PFC, promoting the development of depressive like behaviours.

Demonstration of an ovarian corticotropin releasing factor system in the zebrafish, *Danio rerio*
[P53]

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Corticotropin releasing factor (CRF) is most broadly known for its actions on the hypothalamic-pituitary-adrenal/interrenal axis, where it stimulates the release of adrenocorticotropic hormone from the anterior pituitary gland, which in turn increases secretion of adrenal/interrenal glucocorticoids as a coping response to stress. However, an increasing body of evidence shows the presence of CRF, its associated paralogs, receptors and binding proteins in other peripheral tissues of mammals, including both male and female reproductive tissues. While stress has been shown to negatively affect ovarian function in fish, it is not known if an intraovarian CRF system mediates these effects. The primary objectives of this study were to identify the components of the CRF system expressed in zebrafish ovarian follicles, and to determine whether CRF would modulate basal or gonadotropin-stimulated steroid biosynthesis of ovarian follicles incubated *in vitro*. Using qPCR, significant expression of CRF, its paralogs Urotensin I and Urocortin 3, CRF receptors type 1 and 2, and CRF binding protein was demonstrated in ovarian follicles ranging in maturity from primary to full grown vitellogenic. In full grown follicles, CRF application produces a dose-dependent blocking of human chorionic gonadotropin induced testosterone production. 17β-estradiol production of full grown follicles remains unaffected by CRF, both basally and in the presence of hCG. Collectively, the current studies demonstrate an intraovarian CRF system complete with ligands, receptors and binding proteins in the zebrafish ovary, and suggest that CRF may modulate steroid production and the subsequent reproductive abilites of the zebrafish. (Supported by NSERC Discovery Grants to NJB and GVDK).

Nesfatin-1 modulates ghrelin and leptin in mouse insulinoma (MIN6) cells
[P7]

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Diabetes mellitus (DM) is a major metabolic disorder, partly caused by defects in the hormonal regulation of energy balance. Hormones from the gastrointestinal tract and brain have physiological roles in appetite regulation and energy homeostasis, and represent themselves as useful targets for the development of anti-diabetic therapies. Nesfatin-1 is an 82 amino acid

metabolic peptide. It is co-produced with insulin in rodent pancreatic islet beta cells, and stimulates insulin secretion. The main goal of this study was to analyze whether nesfatin-1 affects two insulin regulatory hormones, leptin and ghrelin in pancreatic islets. We used mouse pancreas section, and mouse insulinoma (MIN6) cells for in vitro studies. Immunofluorescence histochemistry found nesfatin-1/NUCB2 co-localizing leptin in the pancreatic islets of mice. MIN6 cells expresses mRNAs encoding for leptin and ghrelin. Fluorescence microscopy revealed that MIN6 cells are immunopositive for nesfatin-1, leptin and ghrelin. Real-time quantitative PCR found that ghrelin and leptin mRNA levels are increased in vitro in MIN6 cells at 1 hour post-incubation with synthetic nesfatin-1 (100 nM). Additional in vitro and in vivo studies are required to determine the precise role of nesfatin-1 on pancreatic islet derived leptin and ghrelin synthesis and secretion. As leptin and ghrelin are known to affect endocrine functions of the pancreas, nesfatin-1 regulation of intra-islet ghrelin and leptin warrant further consideration.

The synthesis of steroids by *Taenia crassiceps* WFU cysticerci and tapeworms is related to the developmental stages of the parasites
[P113]

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Taeniids tapeworms are hermaphroditic helminths that gradually develop testis and ovaries in their reproductive units. The larval stage of the tapeworms named cysticercus is a vesicle that contains the scolex and proliferates asexually in the mice abdominal cavity. Once in the host they evaginate, attach to the gut and develop an adult organism, the tapeworm. We have shown that *T. crassiceps* ORF and *solium* cysticerci transform steroid precursors to androgens and estrogens. *T. crassiceps* WFU cysticerci can also synthesize corticosteroids. The aim of the present work is to investigate the relationship between steroid synthesis ability and developmental stage of the parasite *T. crassiceps* WFU. For this purpose cysticerci were obtained from the abdominal cavity of female mice, manually separated in non evaginated and evaginated and preincubated for 24h in DMEM plus antibiotics/antimycotics. Thereafter they were moved to new dishes with same media plus tritiated androstenedione (3H-A4) or progesterone (3H-P4) and incubated for 6, 24 or 43h. *Taenia crassiceps* WFU tapeworms were recovered from the intestine of golden hamsters that had been orally infected with cysticerci. The worms were pre-cultured in DMEM plus FBS and antibiotics, moved to new dishes and maintained for different periods without FBS, in the presence of 3H-A4 or 3H-P4. At the end of the experiments the media from cysticerci and tapeworms were analyzed by thin layer chromatography. The production of DOC by cysticerci was also confirmed by RIA. Results showed that testosterone synthesis was significantly higher in the evaginated cysticerci (9.6 ± 2.1 vs 22.9 ± 1.9, $P<0.01$) and increased after 24h in culture ($P<0.01$). Non evaginated and evaginated cysticerci also synthesized small quantities of 17β-estradiol and estrone. The evaginated cysticerci synthesized twice more 3H-DOC than the non-invaginated parasites, the production increased significantly with time in culture. The *T. crassiceps* WFU tapeworms (n=6) synthesized significant quantities of 3H-testosterone (45.25 ± 10.51) and small amounts of estrone after only 3h of culture with 3H-A4. The tapeworms also

synthesized 3H-DOC from 3H-P4 after 12h and significantly increased its synthesis at 24h of culture (39.18±5.61, 52.53±5.07 respectively). In summary our data show that *T. crassiceps* WFU is able to synthesize sexual steroids and corticosteroids in the three stages of development and that their synthesis is related to the developmental stage and time in culture. (Values indicate percent of transformation, media ± SD).

Teneurin C-terminal associated peptide (TCAP) expression and function in the male reproductive system of chordates
[P68]

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The teneurin transmembrane proteins and their associated peptide, teneurin C-terminal associated peptide (TCAP) is a phylogenetically ancient signalling system that is found in most metazoans studied to date. In the brain, the teneurin-TCAP protein interacts with a complex of proteins including α- and β-dystroglycans, neurexins, fibronectin-like leucine rich protein (FLRP) and latrophilin. These proteins form a trans-synaptic adhesion complex that acts to maintain the integrity of the synapse. However, the teneurins and latrophilins are found in a number of tissues, although how they are associated with non-synaptic proteins is not known. Recent studies have established that the teneurins are found in the reproductive system of nematodes (*Caenorhabditus elegans*), tunicates (*Ciona intestinalis*), mice (*Mus musculus*) and monkey (*Cebus apella*). In mice seminiferous tubules, teneurins and TCAP are associated with α- and β- dystroglycans, respectively. Moreover, treatment of mice with synthetic TCAP-1 results in increased testosterone production in a pulsatile manner and enlarges testes diameter. TCAP and teneurin immunoreactivity have also been found in the preoptic area/anterior hypothalamus region and could potentially regulate GnRH action. Further, new in vitro studies using immortalized Leydig and Sertoli cells have established that the key components of TCAP-1 action are present in these cells. We hypothesize that the teneurin-TCAP system represents a novel signaling system in the regulation of the hypothalamic-pituitary-gonadal (HPG) axis.

GnRH-selective regulation of PIP_3-dependent signaling contributes to the differential control of LH and GH secretion in goldfish, *Carassius auratus*
[S7, contributed]

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In goldfish (*Carassius auratus*), pituitary cells are exposed to two endogenous isoforms of gonadotropin-releasing hormone (GnRH), GnRH2 (chicken-II; [His5,Trp7,Tyr8]GnRH) and GnRH3 (salmon; [Trp7,Leu8]GnRH), that both directly stimulate luteinizing hormone (LH) and growth hormone (GH) release. Interestingly, GnRH2 and GnRH3 bind to and activate the same population of pituitary cell-surface receptors (GnRHRs) belonging to the G protein-coupled

receptor (GPCR) superfamily. However, despite using shared GnRHRs and activating common post-receptor mechanisms, GnRH2 and GnRH3 utilize different isoforms of class I phosphoinositide 3-kinase (PI3K) in eliciting acute LH and GH release responses. Although individual isoforms of class I PI3K are known to have distinct intracellular functions, all four isoforms of class I PI3K phosphorylate the 3′-hydroxyl group of phosphoinositide 4,5-bisphosphate to produce the lipid second-messenger phosphoinositide 3,4,5-trisphosphate (PIP_3). Production of PIP_3 facilitates membrane translocation and/or activation of PIP_3-binding domain (pleckstrin homology; PH)-containing effectors such as the AGC family protein kinases and members of the Tec family of non-receptor tyrosine kinases. In the present study, we examined whether GnRH-selective activation of class I PI3Ks results in the differential involvement of PH domain-containing signalling effectors to control GnRH2- and GnRH3-stimulated hormone release responses from primary cultures of dispersed goldfish pituitary cells in column perifusion. Pharmacological mapping of PH domain-dependent signalling was achieved using a small molecule mimetic of PIP_3 to antagonize PIP_3-PH domain interactions in general; as well as through treatments with allosteric or ATP-competitive inhibitors of the classical PIP_3-dependent effectors 3-phosphoinositide-dependent protein kinase 1 (PDK1), Akt (protein kinase B), and Bruton's Tyrosine Kinase (BTK). Results suggest that PIP_3 production by class I PI3Ks contributes to the GnRH2- and GnRH3-selective activation of PH domain-containing effectors and the differential control of hormone release responses from gonadotropes and somatotropes. Taken together, we hypothesize that ligand-selective activation of class I PI3Ks by GnRH2 and GnRH3 results in unique PIP_3-dependent signalling mechanisms that are controlled by the biased coupling of GnRHRs to downstream PH domain-containing effectors.

Nodal and its modulating microRNAs in placental development
[S6, invited]

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Human placenta is a transient organ essential for pregnancy. During placental development, trophoblasts differentiate through two major pathways. First, mononucleated cytotrophoblasts (CTBs) are fused into multinucleated syncytiotrophoblasts (STBs), which are involved in the exchange of gases, nutrients and waste products across the materno-fetal interface. Second, a group of CTBs break through the syncytial layer to create cell columns that anchor onto the decidua. Some CTBs detach from the cell column and form the invasive extravillous trophoblasts (EVTs), which invade the uterus and participate in the remodelling of maternal spiral artery. Defects in placental development lead to pregnancy complications, such as preeclampsia.

Nodal is member of the transforming growth factor-β (TGF-β) family known to play critical roles during embryo development. We have demonstrated that Nodal activated activin receptor-like kinase 7 (ALK7) to inhibit trophoblast proliferation and invasion. We have also found that Nodal induced cell fusion and expression of STB marker genes. These findings suggest that Nodal inhibits EVT differentiation but promotes STB differentiation. Expression and function of Nodal are regulated by several microRNAs (miRNAs). Specifically, miR-378a-5p and miR-218-

5p targeted Nodal while miR-376c-3p inhibits ALK7. Stable or transient overexpression of these miRNAs in an EVT cell line resulted in increased cell proliferation, migration, and invasion. On the other hand, inhibition of endogenous miRNAs using anti-miR-378a-5p, anti-miR-218-5p, or anti-miR-376c suppressed cell proliferation and invasion. In placenta explant culture, force overexpression of these miRNAs promoted the outgrowth of EVT cells. Preliminary results suggest that miR-218-5p induced maternal artery remodelling in a placental explants/decidua coculture model. In preeclamptic placentae, we found that Nodal protein levels were strongly upregulated while miR-378a-5p, miR-218-5p, and miR-376c-3p were significantly down-regulated when compared to gestational age-matched controls. Taken together, these findings suggest that Nodal and its modulating miRNAs play important roles in placental development and disruption of the balance between miRNAs and Nodal signalling may lead to preeclampsia.

Neuroendocrine control of ionic balance in larval zebrafish (*Danio rerio*)
[S13, invited]

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Prior to gill development, ionic balance in larval zebrafish (*Danio rerio*) is achieved by regulated adjustments of ion influx and/or efflux across (or between) ion-transporting cells (ionocytes) localised primarily to the yolk sac epithelium. At least four sub-types of ionocytes have been described including those involved in Na^+ uptake and acid excretion [H^+-ATPase-rich (HR) cells], Na^+ and Cl^- uptake [Na^+-Cl^- co-transporter (NCC) expressing cells], Ca^{2+} uptake [Na^+/K^+-ATPase rich (NaR) cells] and K^+ secretion [KS cells]. Depending on the prevailing environmental conditions, the rates of ionic uptake and/or losses are adjusted acutely and chronically by a suite of hormones and signalling molecules that includes catecholamines, angiotensin II (ANG II), parathyroid hormone (PTH), cortisol and the gaseous endocrine factors hydrogen sulphide (H_2S), nitric oxide (NO) and possibly carbon monoxide (CO). Na^+ uptake is stimulated acutely by catecholamines and angiotensin II by specific interaction with HR cells. We suggest that under conditions of low pH or exposure to water deficient in Na^+, nerves innervating the HR cells release adrenaline and/or noradrenaline which interact with ß-adrenergic receptors to promote cyclic AMP production and activation of protein kinas A (PKA); ultimately, Na^+ uptake is stimulated by activation of the Na^+-H^+ exchanger, NHE3b. In response to ion-poor or acidic water, the renin-angiotensin-system (RAS) is activated and ANG II levels are elevated. Unlike catecholamines, ANG II appears to activate Na^+ uptake by interacting with the NCC expressing cells. In contract to the acute stimulatory effects of catecholamines and ANG II on Na^+ uptake, the bioactive gases H_2S and NO, are inhibitory and may play a role in reducing Na^+ uptake when fish are exposed to Na^+-enriched water. While the situation for NO is unresolved, it is likely that H_2S is synthesized within the HR cells by the enzymes cystathionine-ß-synthase (CBS) cystathionine-γ-lyase. Under chronic conditions of low pH or low Na^+ exposure, Na^+ uptake is enhanced by cortisol after interacting specifically with glucocorticoid receptors (GRs) localised to HR cells. Concomitantly, Na^+ losses are minimized owing to cortisol-mediated "tightening" of paracellular pathways. These findings suggest that cortisol may help to minimize the negative consequences of acid exposure on Na^+ homoeostasis

via GR-mediated reductions in epithelial permeability and paracellular Na^+ loss. Two key endocrine agents involved in stimulating Ca^{2+} uptake are PTH and H_2S.

Effects of progesterone and norethindrone on female fathead minnow (*Pimephales promelas*) steroidogenesis
[S3, contributed]

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The widespread use of oral contraceptives contributes to the near ubiquitous presence of synthetic progestins in the aquatic environment. Currently, there is limited information as to the potential endocrine disrupting effects of synthetic progestins in the aquatic environment. The objective of the present study was to compare the effects of a synthetic (norethindrone or NET, 100 ng/L) and endogenous progestin (progesterone or P4, 100 ng/L) on female fathead minnow (Pimephales promelas) steroidogenesis. In vivo exposure to either compound lowered gene expression of luteinizing hormone (LH) levels in the brain along with down-regulation of membrane progesterone receptor isoforms (mPRα and mPRβ) in fish ovary tissue. This association indicated a close functional coupling between LH productions and ovary membrane progestin receptor expressions/activations. In vitro exposure of ovary tissue to progesterone resulted in elevated progestogen (pregnenolone, 17α-hydroxyprogesterone, and 17α,20β-dihydroxypregnenone) and androgen (testosterone) productions, indicating that progesterone was capable of acting as substrate for steroid productions. In vitro exposure of ovarian tissue to norethindrone showed a dose-dependent decrease in testosterone and 11-ketotestosterone productions. Lowered androgen production was likely due to in vivo observed reduction of 3β-HSD gene expression in ovaries. These results suggest anti-androgenic activities of norethindrone. Overall, our study showed that exposure to a natural progestogen (progesterone) and synthetic progestin (norethindrone) were capable of modulating LH (in brain) and mPRα and mPRβ expressions (in ovary).

The fire ant (*Solenopsis invicta*) short neuropeptide F and insulin-like peptides signaling systems: toward elucidating their role in reproduction, colony nutrition and worker division of labor
[S2, invited]

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The short neuropeptide F (sNPF) and insulin-like peptides signaling pathways are conserved mechanisms involved in nutritional regulation in insects. In social insects, nutrition has profound effects on reproduction, caste determination, worker division of labor, and in sociality-mediated

behavioural plasticity. Therefore, to investigate the regulatory cascades controlled by the sNPF peptide and insulin-like peptides in the red imported fire ant (*S. invicta* Buren) we began by characterizing their cognate receptors. Two insulin receptors (InR1 and InR2) were cloned and their relative transcript levels in different developmental stages of queen and workers were analyzed. In addition, two *S. invicta* insulin-like peptides were predicted from the genome. The sNPF receptor (sNPFR) was cloned and immunolocalized in the brain of mated and virgin queens, and of workers, and in the queen ovaries. A different distribution pattern of sNPFR labeled neurons was observed in brains of workers from different subcastes. Additionally, within each subcaste, differences were observed in immunolabeled neurons in brains from workers obtained from colonies with or without brood present. These observations suggested that the sNPF signaling system is involved in regulating behaviours associated with specific subcastes and tasks. To understand the neuropeptide receptor downstream signaling cascade it is necessary to deorphanize the receptor. However, the cognate ligand(s) for this receptor had not been identified. Previous attempts to deorphanize the putative sNPFR with sNPF peptides from other insect species which ended in the canonical sequence LRLRFamide, failed. We mined the genome and cloned the full length cDNA of the putative S. invicta sNPF prepropeptide and identified the putative ligand(s) from its deduced sequence. Receptor functional expression analyses with these peptides indicate that fire ants have a unique sNPF signaling system in that the functional ligands end in Y, not F (sNPY). This discovery will now allow us to investigate the function of the identified neuropeptide(s) and advance neuroendocrinology of the fire ant.
References: PLoS ONE 9(10): e109590, 2014; PLoS ONE 8(12): e83966, 2013.
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Characterization of appetite-regulating factors in platyfish, *Xiphophorus maculatus* (Cyprinodontiformes Poeciliidae)
[P14]

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The regulation of energy in fish, like most vertebrates, is a complex process that involves a number of chemical signals originating and networking in different parts of the brain and throughout the body. These signals include anorexigenic hormones that suppress feeding and hunger [e.g. cholecystokinin (CCK) and cocaine-and amphetamine-regulated transcript (CART)] as well as orexigenic peptides that stimulate feeding and food intake [e.g. orexin and neuropeptide Y (NPY)]. Platyfish, *Xiphophorus maculatus*, are freshwater viviparous fish found in tropical waters from South America to northern Mexico. Although these fish have been the object of numerous physiology and behavioural studies, very little is known about the endocrine mechanisms regulating their feeding. In order to elucidate the role of these peptides in the regulation of feeding, we examined the effects of peripheral injections of CCK and orexin on feeding behaviour and food intake. Injections of CCK decreased both food intake and searching behaviour, while injections of orexin increased searching behaviour but did not seem to have an effect on food consumption. In order to better characterize these peptides, we performed tissue

distribution and gene expression studies. Tissue distribution studies show that CCK, CART, NPY and orexin all show a widespread distribution in brain and several peripheral tissues, including gut. In addition, we compared the expression of these peptides in brain and gut between fed and 10-day fasted platyfish using qPCR. Fasting induced increases in both orexin and NPY mRNA expressions and a decrease in CART expression in both brain and gut. There were no significant differences in the expression of CCK between fed and fasted fish in either brain or gut. The widespread distribution and the fasting-induced changes in expression of these peptides suggest that they might have several physiological roles in platyfish, including the regulation of feeding.

The molecular basis of skin-specific modifications associated with metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus*)
[S10, contributed]

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Thyroid hormones are the mediators of flatfish metamorphosis and trigger a cascade of events that lead to amongst other things the maturation of skin. The present study takes advantage of the large size of the Atlantic halibut to study the molecular and cellular modifications in skin from individual larvae during metamorphosis. The working hypothesis is that since THs act as transcription factors the overt change in the skin during metamorphosis will be preceded by significant modifications in the transcriptome. 454 pyrosequencing technology was used to generate a halibut skin specific transcriptome and SOLiD technology was used to profile transcriptional changes in individuals at stages 7, 8 and 9. The skin in stage 7 halibut was a simple epithelia and developed into a more complex multi-layered tissue during metamorphosis. TH responsive transcripts and candidate genes underpinning remodelling and maturation during metamorphosis were identified. Abundant transcripts in skin that were modified during metamorphosis included collagens (col1a1, col1a2), genes involved in skin morphogenesis, pigmentation (e.g. melacortin 1 receptor, dopachrome tautomerase, lysosomal-trafficking regulator), pigment cell development, melanocyte differentiation and melanosome transport. The skin transcriptome contained 113 putative TH-responsive genes some of which were significantly modified during metamorphosis. Asymmetry in thyroid receptor beta (TRβ) and monocarboxylate transporter 10 (MCT10) was detected between the ocular and abocular skin. Asymmetry was also detected in some of the TH responsive genes. The small proportion of differentially expressed TH responsive genes relative to the total number of differentially expressed genes (~8000) during metamorphosis leads us to hypothesis that a core group of TH responsive genes trigger a cascade of events that lead to the overt modifications in the skin.
Acknowledgement: This project was supported by the European Community FP7 (LIFECYCLE-No. 222719). RNA was in receipt of a PhD fellowship (BD/69209/2010) from the Science Foundation of Portugal. We thank H. Smáradóttir for providing the Atlantic halibut samples and M.A.S Thorne for preliminary data processing.

Tectal corticotropin-releasing factor (CRF) neurons respond to fasting and a reactive stressor in the African clawed frog, *Xenopus laevis*
[C15]

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Several lines of evidence suggest that the neuropeptide CRF modulates neuronal circuitry involved in foraging/predator avoidance tradeoffs. For example, ICV or peripheral administration of CRF inhibits visually guided prey capture in anurans. The optic tectum, a midbrain area critical for integrating visual information to elicit a motor response, houses CRF-producing interneurons and we have previously shown that it contains the largest pool of releasable CRF in the anuran brain. In mammals, activity of hypothalamic CRF neurons is closely tied to stressor exposure and energy balance. Whether tectal CRF content changes in response to stressors or changes in energy status is unknown. We hypothesize that tectal CRF plays a critical role in mediating the feed or flee tradeoffs, and we predict that food deprivation will decrease and stressors will increase CRF concentration in the optic tectum. We examined the effects of food deprivation and categorically distinct (reactive and anticipatory) stressors on CRF content in the optic tectum (OT) and compared changes to those occurring in the hypothalamus/thalamus (H/T), telencephalon (Tel), and brainstem (BS) of juvenile *X. laevis*. For the food deprivation studies, frogs received no food or normal food rations for 8 d. For the stressor studies, frogs were untreated or exposed to ether vapors (1 min) or shaking stress (4 h). CRF content was assessed using a homologous radioimmunoassay. The rank order for CRF tissue concentration in controls was H/T > OT > Tel > BS. Food deprivation decreased CRF OT content but did not alter CRF content of the Tel or H/T when compared to controls. Interestingly, CRF content of the BS increased in response to food deprivation relative to controls. Exposure to a shaking stressor increased CRF in the H/T but did not alter CRF in the OT. In contrast, exposure to ether elevated CRF in the OT relative to untreated controls but had no effect on CRF content in the H/T. Decreased CRF content in the OT following food deprivation suggests that the CRF OT signaling system can be modulated by satiety neuropeptides. Ether vapors, a reactive stressor, elicited a response from tectal CRF neurons suggesting that ascending noradrenergic neurons from the A2 nucleus may participate in tectal regulation. Our data suggest that tectal CRF neurons may play a role in modulating visually guided behaviour during reactive, but not an anticipatory, stress and in response to changes in energy balance.

Interplay between juvenile hormone, ecdysone and microRNAs in mosquito reproduction
[Plenary]

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Mosquitoes, in addition to being vectors of numerous devastating human diseases, represent outstanding research models because their reproductive events are synchronized by the intake of

blood and occur within a short time span. We investigated the network of regulatory factors responsible for sequential gene expression in the mosquito fat body, a tissue analogous to the vertebrate liver. We show that systemic factors, juvenile hormone (JH), Ecdysone (20E) and nutritional amino acids (AAs), differentially regulate this gene expression program. JH and 20E signaling is mediated by their respective receptors Met and EcR. The orphan nuclear receptor HR3, previously implicated as a developmental switch in Drosophila, plays an important role in controlling sequential repression and activation of gene cohorts during the mosquito reproductive cycle. It points to its conserved role as a reprogramming switch in insect development and reproduction. We have demonstrated the repressive function of 20E/EcR, which down-regulates large cohorts of PBM genes. We characterized microRNAs that play significant roles in regulating critical physiological functions during mosquito reproduction. Our studies provide new insights into the complexity of regulatory mechanisms responsible for temporal coordination of gene expression during mosquito reproduction.

Molecular mechanisms of hormone synergy at the Krüppel-like factor 9 nuclear receptor synergy module
[P90]

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Cooperative, synergistic gene regulation by nuclear hormone receptors (NRs) can increase sensitivity and amplify cellular responses to hormones. We investigated the molecular basis for thyroid hormone (TH) and glucocorticoid (GC) synergy on the Krüppel-like factor 9 (*Klf9*) gene, which codes for a zinc finger transcription factor involved in development and homeostasis of diverse tissues. Using transfection-reporter assays we identified regions of the *Xenopus* and mouse *Klf9* genes 5 to 6 kb upstream of the transcription start sites that supported synergistic transactivation by TH plus GC. Within these regions we found an orthologous sequence of ~180 bp that is highly conserved among tetrapods, but absent in fishes, and possesses chromatin marks characteristic of an enhancer element. The *Xenopus* and mouse ~180 bp DNA element conferred synergistic transactivation by hormones in transient transfection assays, so we designated this region the *Klf9* Synergy Module (KSM). Computational, mutagenesis, gel shift, DNAse I footprinting and chromatin immunoprecipitation analysis identified binding sites within the mouse KSM for TH receptor (TR), GC receptor (GR) and nuclear factor kappa B (NF□B); the TR and NF□B sites overlap to form a composite element, and we found interactions among these signaling pathways. Thyroid hormone strongly increased recruitment of liganded GR and serine 5 phosphorylated (initiating) RNA polymerase II to chromatin at the KSM; whereas, forced expression of a dominant negative TR partially blocked the response to GC and hormone synergy. Taken together, our findings support a

mechanism for transcriptional synergy whereby TR modulates access of GR (and possibly other transcription factors like NF□B) to chromatin. The KSM is bi-directionally transcribed to generate noncoding enhancer RNAs which are also synergistically induced by combined hormone treatment, and interacts with the *Klf9* promoter and a far upstream region through chromosomal looping. Our findings support that the KSM plays a central role in hormone regulation of vertebrate *Klf9* genes, it evolved in the tetrapod lineage, and has been maintained by strong stabilizing selection.
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Insulinotropic actions of a nesfatin-1-like peptide encoded in nucleobindin-1
[P17]

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Nesfatin-1 (82 amino acid) is a peptide encoded in a secreted precursor, nucleobindin-2 (NUCB2). It is an anorexigenic, insulinotropic peptide found abundantly in brain, gastric oxyntic mucosa, and pancreas. Exogenous nesfatin-1 decreases food intake, and stimulates insulin secretion. Nucleobindin-1 (NUCB1) is a protein with very high similarity to NUCB2. NUCB1 also encodes a very conserved nesfatin-1 like peptide (NLP). The NUCB1 sequences also retain the proposed prohormone convertase (PC) cleavage sites. The cytoplasmic presence of NUCB1 and that it is a secreted protein suggests a potential endocrine function for NUCB1 and/or its encoded peptides. We hypothesized that the NLP region of NUCB1 is bioactive. The main objectives of this research are to determine if NUCB1 is present in pancreatic islets of mice, and to test the effects of synthetic NLP on insulin secretion. In silico analysis using SignalP 4.1 server found a signal peptide cleavage site at position 25, 26 (Arg, Val) preceding the NLP region in NUCB1 sequence. Further analysis using ProP 1.0 server showed potential proprotein convertase cleavage sites at Lys-Arg (KR), forming a 77 amino acid NLP. This was reaffirmed using NeuroPred™ cleavage site/protein mass prediction tool. Immunofluorescence analysis detected NUCB1-like immunoreactivity in a murine pancreatic beta cell line (MIN6 cells). NUCB1 is co-localized with insulin in pancreatic beta cells. RT-PCR analysis found NUCB1 mRNA in both pancreas and MIN6 cells. To assess the effects of synthetic NLP on preproinsulin mRNA expression and insulin secretion, MIN6 cells (2X105 cells/well, n=8 wells/treatment; 3 separate studies) were incubated for 1 hour with synthetic rat NLP. At 1 hour post-incubation, insulin secretion (ELISA/RIA) into media, and preproinsulin mRNA expression were determined. NLP significantly upregulated preproinsulin mRNA expression at 10 and 100 nM doses. Concurrently, insulin secretion was also stimulated at 10 nM (87.7±2.57 ng/mL) and 100 nM (92.7±4.5 ng/mL) NLP compared to controls (67.8±2.5 ng/mL, $p<0.05$). In identical experiments using MIN6 cells and a scrambled peptide from NLP, the scrambled peptide at doses tested above did not elicit any effects on preproinsulin mRNA expression or insulin secretion. Based on our results, NLP appears as another endogenous insulinotropic peptide. Future studies examining endogenous NLP and its mechanism of action in regulating glucose homeostasis and insulin secretion warrant consideration.

Does nesfatin-1 regulate enteric hormone secretion in mice?
[P16]

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The hormones secreted by intestinal mucosal enteroendocrine cells regulate insulin secretion. These include GLP-1 and PYY, GIP, and CCK. While GLP-1, GIP and CCK are insulinotropic, PYY is insulinostatic. Nesfatin-1 is an anorexigenic, insulinotropic peptide (82 amino acids) found abundantly in gastric oxyntic mucosa, brain and pancreas. Exogenous nesfatin-1 decreases food intake, and stimulates insulin secretion. The main objectives of this research are to determine whether nesfatin-1 is in the intestine of mice, and it regulates enteric hormone secretion. Immunofluorescence microscopy detected NUCB2/nesfatin-1 immunoreactivity in STC-1 cells, and in the mucosal cells of small and large intestines of male C57BL/6J mice. NUCB2/nesfatin-1 is co-localized with CCK, GLP-1 and PYY immunoreactive cells in intestine, indicating that enteroendocrine cells are a source of endogenous nesfatin-1. To assess the effects of nesfatin-1 on intestinal hormones, STC-1 cells (2X106 cells/well, n=8 wells/treatment; 2 separate studies) were incubated for 1 hour with synthetic rat nesfatin-1. At 1 hour post-incubation, secretion (ELISA/RIA) of GLP-1, GIP, CCK and PYY into the media, and expression of mRNAs (qPCR) encoding these peptides were measured. Nesfatin-1 upregulated preproglucagon mRNA at 0.01, 0.1, 1 and 10 nM. Concurrently, total GLP-1 levels were augmented at 0.1 nM (10.9±0.8 pM), 1 nM (10.5±0.8 pM) and 10 nM (9.98±0.8 pM) nesfatin-1 compared to controls (6.6±0.9 pM, $p<0.01$). Nesfatin-1 increased GIP mRNA expression at 0.1, 1 and 10 nM. Total GIP levels were also elevated at 0.1 nM (7.7±0.4 pg/mL), 1 nM (9.4±0.3 pg/mL) and 10 nM (8.2±0.4 pg/mL) when compared to controls (5.8±0.1 pg/mL, $p<0.1$). Nesfatin-1 enhanced CCK mRNA expression at 1 and 10 nM, while PYY mRNA was decreased at all doses tested. A corresponding stimulation in CCK secretion was found in 0.1 nM (36.28±2.9 pg/mL), 1 nM (40.14±1.94 pg/mL) and 10 nM (41.50±1.7 pg/mL) doses compared to controls (23±1.06 pg/mL, $p<0.01$). Similarly, nesfatin-1 attenuated PYY secretion at 0.01 nM (3.95±0.55 pg/mL) and 0.1 nM (5.33±2.35 pg/mL) doses compared to controls (18.45±2.74 pg/mL, $p<0.001$). These novel results demonstrate a direct action of nesfatin-1 on enteroendocrine cells in regulating hormone secretion in vitro. While nesfatin-1 stimulates GLP-1, GIP and CCK, it inhibits PYY secretion. Whether intestinal metabolic hormones modulated by nesfatin-1 mediate its role in the regulation of energy balance remains to be elucidated.

Postnatal vanishing testis-like syndrome in a 38XX/38XY agonadic hog (*Sus scrofa*)
[P114]

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Mammalian sex determination depends on the chromosomal complement: XY in males, XX in females. In most eutherian mammals, the Y-linked gene SRY (Sex determination region on Y chromosome) specifies the fate of the gonadal primordium by up-regulating male-specific developmental networks to initiate testis differentiation. In absence of SRY, the bipotential gonad develops into ovary. Besides that, chromosomal alterations such as XY/XX chimerism, result in chimeric, ovotesticular disorder of sexual development (DSD); an identity in which the coexistence of testicular and ovarian tissue in the same individual is observed. Here, we report some endocrine and molecular findings from an unusual case of DSD, in a 38,XX[53%]/38,XY[47%] hog who displayed postnatal phenotypic changes including, delayed growth and late-onset vanishing testis-like syndrome. At birth, it exhibited genital ambiguity characterized by female external genitalia and two prominent scrotal masses resembling testis. Circulating levels of testosterone, estradiol and progesterone were undetectable at one- and seven-months of age, while cortisol was normal at both ages. DNA analysis excluded alterations in SRY, SF1, SOX9, NR3C4 and SRD5A2. At 7 months-old, the XY/XX pig showed a marked growth delayed, but the most striking phenotypical finding was that the testes-like structure, that had once existed, was vanished completely in a two week period. After slaughtering the animal, the internal genitalia exclusively consisted of urethra and the lower two thirds of the vagina. No fallopian tubes, uterus, upper third of the vagina or Wolffian derivatives were found. More importantly, testes, ovaries, ovotestes or gonadal streaks neither could be identified. In the present case, the absence of Müllerian ducts suggests expression of AMH by embryonic Sertoli cells, while the absence of Wolffian duct would indicate an insufficient or null production of androgens by embryonic Leydig cells. The XX/XY sex chromosome dosage, and/or duplication of the DAX1 gene on the X chromosome, in presence of a normal SRY gene, may have caused the predominant female phenotype. A likely mechanism involving DAX1-induced inhibitory effects on SRY, SF1 (NR5A1) and androgen receptor (NR3C4) gene transcription is proposed. To our knowledge, this is the first reported case of XX/XY chimeric, ovotesticular DSD, associated to agonadism in pigs.
Study supported partially by the CONACYT, México (Grant # 166408).

Effects of maternal social stress on offspring survival, anxiety behaviour, and responsiveness to stress in zebrafish (*Danio rerio*)
[P54]

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Fish are experiencing multiple stressors both in agriculture and in natural settings. Maternal stress can have a significant effect on offspring, and hence the health of fish populations, because developing eggs are exposed to the maternal environment, allowing the deposition of maternal mRNA, proteins and lipids into the oocytes. In particular, increased maternal cortisol as a result of stress prior to spawn may affect offspring later in life because cortisol plays an organizational role during early development in teleost fish. The present study tested the hypothesis that maternal social stress in zebrafish (*Danio rerio*) affects offspring behaviour and responsiveness to stressors by hormonal mediation (via cortisol). Even though *Danio rerio* is a social species that prefers swimming in shoals, behavioural studies have reported the formation of social

hierarchies owing to competition for limited resources such as food, territory, and mates. The formation of dominance hierarchies results in subordinate female zebrafish that have higher circulating cortisol levels than dominant females. For these reasons, we compared offspring phenotypes for dominant versus subordinate mothers. Size-matched, adult female zebrafish were paired for two days, and observed twice daily to score behaviour indicative of social status. After two days, subordinate, dominant and sham (unpaired) females were bred with male zebrafish of similar mass and fork length. To distinguish between effects of increased maternal cortisol and maternal social status, a separate subset of adult females was exposed to exogenous cortisol by intramuscular injection prior to breeding. Offspring survival and hatching success were assessed daily until 6 days-post-fertilization. Embryos were sampled for analysis of cortisol concentrations, and the remaining viable embryos were raised to appropriate time-points when behavioural and stress-responsiveness tests were conducted. Specifically, anxiety-related behaviours were quantified using a novel tank diving test, a black/white preference test, and an open-field test; and stress responsiveness was assessed by measuring baseline and stress-induced whole-body cortisol levels following air exposure. The anxiety-inducing effects of maternal social stress on offspring, and the role of cortisol will be discussed.

Evaluation of the histological and functional effects of estrogen and atrazine on the thymus gland of *Xenopus laevis* tadpoles
[P40]

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Introduction of estrogens and estrogen-like pollutants into the natural habitats of aquatic species has been shown to adversely influence the physiology and ecology of these species. We have previously shown that treatment of tadpoles with exogenous (Dexamethasone 2uM), estradiol (10uM), or estrogen mimetic pesticide, Atrazine (50ug/L) induces apoptosis and involution of the thymus gland in *Xenopus laevis* at Nieuwkoop-Faber (NF) stages 50 and 54. Since the thymus plays an important in the development of larval and adult immune cells in vertebrates, we sought to assess the consequences of these changes on immune function in metamorphosing *Xenopus laevis*.

We compared response of tadpole at different stages of natural or induced metamorphosis with to live yeast cells. Tadpoles were injected intraperitoneally with yeast after 1 week to 1 month pre-exposure to atrazine or estradiol. We report that tadpoles at the prometamorphic to climax natural metamorphosis were generally less susceptible to yeast compared with premetamorphic tadpoles (NF54 and earlier). NF50 stage tadpoles pre-treated with dexamethasone (1uM) or estradiol (2.5uM) were more susceptible to intraperitoneal yeast injections compared with sham injected controls. NF54 tadpoles pre-treated with either estradiol (10uM) or atrazine (50ug/L) (doses are associated with thymus involution) died faster and to a greater extent compared with controls or lower doses of estradiol (2.5uM) or atrazine (5ug/L).

The data suggests exogenously administered hormones, may impact immune tolerance of tadpoles in a manner related to the developmental stage of tadpoles and concentrations of the endocrine disruptor.

Hormone regulation of maternal care in the mouth brooding cichlid *Astatotilapia burtioni* [P115]

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Steroid hormones are important regulators of aggression, parental care, and courtship in many vertebrates. Because these three behaviours often co-occur in male teleosts, the relationships between individual steroids and both aggression and reproduction may be more easily addressed in females, for whom courtship display is usually not a confounding factor. We observed the behaviours and measured secreted testosterone, 11-ketotestosterone, progesterone, and estradiol in female mouth-brooding fish both during the incubation of developing embryos and during the defense of free-swimming offspring (fry.) We found that all hormone levels were lower during the incubation period than during the defense period, though testosterone, 11-ketotestosterone, and estradiol peaked more rapidly after release of fry and subsequent increase of aggression than did progesterone. Levels of both testosterone and progesterone were lower in brooding mothers who later engaged in brood cannibalism; these hormones may therefore be important regulators of maternal care. Progesterone was also directly correlated with aggression and may play a role in brood defense; females who later engaged in brood cannibalism defended fry less aggressively than did females who did not later engage in cannibalism. These effects were sufficiently robust to be observed even in small sample sizes, illustrating the advantages of using female teleosts to study the relationships between steroid hormones, parental care, and aggression.

Expression analysis of the heterodimeric glycoprotein hormone receptor, LGR1, in the dengue fever vector, *Aedes aegypti*
[P32]

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GPA2/GPB5 is a relatively novel-discovered, yet evolutionarily ancient glycoprotein hormone found in bilateral metazoans including the mosquito, *Aedes aegypti*. Contrary to its vertebrate glycoprotein hormone relatives, which include the gonadotropins and thyroid-stimulating hormone, the exact physiological role of GPA2/GPB5 in both vertebrates and invertebrates remains unclear. Based on the transcript expression profile of its receptor, the leucine-rich repeat-containing G protein coupled receptor 1 (LGR1), along with the glycoprotein hormone subunits (GPA2 and GPB5), a physiological role linked to the adult developmental stage seems likely. Moreover, electrophysiological studies measuring ion transport across gut epithelia treated with recombinant GPA2/GPB5 suggests an ionoregulatory function. However, in order to further delineate the function of GPA2/GPB5 in the mosquito and homologs native to other

organisms, characterizing the LGR1 expression profile at the protein level (e.g. tissue-specific, developmental and sex-specific distribution patterns) is required since LGR1 transcript abundance may not accurately correlate with protein activity. Following an apparent ionoregulatory and potential osmoregulatory role, we have investigated LGR1 protein expression in adult gut epithelia involved in ionic and osmotic homeostasis and examined if differences occur in adult males relative to females. Immunocytochemical techniques using mammalian cell culture models has confirmed *A. aegypti* LGR1 localizes to the plasma membrane and western analysis confirms that membrane protein fractions, but not cytosolic protein fractions, contain an expected-sized band of ~105 kDa. Immunohistochemical techniques on wholemount tissues have been performed to visualize the LGR1 tissue distribution pattern in adult *A. aegypti*. Based on these qualitative findings, our future studies will focus more specifically on tissues expressing LGR1 and quantify the spatial, temporal, sex-specific and feeding-state related LGR1 expression profile. This research will help to elucidate the unknown function of the glycoprotein hormone GPA2/GPB5 in *A. aegypti* and may also reveal physiological roles of its homologs in other organisms, including both invertebrates and vertebrates.

Adolescence and the shaping of the stress response
[S12, invited]

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Interest in adolescence as a crucial stage of neurobehavioural maturation is growing, as is the concern of how stress may perturb this critical period of development. Notably, stress reactivity changes significantly during adolescence. These changes may contribute to the increase in stress-related dysfunctions often associated with this stage of development, such as anxiety, depression, drug use and abuse, and obesity. My talk will examine the effects of pubertal and adolescent development on hormonal and behavioural stress reactivity, as well as the similarities and differences that exist between males and females in the context of stress responsiveness. Studying how pubertal development shapes stress reactivity will not only increase our basic understanding of how adolescence affects the functioning of key neuroendocrine systems, but may also help shed light on the developmental vulnerabilities associated with puberty.

Age and sex differences in serum cytokine levels following exposure to a bacterial endotoxin
[P101]

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Exposure to stressors can have long lasting consequences, both on the brain and behaviour, especially during critical periods of development. Puberty is a critical period of development during which sexual maturity is attained. During this period, the brain undergoes important

reorganization and developmental processes. Previous research has shown that an injection of the bacterial endotoxin, lipopolysaccharide (LPS), can cause pubertal mice to be less responsive to estradiol and progesterone treatments in adulthood compared to mice that were not injected with LPS during puberty. The objective of the current study is to examine age- and sex-specific differences in serum cytokine levels following exposure to this endotoxin to investigate differences in immune response. We hypothesized that pubertal mice will show greater serum cytokine concentration levels than adults and that females will show greater cytokine concentrations than males. To test this hypothesis, six and ten week old male and female CD-1 mice were injected with either saline or LPS, and then euthanized for trunk blood collection at two, eight, and twenty four hours post treatment. These samples were analyzed with a multiplex Luminex immunoassay to determine serum concentrations of TNFα, IL-10, IL-12(p70), IL-1β, and IFNγ. Contrary to our hypotheses, the results showed that, following LPS treatment, adult females had significantly higher serum cytokine levels than pubertal females as well as adult and pubertal males and took longer to return to baseline cytokine levels than did the other groups. This suggests that an acute immune response may be more adaptive, since pubertal mice that do not express such immediate and severe reactions express long-term alteration to their brains and behaviour.

A multi-taxa comparative animal model approach to provide mechanistic insight into neuroendocrine disruption
[S4, invited]

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Developmental exposure to estrogenic endocrine disrupting chemicals (EDCs; i.e., chemicals that mimic and can interfere with natural hormones) may induce neurodisruptive effects in wildlife and humans. As recognized over two centuries ago, "*Between animal and human medicine there is no dividing line – nor should there be*". This concept has reemerged as the "*one health, one medicine*" concept. All complex animal taxa share commonalities in epigenetic and gene-expression pathways and neurophysiology. Therefore, neuroendocrine disruptions identified in animals are relevant to human populations. Two prevalent EDCs are bisphenol A (BPA) and ethinyl estradiol (EE, estrogen present in birth control pills). BPA, which is used in the production of a wide range of commonly used household items. These have been identified in a wide range of habitats, thus ensuring promiscuous and persistent exposure. We have undertaken long-term studies to determine if developmental exposure to BPA induces similar neurobehavioural deficits across species ranging from fish to turtles to Native American California mice (*Peromyscus californicus*), who are otherwise highly social, monogamous, and biparental, as observed in most human societies. We have determined that developmental exposure to BPA and EE results in spatial navigational learning and memory deficits in related male deer mice (*P. maniculatus bairdii*). Early contact to BPA and EE leads to behavioural effects in California mice, as evidenced by social and parenting deficits in males and females and anxiogenic effects in females. We are currently testing similar behaviours in painted turtles (*Chrysemys picta*) exposed *in ovo* to BPA and EE to determine if similar neuroendocrine

disruptive changes are observed in this aquatic species. We showed developmental exposure to BPA results in males possessing ovarian structures in their gonad, suggestive of partial sex-reversal. Treated males may also be feminized in their behavioural patterns. For all three species (fish, turtles, and California mice), we are currently comparing neural transcriptomic profiles after early BPA and EE-exposure to determine if similar transcripts are altered. Our initial data in the hypothalamus of California mice indicate BPA and EE result in unique, sex-dependent transcriptomic signature patterns. Our cross-taxa comparison approach will hopefully yield mechanistic insight into how such chemicals lead to neurodisruptive effects in wildlife species and humans.

Age and sex differences in c-fos expression following Poly I:C treatment in pubertal and adult CD1 mice
[P102]

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Puberty, a critical period for brain development and reorganization, is sensitive to exposure to stressors and immune challenges. Previous studies have shown that exposure to the immune stressor lipopolysaccharide (LPS), a bacterial endotoxin, during puberty (at 6 weeks of age), results in enduring changes in reproductive and non-reproductive behaviours, such as depression, anxiety, and cognitive function. The mechanism underlying these alterations remains unknown. Recent findings from our laboratory show that a single injection of LPS induces age and sex differences in c-fos expression. More specifically, LPS treatment increased the expression of c-fos in the arcuate and ventromedial nuclei of the hypothalamus, the CA1 and dentate gyrus of the hippocampus, the lateral septum and the lateral habenula in adult mice compared to their saline-treated counterparts. However, LPS treatment failed to increase c-fos expression in these brain regions in pubertal mice. The objective of the current study was to examine whether treatment with a viral vector, polyinosinic-polycytidylic acid (poly I:C), also induces age and sex differences in c-fos expression in mice. To examine this question, 80 pubertal (6 weeks old) and adult (10 weeks old) mice were treated either with poly I:C (12mg/kg) or saline vehicle. Tissue samples were collected two hours following treatment and stained for c-fos using immunocytochemistry. Pubertal male mice treated with poly I:C showed a significant increase in c-fos expression in the CA3 and dentate gyrus regions of the hippocampus compared to saline controls. In contrast, in pubertal females, and in adult males and females, poly I:C treatment failed to increase c-fos expression in these regions compared to saline controls. These results show that the viral-mimetic poly I:C induces a different pattern of brain activation compared to the bacterial endotoxin LPS. Taken together, these results will contribute towards a better understanding of the mechanisms underlying the enduring effects of exposure to an immune challenge during puberty on reproductive and non-reproductive behaviours.

Investigation of a novel endocrine axis in male-pregnant syngnathid fish: the hypothalamic-pituitary-brood pouch axis
[P69]

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Although viviparity (extended embryonic development within the parent) occurs across all taxa, male viviparity is exceedingly rare. All members of the teleost fish Family Syngnathidae (seahorses, pipefish, and seadragons) reproduce via male pregnancy. The morphological and physiological changes that occur during pregnancy in male syngnathids exhibit similarities with those of female mammals, making syngnathids a promising model for understanding the convergent evolution of mechanisms mediating pregnancy. Research on the endocrinology of male pregnancy has been scant, in part, due to the limitations of working with such non-model, small-bodied fishes. Modern neuroanatomical and molecular genetic techniques provide a way to supersede such technical difficulties. We have paired traditional histological staining with multi-fluorescent immunohistochemistry to investigate the anatomy of a novel endocrine axis: the hypothalamic-pituitary-brood pouch axis, providing the first detailed analysis of syngnathid neuroendocrine anatomy. We sampled males of two species of syngnathids, the Northern pipefish (*Syngnathus fuscus*) and the lined seahorse (*Hippocampus erectus*), over several stages of pregnancy. We have discovered a unique placement of the pituitary in the pipefish, which is situated between the lower hypothalamic lobes of the brain. In contrast, the seahorse has a pituitary that is ventrally offset from the hypothalamus, similar to that of other vertebrates. We have also demonstrated that the brood pouch epithelium of the Northern pipefish contains ionocytes, similar to those found in the gills of teleost fish. These ionocytes undergo morphological changes across the pregnancy cycle, providing support for their role in the osmoregulation of the brood pouch fluid. Future investigations will focus on the hormonal regulation of male pregnancy in syngnathids. We hypothesize that prolactin released from the pituitary acts via prolactin receptors in the brood pouch epithelium to regulate epithelial proliferation and ionocyte activity during early pregnancy.

Characterization and cloning of the long neuropeptide F (NPF) receptor in the Chagas disease vector, *Rhodnius prolixus*
[P33]

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Long neuropeptide F (NPF), a member of the FMRFamide-related peptidec family, is a neuropeptide implicated in the control of feeding, digestion and reproduction in various insect species. Here we have isolated the cDNA sequence encoding the NPF receptor (RhoprNPFR) in *Rhodnius prolixus.* RhoprNPFR is a rhodopsin-like G-protein coupled receptor (GPCR), with the characteristic 7 transmembrane domains. RhoprNPFR is 1277bp long and is composed of 3 exons and 2 introns. This receptor is conserved with other NPF receptors in various insect species, with 100% conservation in a DRY motif after the third transmembrane domain in the cytoplasmic loop. Two cysteine residues, most likely used to form a disulfide bond between the

second and third extracellular loops, are also conserved. Quantitative PCR (qPCR) shows that RhoprNPFR is a brain-gut receptor with expression throughout the central nervous system and the digestive tract. Expression of the receptor was also seen in the oviduct/spermathecae (of females) and seminal vesicle/ejaculatory duct (of males), suggesting that RhoprNPFR may play an important role in sperm storage and release. Injection of RhoprNPF into mated, fed, female adult *R. prolixus* resulted in an increase in egg production coupled with egg retention within the ovaries. This suggests that RhoprNPF is also involved in regulating ovulation in female *R. prolixus*.

Effects of Poly(I:C) on thermoregulation in CD1 mice
[P103]

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Exposure to stress during critical periods of development can lead to enduring changes in the functioning of the brain and body that can impact physical and mental health. The objective of the current study is to examine age and sex differences in immune response following exposure to the viral mimetic polyinosinic:polycytidylic acid (Poly(I:C)). We hypothesized that there will be age and sex differences in Poly(I:C)-induced sickness behaviour and changes in body temperature. To test this hypothesis, male and female mice received an intraperitoneal injection of either saline or Poly(I:C) at 6 (puberty) or 10 (adulthood) weeks of age. We also examined the effect of circulating gonadal hormones by gonadectomizing half of our mice. We predicted that Poly(I:C) would induce a significant rise in body temperature compared to saline treated controls based on previous literature. We predicted that adolescent mice would show a distinct body temperature profile and sickness behaviours in comparison to the adult mice. We also predicted that female mice would significantly differ from male mice in each age category. Finally, we predicted that gonadectomized females and intact males would display the same temperature profiles and sickness behaviours in each age category. These findings provide a better understanding of age and sex differences in immune response to a viral infection and the effect of circulating gonadal hormones in these differences. These results also suggest potential mechanisms of interactions between stress, immune response and gonadal hormones that cause enduring changes in brain functioning and behaviour. Future research will attempt to identify age and sex differences in cytokine and corticosterone levels as well as cytokine expression in significant brain regions following exposure to Poly(I:C).

The role of cortisol in osmoregulation and thermal tolerance in brook trout (*Salvelinus fontinalis*) during seawater acclimation
[P110]

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Migratory fish encounter a wide range of environmental conditions, including changes in salinity, temperature, and dissolved gases, yet how the these stressors interact on the physiology of the fish during migration is not well-understood. The aims of this study were: (1) to assess how thermal tolerance of brook trout (*Salvelinus fontinalis*) is affected during seawater acclimation, and (2) to investigate the role of cortisol on osmoregulation and thermal tolerance in brook trout throughout seawater acclimation. Freshwater-acclimated (FW) brook trout were examined for critical thermal maximum (CTmax), then transferred to 25 ‰ salinity (SW), while maintaining a FW control. Additional fish were administered a cortisol implant (5 or 25 μg/g) prior to SW transfer. Fish were examined for CTmax on days 2, 5, and 16 after SW exposure, and sampled at both rest and at their thermal maximum for analysis of plasma cortisol, glucose, and [Cl^-], gill Na^+/K^+-ATPase (NKA) activity and heat shock protein 70 (HSP70) expression, and white muscle moisture. Osmoregulatory indicators suggested fish had fully acclimated to SW by day 16. By day 2 of SW exposure, CTmax in the SW was significantly reduced (from 31 to 26 °C), then returned to FW values by day 16. Plasma cortisol concentrations were significantly elevated (8-fold) by day 2 after salinity transfer but had returned to FW control values (10 ng mL^{-1}) by day 5. Both FW and SW fish had significantly elevated concentrations (2.5-fold) of cortisol at CTmax compared to respective resting values. Cortisol implant significantly reduced (by 60 %) the salt-load incurred by day 2 of SW exposure, but there was no difference between cortisol doses. In summary, these findings indicate that brook trout are much more sensitive to temperature change during (although not after) salinity acclimation, and suggest a possible involvement of cortisol in the interaction of osmoregulatory and thermoregulatory status.

Chromatin remodeling and histone modification underlying the regulation of *Xenopus* growth and development by thyroid hormone receptor
[Plenary]

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Thyroid hormone (TH) plays an important role during postembryonic development in vertebrates, a period around birth in mammals or of metamorphic transformation in amphibians. TH regulates gene transcription through nuclear TH receptors (TRs) that bind to specific DNA sequences in target genes. TRs can repress or activate TH-inducible genes in the absence or presence of TH, respectively. Interestingly, TRs are expressed earlier than the synthesis of endogenous TH during vertebrate development, suggesting developmental roles for both liganded and unliganded TRs. We have been investigating such potential roles by using *Xenopus* metamorphosis as a model. We have shown previously that TH plays a causative role in amphibian metamorphosis via binding to TRs. More recently, we have investigated the developmental roles of TRα, which, unlike TRβ, is highly expressed prior to the synthesis of endogenous TH, by using TALEN (transcription activator-like effector nuclease)-mediated gene knockdown approach. We show that TRα knockdown has no effect on embryogenesis but leads to accelerated growth and development of the tadpoles, indicating that unliganded TRα controls both premetamorphic tadpole growth and metamorphic timing. On the other hand, such

knockdown animals are also resistant to exogenous TH treatment and have delayed natural metamorphosis, demonstrating a critical role of TRα in mediating TH effect on metamorphosis. Toward understanding the underlying mechanisms, we have shown that unliganded TR recruits histone deacetylase (HDAC)-containing N-CoR/SMRT complexes in premetamorphic tadpoles while liganded TR recruits histone modification complexes during metamorphosis. We have further shown that liganded TR induces histone modifications and the removal of core histones at target promoters in vivo. Collectively, our data indicate that TR recruits histone-modification complexes to alter the dynamics of nucleosomal structure and histone modifications at the target genes in a TH dependent manner, thereby regulating growth and metamorphic timing in premetamorphic tadpoles and the rate of metamorphic progression during metamorphosis. Similar mechanisms are likely responsible for TR function in other vertebrates, including mammals, as the maturation of many organs into the adult form during postembryonic development is dependent upon TH and resembles organ metamorphosis in amphibians.

Behavioural aspects of locomotion in rodents with teneurin C-terminal associated peptide (TCAP) administration
[P19]

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The synthetic version of teneurin C-terminal associated peptide is highly effective at inhibiting behavioural arousal in rodents as shown by studies in acoustic startle reflex, elevated plus maze, H-maze, open field and cocaine reinstatement studies. TCAP and its proprotein, teneurin, are expressed in a number of regions associated with sensory and motor regulation in rodents and primates. Recent in vitro studies on the cellular actions of synthetic TCAP-1 indicate that it inhibits the signal transduction system associated with its receptor, latrophilin-1, a GPCR with three described isoforms. Disruption of latrophilin-3 in zebrafish has been associated with increased locomotor behaviour. However, because endogenous administration of synthetic TCAP-1 in mice and rats does not promote a significant increase in locomotion, per se, it is plausible that TCAP-1 affects arousal behaviours associated with locomotion. As a result, we have examined the role of TCAP-1 on the running behaviour of golden hamsters (Mesocritus auratus). Central injection of synthetic TCAP-1 into and around the suprachiasmatic nucleus of this species results in a disruption of running wheel activity. These studies indicate a novel action of TCAP-1.

Prolactin and parental care in the zebra finch (*Taeniopygia guttata*)
[S14, contributed]

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Parental care, defined as any behaviour that increases an offspring's fitness, is a widespread phenomenon observed in many diverse taxa which has evolved independently numerous times resulting in species-specific parental behaviour. While it is clear that species have converged on parental care in order to increase fitness, it is less clear whether the diversity in parental care behaviour has resulted from species-specific mechanisms or whether species have co-opted similar mechanisms to promote parental behaviour. Birds are the most parental vertebrate clade, with ~99% of species showing some form of parental care. However, few causal studies exist on the neuroendocrine mechanisms of avian parental care. The hormone prolactin (PRL) and its central receptors are a promising candidate mechanism of parental care to investigate in birds. PRL has a well-established role in maternal care in mammals and ring doves and has been suggested to be involved in parental care in birds, including songbirds. The zebra finch, a socially monogamous, biparental songbird, is an exceptionally useful animal model to study parental care and other close social relationships. Both sexes share parental care equally, exhibit the same parental behaviours, and show a marked improvement in breeding success with experience. Currently, there are no published studies looking at PRL's involvement in the expression of zebra finch parental behaviour or the mechanisms underlying the improvement in breeding success. We have found that plasma PRL significantly elevates from non-breeding baseline concentrations during late incubation and early post-hatch care and that this elevation is greater in reproductively experienced birds, compared to inexperienced birds. Plasma samples were assayed using an ELISA that we validated in zebra finches. In addition, plasma PRL concentrations are highly correlated with the amount of parental care behaviour displayed during days 2 and 3 post-hatch, the number of chicks that successfully hatched, as well as chick survival to fledging. Findings from these studies will be used to inform hypotheses and predictions for future work involving experimental manipulations of PRL during parental care.

Evolution of the GnRH and GnRH receptor families: Insight from a basal vertebrate and mollusk
[S7, invited]

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The hypothalamic-pituitary system is considered to be a vertebrate innovation that emerged prior to or during the differentiation of the ancestral agnathans likely due to one or two whole rounds of genome duplication. Reproduction in vertebrates is controlled by a hierarchically organized endocrine system. Gonadotropin-releasing hormone (GnRH) and its receptor (GnRH-R) play pivotal roles in the regulation of reproduction in vertebrates. In spite of the very diverse patterns of life cycles, reproductive strategies, and behaviours, this endocrine system is remarkably conserved throughout the vertebrate lineages. Several GnRH-like and GnRH-like receptor molecules have been identified in representatives of invertebrates. However, the physiological functions of these GnRH like molecules are poorly understood. The function(s) of GnRH-like peptide and related peptides including corazonin (Crz), adipokinetic hormone (AKH) and AKH/Crz-related peptide (ACP) in reproduction have not been established. In fact, it may be that

the ancestral function of the GnRH peptide family in invertebrates was not directly related to reproduction. In the mollusk, the sea hare (*Aplysia californica*), GnRH and AKH were identified and shown to be involved in physiological functions other than reproduction such as feeding (Johnson et al., 2014). We will summarize our latest findings on the GnRH systems in a basal vertebrate, the sea lamprey, and an invertebrate, the sea hare, that provide a unique insight into the evolution of the GnRH and GnRH like systems. We will provide phylogenetic, syntenic and functional analyses summarizing the evolutionary relationships of the GnRH and its receptors that evolved divergent structure and functions in the vertebrate lineages. Support: NSF IOS-1257476, AES NH00624 to SAS and NSF IOS 1352944 to P-ST.

Expression and functional roles of gonadotropin-inhibitory hormone (GnIH) in the zebrafish (*Danio rerio*)
[P70]

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Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic neuropeptide capable of inhibiting the release of pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), in birds and mammals. However, the functional role of GnIH is not clear in fish. In particular, data on GnIH localization and functions within the well-accepted vertebrate model, the zebrafish (*Danio rerio*), is scarce. Therefore, the goal of this study is to determine the functional roles of GnIH in zebrafish reproduction by determining GnIH's neuronal localization and interactions within the brain-pituitary axis and by knockout techniques. Using adult *FSH:eGFP* and *LH:mCherry* transgenic fish pituitaries, we conducted immunocytochemistry for GnIH and found that GnIH-immunoreactive fibers are indeed located in the neurohypophysis, but the GnIH signal in the pituitary is greatly reduced compared with that of the mediobasal hypothalamus. Furthermore, the interactions between GnIH neurons and those of GnRH2 and GnRH3 are currently being investigated in the *GnRH2:eGFP GnRH3:tdTomato* double transgenic adult brains to determine GnIH's structural relationships to the GnRH systems. Finally, we achieved targeted, heritable mutations in GnIH gDNA by developing a *gnih*$^{-/-}$ knockout line, using the TALEN technology. In-crossing of *gnih*$^{-/-}$ fish have demonstrated that these fish are capable of spawning and producing viable offspring, indicating that *gnih* is not essential for reproduction. We are currently testing reproductive fitness and general performance in the *gnih*$^{-/-}$ line by evaluating larval behaviour, gonad development, and developmental expression of other genes (e.g., *gnrh2*, *gnrh3*, *lhβ*, etc.) along the zebrafish reproductive axis. A multi-level approach to studying GnIH's functional role within the reproductive axis of zebrafish has shown promising results to date. The addition of data from the *gnih*$^{-/-}$ line will enable future studies to elucidate the degree to which GnIH regulates neuroendocrine control of reproduction, as well as the mechanisms through which regulation occurs.

Nucleobindin-1 encoded nesfatin-1 like peptide (NLP) decreases food intake and downregulates preproghrelin mRNA expression in fish
[P18]

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Nesfatin-1 is a multifunctional metabolic peptide encoded in the precursor, nucleobindin-2 (NUCB2) of mammals and non-mammals. Nesfatin-1 play a crucial role in regulating whole body energy homeostasis and blood glucose levels in vivo. NUCB2 is closely related to another protein nucleobindin-1 (NUCB1). We hypothesized that NUCB1, like NUCB2, encodes a nesfatin-1 like biologically active peptide. The main objectives of this research are to determine the tissue distribution of nesfatin-1/NUCB1 and also to study the effect of exogenous nesfatin-1 on food intake in fish. Our in silico analysis found a nesfatin-1 like peptide (NLP) in NUCB1. NUCB1 mRNA expression were detected in the brain and in peripheral tissues including the gut, liver, gonads and eye of goldfish and zebrafish. Immunofluorescence microscopy detected the NUCB1/NLP immunoreactivity in the pituitary, testis, ovary and gut of goldfish. Intraperitoneal injection of 100 ng/g bodyweight of synthetic NLP decreased food intake when compared to saline injected controls. In the hypothalamus and gut of these fish that had reduced food intake, NLP also downregulated ghrelin (an orexigen) mRNA expression (RT-quantitative PCR). Our preliminary results suggest that NUCB1/NLP is present in fish and has metabolic effects. Further studies are required to better our understanding on NLP biology in fish.

Answering big questions in evolutionary endocrinology: combining field and lab research, high-throughput molecular genetics tools, and modeling
[S14, invited]

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Molecular signals such as hormones regulate the phenotypic outcomes of development in response to genetic and environmental variation. As such, molecular signals represent the underlying targets of selection on phenotypic traits. In a general sense, we now have a strong understanding of the genetic basis of these signals, as well as their functional roles during development. Nevertheless, our understanding of the how molecular signals evolve in concert with the phenotypes they regulate is still rudimentary. For example, hormonal pleiotropy generates correlations among phenotypic traits and plays an important role in phenotypic integration. But is hormonal pleiotropy the outcome of a constraint on physiological variation, or is pleiotropy itself is adaptive, facilitating appropriate correlated responses to selection or environmental variation? Many similar questions remain. One of our major research goals is to understand the how the mechanistic bases of phenotypic development evolve, and what implications this has for the outcomes of phenotypic evolution. We take three main approaches to address this goal. First, we are measuring standing variation in life history traits and trade-offs

in a number of Lepidopteran species. Further, we measure plastic phenotypic responses in these species to hormone application experiments. We then use gene expression data to better understand the genetic basis of the developmental mechanisms underlying the measured phenotypic variation in these species, and how these mechanisms themselves evolve. Finally, we are using individual-based evolutionary simulations to investigate general mechanisms of life history evolution, and to test hypotheses about molecular signaling evolution that are otherwise difficult or impossible to test. Our results thus far suggest that juvenile hormone application increases fecundity in all species tested, but its effect on other traits and trade-offs varies among species. This suggests that the pleiotropic role of juvenile hormone uncouples among some butterfly species. We will present results from our experiments on life history variation as well as results from models and preliminary analyses of genome-wide expression data from RNAseq to better understand the mechanistic and evolutionary basis of this variation.

Introduction to droplet digital PCR (ddPCR) and application to molecular medicine
[workshop]

Taylor, S. (Bio-Rad Field Application Manager) and Gumley, A. (Instrument Specialist/Territory Manager)

Droplet Digital PCR (ddPCR) uses emulsion chemistry to partition 20uL qPCR samples into 20,000 oil encapsulated nanodroplets for ddPCR. This serves to significantly increase precision and sensitivity while eliminating many of the requirements for optimization and validation that are associated with qPCR. The technology is ideally suited for samples with low abundant targets (ie: microRNA and low expressing targets in cDNA), copy number variance and SNP mutation abundance experiments and to achieve statistically significant data from contaminated samples. Since each well is an independent and absolute measure of nucleic acid concentration, standard curves are not required with ddPCR and replicates (either biological or technical) can be tested on different plates and days with a high level of precision and reproducibility. This permits the testing of samples as they are received during longer term projects to assess the potential success of a given study throughout the sample collection period without introduction of interplate variability associated with RT-qPCR. Here we introduce ddPCR technology and its application to molecular medicine in the detection and absolute quantification of DNA-based markers for the diagnosis and treatment of disease. The application of ddPCR to CRISPR as a new genome editing tool will also be discussed.

Physiological roles, molecular regulation, and disruption of the progesterone receptor signaling pathways in amphibians
[P38]

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It is now recognized that certain contaminants (e.g., pesticides, pharmaceuticals, industrial by-products) that are present in the environment have the ability to act on the endocrine system of

vertebrates at very low concentrations (ng/L). These endocrine disrupting chemicals (EDCs) alter hormonal homeostasis via the disruption of molecular controls. Among other actions, EDCs can modulate biosynthesis and hormonal actions, which can result in mild to severe health defects. Given that endocrine systems are well conserved among all classes of vertebrates, a hormonal disruption in one species is likely to occur in other species.

Gestagens, which include endogenous and synthetic progesterone receptor ligands used in human and veterinarian drugs, are an emerging class of contaminants that have been recently measured in surface water in North America. Endogenous progestogens are essential in the regulation of reproduction in mammalian species and recent studies have demonstrated that environmental gestagens have the ability to also interfere with the reproduction in aquatic vertebrates. This project aims to understand the roles and the regulation mechanisms of progesterone in amphibians and to assess the consequences of exposures to environmental gestagens on the progesterone receptor signaling pathways in frogs. This study 1) will establish the developmental profile of the progesterone receptors in *Silurana tropicalis* embryos using *in situ* hybridization and real-time RT-PCR techniques and 2) will assess the effects of progesterone and melengestrol acetate in amphibian early development and at metamorphosis in order to analyze change in the expression change of a suite of gene targets of interest (including, *pr, 3β-hsd, ar, er, fshβ, prl,* and *srd5a*).

Cortisol effects on local GH/IGF signaling in rainbow trout myogenesis
[P55]

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Cortisol is naturally produced by the adrenal cortex when vertebrates are under stressful conditions. In mammals, cortisol up regulates the production of myostatin, resulting in the loss of muscle mass. In teleosts, this role of cortisol is not clear as recent evidence suggests that cortisol may have little or no effect on myostatin. Stressful conditions, including crowding and handling, increase circulating cortisol levels, and chronic stress has been shown to decrease overall growth. The growth hormone/insulin-like growth factor axis (GH/IGF) plays an important role in regulating organismal growth. This study examines whether cortisol down regulates IGF, GH, and their corresponding receptors in isolated muscle myotubes from *Oncorhynchus mykiss* (rainbow trout). Additionally, we are investigating the effects of cortisol on the regulation of IGFBP in trout, as some binding proteins increase the half-life of IGF and therefore the probability of active IGF. Muscle cells were isolated from juvenile rainbow trout and cultured for three days in DMEM supplemented with 10% FBS. The cells were treated on day four with 10, 100, 1000 ng/µL of cortisol and collected after 24 and 48 hours. We hypothesized that increased exposure to cortisol will down regulate mRNA expression in the IGF, GH, and their corresponding receptors compared to the control muscle cells. In line with this hypothesis, increasing cortisol levels should increase IGFBP levels, which will have a negative impact on the gene expression of GH, IGF, and their receptors. In rainbow trout, cortisol treatment does not affect the negative growth regulator myostatin, but cell proliferation is decreased. Therefore, we hypothesize that cortisol regulates myogenic cell proliferation through local GH/IGF signaling.

Applying the principles of evolutionary endocrinology to develop spawning induction methods in amphibians
[S8, invited]

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The rapid decline of amphibians globally means that there is an urgent need to develop new reproductive technologies to establish captive colonies followed by reintroduction into suitable habitats. While the basic HPG plan is similar from teleosts to tetrapods, our knowledge of the neuroendocrine control lags by decades in Amphibia. Evolutionary principles must therefore be applied to rapidly advance captive breeding strategies. Many attempts have been made with mammalian gonadotropin preparations. Phylogenetic analysis has shown that hCG and PMSG are protein products of genes only distantly related to the amphibian gonadotropin subunits LH and FSH beta. This relationship may explain the wide range of hCG-sensitivities, i.e., little to no response in *Lithobates pipiens*, to spawning in *Bufo* and *Xenopus* at high doses. Amphibians express mammalian GnRH and there are numerous analogues available. Almost nothing is known about the control of gonadotropins, given the extremely limited availability of RIAs for Amphibia. In some species GnRH alone will stimulate LH but not spawning, indicating the existence of inhibitory factors. Licht and Sotowska-Brochocka revealed an inhibitory role of dopamine (DA) on the release of LH in *Rana temporaria*, a mechanism known for various teleosts and mammals. This role is suggestive of a conserved action that can be extrapolated to amphibians. The combination of a GnRH agonist with a DA antagonist has proven effective in both abundant and endangered species where there is limited information on their physiology. We have named this the AMPHIPLEX method, because it induces amplexus and spawning. Large-scale spawning has been successful in *L. pipiens* where reintroductions have begun in British Columbia. The production of fertile eggs followed by healthy tadpole development has been seen in *L. pretiosa*, *Ceratophrys ornata*, *Odontophrynus americanus* and *Pseudacris triseriata*. Critically endangered Puerto Rican crested toads, *Peltophryne lemur*, were spawned by the Nashville Zoo and >7,000 tadpoles have been sent back to Puerto Rico for release by the USFWS. Viable hellbender salamanders, *Cryptobranchus alleganiensis*, were obtained following *in vitro* fertilization using gametes collected post-injection. Critically missing is rigorous assessment of the effects of GnRH alone and in combination with DA antagonists on gonadotropin production. New research must be directed at uncovering reproductive neuroendocrine control mechanisms in Amphibia.

Plasticity in the postnatal gonadotropin-releasing hormone system
[S7, contributed]

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Vertebrate neuroendocrine systems respond dynamically to environmental cues to enhance adaptation and survival. In this study, we examined if a defective postnatal neuroendocrine system that secretes gonadotropin-releasing hormone (GnRH) can respond positively to environmental cues to restore the organism's declining reproductive function. Transgenic mice with GnRH neuron-specific deficiency in fibroblast growth factor (Fgf) signaling exhibited significant age-dependent decline in GnRH neurons and gonadal functions. However, if they were housed with opposite-sex (OS) littermates and allowed the opportunity for sexual interaction, their GnRH neurons and downstream gonadal functions were restored to normal levels. We then used RNA-seq to interrogate genes that may mediate this experience-dependent restoration of the GnRH system. A total of 1,485 genes in the preoptic area (region housing GnRH neurons) were differentially expressed between same-sex (SS) and OS-housed transgenic mice. These included candidates likely to be neuroprotective of GnRH neurons (Bdnf, Fgf10, and Fgfr3) and stimulatory to GnRH neuronal activity (R type calcium channel). The upregulation of Bdnf and Fgf10 in OS-housed transgenic mice was confirmed by quantitative PCR. In sum, we have identified an array of genes whose expression is altered by sexual experience and may participate in the restoration of a suboptimal GnRH system. These results strongly support the extraordinary plasticity of the postnatal GnRH system. (Supported by NIH HD042634)

Estrogenicity of captive southern white rhinoceros diets and their association with fertility [P78]

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Over the past century the southern white rhinoceros (SWR) population has grown from approximately 100 individuals to a current estimated population of 20,000. This remarkable recovery is a result of both *in situ* protection of wild stocks and the establishment of *ex situ* captive breeding programs. Although captive breeding programs were initially successful, poor reproduction of captive-born females has left the captive population unsustainable in its current state. Many captive female SWR exhibit pathologies that impair reproduction and are consistent with prolonged exposure to estrogenic chemicals. In our lab, we have investigated the potential role of dietary phytoestrogens in this phenomenon, finding that SWR estrogen receptors (ESRs) are more sensitive to phytoestrogens than ESRs of greater one-horned rhinoceros; a species that receives similar diets in captivity yet reproduces well. In the present study, we measured SWR ESR activation to determine estrogenicity of extracts of individual feeds and whole diets from nine North American SWR breeding institutions. Our results indicate that most grasses and hays, with the exception of alfalfa, do not contain significant levels of phytoestrogens. However, all commercial pellets tested contained concentrations of phytoestrogens that significantly stimulate activation of ESRs. As a result, the level of diet estrogenicity was found to be associated with the percentage of pellets fed in the whole diet. Finally, we used studbook data to calculate historical fertility of female SWR at each institution whose diets we tested and determine if a relationship exists between estrogenicity of diets and reproductive success. Our findings indicate that for wild-born females brought into captivity, there is no relationship between diet estrogenicity and

fertility. However, for captive born females there is significant negative relationship between institutional diet estrogenicity and fertility, suggesting that developmental exposure to dietary phytoestrogens is contributing to the reproductive failure of captive-born female SWR. Taken together, these data can be used to identify specific food items and develop whole diets that are low in phytoestrogens that may be used to resolve low fertility issues in future captive-born female SWR.

Comparative endocrinology of nesfatin-1: Update 2015
[S1, invited]

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Nesfatin-1 is an eighty two amino acid anorexigen encoded in nucleobindin-2 (NUCB2). Prohormone convertases 2 and 1/3 (PC2 and PC1/3), the same enzymes that process precursors of many endocrine factors, including insulin, cleaves nesfatin-1 from its precursor. Since its discovery in 2006, nesfatin-1 gradually emerged as a multifunctional metabolic peptide with significant effects on whole body energy homeostasis and the regulation of endocrine, reproductive and cardiovascular systems. This talk will focus to highlight the major findings on the comparative endocrinology of nesfatin-1 in non-mammals and mammals during the past two years (since NASCE 2013). Specific focus will be on findings arising from the speaker's laboratory. In non-mammals, new discoveries include the roles of nesfatin-1 in regulating cardiac functions, and growth hormone secretion. Nesfatin-1 was also identified in several fishes and at least in one amphibian. Emerging evidences show that steroids and nutrients are major regulators of nesfatin-1 expression and secretion. It was also convincingly shown that both small and large intestines are sources of nesfatin-1. Nesfatin-1 regulates stomach derived ghrelin, and intestinal hormones including glucagon like peptide-1. From a domestic animal endocrinology perspective, nesfatin-1 and its biological activity have been identified from a number of domestic species including pigs, cats and dogs. Overall, the past two years witnessed significant growth in our understanding on nesfatin-1, an orphan peptide still in its infancy.

Sex steroid hormones modulate NUCB2/nesfatin-1 and ghrelin in goldfish
[P20]

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Nesfatin-1 (encoded by nucleobindin2/NUCB2 gene) is an appetite inhibitory peptide, while ghrelin is an appetite- stimulatory hormone in goldfish. Both peptides are meal responsive, and are involved in the regulation of reproduction in fish. However, the regulation of these peptides

in vivo is poorly understood. Considering the abundant expression of these peptides in the gonads of goldfish, and its reproductive functions, we hypothesized that sex steroids regulate both ghrelin and nesfatin-1. The aim of the present study was to characterize the effects of estradiol (E2) and testosterone (T) on the expression mRNAs encoding NUCB2/nesfatin-1, ghrelin, ghrelin receptor and ghrelin O-acyl transferase (GOAT), the enzyme that acylates ghrelin in goldfish (Carassius auratus). First, a dose-response assay was performed in which fish were intraperitoneally (ip) implanted with pellets containing 25, 50 and 100 µg/g body weight (BW) of E2 or T. After 2.5 and 5 days, serum samples were collected, and E2 and T levels were determined by immunoassays. Observations from this first experiment allowed us to determine the appropriate steroid hormone doses (100 µg/g BW) and implantation period (2.5 days) that achieves the highest E2 or T serum levels. In a second experiment, fish were ip implanted with pellets containing 100 µg/g BW of E2, T or without hormone (control). After 2.5 days, gene expression in gut, forebrain, hindbrain, hypothalamus and pituitary was measured using real-time qPCR. NUCB2/nesfatin-1 mRNA expression was increased in the forebrain for T group and reduced in the gut and pituitary under both treatments. Ghrelin and GOAT expression was upregulated in the gut by both E2 and T treatments, while the same effect was observed for GHSR in the pituitary. Both treatments also led to a reduction in ghrelin mRNA levels in the hypothalamus. These results show, for the first time in fish, a modulation of NUCB2/nesfatin-1 and ghrelin by sex steroid hormones. The interaction between sex steroids and genes implicated in food intake regulation provides a possible explanation for seasonal changes in the endogenous metabolic hormone milieu in goldfish.

Circadian pattern of the ghrelinergic system and NUCB2/nesfatin-1 in goldfish
[P21]

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Living organisms have endogenous clocks that synchronize biological processes to the 24-h light/dark cycle, leading to the development of daily rhythms in behaviour and physiology. Among other processes, the circadian system regulates food intake but very few studies have described a daily oscillation of hormones involved in metabolism and appetite regulation. The aim of the present study was to characterize the possible circadian variations in appetite regulatory hormones nesfatin-1, and ghrelin, and its related genes (ghrelin receptor/GHSR and the enzyme that activates ghrelin, ghrelin-O-acyl transferase/GOAT) in goldfish (Carassius auratus). Fish were maintained under a 12L:12D photoperiod and scheduled feeding, and samples of blood, forebrain, hindbrain, hypothalamus, pituitary and gut were collected throughout a 24 hour cycle. Circulating hormone levels were measured by immunoassays, and gene expression was determined by real time-qPCR. Circulating levels of nesfatin-1 was highest around daily feeding time. Circadian patterns are observed for ghrelin, GOAT and ghrelin receptor mRNA expression in the hypothalamus and pituitary, all of them with the acrophase occurring during the night. Ghrelin, but not GOAT and ghrelin receptor, is also found to display a daily expression rhythm in the gut. No significant rhythmic oscillations are observed in the

forebrain and hindgut for any of the ghrelin-related genes. NUCB2/nesfatin-1 expression shows a rhythmic profile in the pituitary with one peak during the light time and another during the night. In the hindbrain and hypothalamus, a higher expression of NUCB2 is found during the early daytime, although no rhythmical pattern is detected. These results provide the first evidence for circadian patterns of NUCB2/nesfatin-1 and the ghrelinergic system in both central and peripheral tissues of goldfish.

Macronutrient composition of diet modifies tissue specific abundance of endogenous ghrelin and NUCB2/nesfatin-1 in goldfish (*Carassius auratus*)
[P22]

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The macronutrient composition of diet is a very important factor in the regulation of body weight and metabolism. Several lines of studies in mammals have shown that the macronutrient contents of diet regulate metabolic hormones, but little is known about this aspect in fish. Therefore, the aim of this research was to determine the possible short- and long-term effects of macronutrients on the expression of two appetite-regulating hormones, ghrelin (orexigenic) and nesfatin-1 (anorexigenic, encoded in the nucleobindin2/NUCB2 gene), in a teleost model, goldfish (*Carassius auratus*). Fish were fed once daily on control, high-carbohydrate, high-protein, high-fat and very high-fat diets for 7 (short-term) or 28 (long-term) days. At the end of the study period, fish were sacrificed at the scheduled feeding time, and hypothalamus, pituitary, gut and liver were collected. Ghrelin, ghrelin O-acyl transferase (GOAT), ghrelin receptor (growth hormone secretagogue receptor 1a, GHSR1a) and NUCB2/nesfatin-1 gene expression was determined using real-time-quantitative PCR. Short-term feeding with a high-protein diet resulted in a significant decrease in ghrelin and GHSR, but not GOAT mRNA expression in liver. Feeding of high- and very high-fat diets significantly increased GHSR expression in the gut. Similarly, fish fed on high-fat and a very high-fat diets had significantly high expression of ghrelin, GOAT and GHSR mRNAs in the pituitary compared to those fed on a high-carbohydrate diet, but not to the control group. Fish fed on a high-fat diet for one week showed high levels of NUCB2 gene expression in the hypothalamus and liver. After the long-term feeding with macronutrients-enriched diets, fish fed on high-carbohydrate and very high-fat diets exhibited significantly higher levels of mRNAs encoding ghrelin, GOAT and GHSR in the pituitary. GOAT and GHSR mRNAs, but not ghrelin mRNA expression was higher in the gut of fish fed on a high-carbohydrate diet compared to fish fed on any of the other diets. A high-fat diet significantly increased GHSR expression in liver. Diet composition did not produce any short-term or long-term effect on ghrelin, GOAT and GHSR expression in the hypothalamus. Long-term feeding with a high-protein diet significantly increased NUCB2 mRNA expression in the pituitary, but decreased it in gut. Taken together, these results show for the first time that nutrients differentially modulate the expression of ghrelin (and related genes GOAT and GHSR) and NUCB2/nesfatin-1 in both central and peripheral tissues of goldfish.

Fish endocrine disruption responses along complex land-use gradients: opportunities and limitations for mitigation by regulation and treatment technology
[P46]

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The urban-water cycle modifies natural stream hydrology, and domestic and commercial activities increase the burden of steroidal and non-steroidal, natural and synthetic estrogenic endocrine-disrupting chemicals, that can disrupt endocrine system function in aquatic organisms. This paper presents results from a series of integrated field and laboratory, chemical and biological investigations into the occurrence, fate, and effects of endocrine-disrupting chemicals in the headwater reaches of major river systems in Colorado, the Chesapeake Bay, and Australia. Our long-term, continental-scale studies show that the occurrence and effects of endocrine disrupting chemicals are relatively low in river headwaters, and increase downstream with increasing anthropogenic activity. We have demonstrated that exposure to environmentally-relevant exposure to wastewater treatment facility (WWTF) contaminants has adverse implications for sexual selection in native fish by disrupting female-choice. We show that exposure to non-estrogenic antimicrobial WWTF contaminants disrupt the diversity and abundance of microbial communities in the fish gut with potential adverse implications for host fitness. Through a long-term site-specific integrated field and laboratory investigation we demonstrate significant recovery of reproductive health in wild and experimentally-exposed fish following a full-scale upgrade of WWTF treatment process. Through multi-generational studies we have identified transgenerational consequences of estrogen exposure on fertility that may impact the long-term recovery of exposed populations. Our studies demonstrate the impacts of human populations on the health of aquatic ecosystems can be mitigated by regulatory action and implementation of appropriate WWTF treatment technologies.

The multiple regulatory networks controlling ovulation in teleosts
[Gorbman-Bern Memorial Lecture]

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The endocrine control of ovulation in teleosts has been of interest for more than 50 years. Much of the initial focus was on methods to induce and synchronize ovulation in species that were of commercial importance. More recently ovulation and the successful spawning of eggs have been used as measures of reproductive success in toxicological studies that are examining the effects of chemicals in the environment that interfere with endocrine function. These studies have led to the characterization of adverse outcome pathways that describe the mechanisms involved in mediating the ovulatory process. A series of studies have shown established the pivotal roles that luteinizing hormone (LH), progestins and eicosanoids, notably the prostaglandins, play in the ovulatory process. Subsequent studies have implicated a suite of other processes and pathways

as being critical to ovulation including the induction of apoptosis, contraction of smooth muscle tissue, activation of the immune system and changes in the activity of a suite of proteases and protease inhibitors that control the degradation of the apex of the follicle layer, all of which play a role in the successful rupture of the ovarian follicular tissue and release of the mature egg into the oviduct. Despite the progress in describing these pathways, major gaps in our knowledge remain. These are as basic as defining what factors control the synthesis of prostaglandins given that LH does not appear to directly mediate their production. The precise eicosanoids involved in ovulation have remained elusive given that products of both cyclooxygenase and lipoxygenase dependent metabolism seem to be involved. More than 20 different proteases and protease inhibitors have been implicated in ovulation and defining which are critical will require new experimental approaches. We also know very little of the factors that control the competency of the ovarian follicles to respond to an ovulatory signal. This presentation will attempt to characterize the state of our knowledge of the regulation of ovulation and provide some insights into how we may continue to advance this fundamental area of reproductive biology.

Age and sex differences and programming effects of LPS treatment on thermoregulation, sickness behaviour, cytokine levels, and arginine-vasopressin expression
[P104]

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Puberty is an important developmental event that is marked by the remodelling of the nervous, reproductive, and immune systems. Exposure to stressors during this critical period of development can have enduring effects on behaviours and all the aforementioned systems. The purpose of the study is to investigate age and sex differences in sickness behaviour and body temperature changes following exposure to the bacterial endotoxin, lipopolysaccharide (LPS). We also examined the effects of circulating gonadal hormones on these differences by performing gonadectomies on male and female mice from both age groups. Results showed that male mice display more sickness behaviour and greater fluctuations in body temperature following LPS treatment than do female mice. Moreover, adult mice display more sickness behaviour and greater changes in body temperature following LPS treatment compared to pubertal mice. Gonadectomized pubertal female mice display steeper and longer temperature drops than sham-operated animals, and this was more similar to the male mice temperature profile. The removal of the gonads did not eliminate adult sex differences suggesting that additional factors contribute to these differences. Male mice experience greater attenuation of the hypothermic response after a second LPS treatment compared to their female counterparts. There were also age differences in the effect of LPS on cytokine levels and arginine-vasopressin expression. These findings contribute to a better understanding of the mechanisms involved in the enduring behavioural alterations seen after exposure to an immune challenge during puberty.

Characterization of nestin protein in the goldfish brain
[S7, contributed]

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Nestin is an intermediate filament protein involved in neurogenesis, neuroendocrine plasticity and regeneration in fish, mice, and humans. It has been extensively used as a marker for proliferating and migrating neuronal stem cells but is not expressed in mature neurons. In this study we used a multi-antigenic peptide (MAP) strategy to generate a goldfish-specific nestin antibody. Western blotting experiments using a telencephalon extract revealed the presence of four proteins of differing molecular weights (~90, ~70, ~37, ~30 kD). These 4 bands were extracted and analysed using mass-spectrometry to determine their identity. Rapid amplification of cDNA ends PCR was performed to obtain the goldfish (gf) *nestin* full-length transcripts and investigate the existence of different mRNA isoforms. We uncovered 2 transcripts with two distinct 5'-UTRs. Both contained the same initiation codon but one had a deletion of 66 nucleotides. Two different 3'-UTR ends were also evident. To elucidate how many different mRNA isoforms exist in *gf nestin*, specific primers to both 5' and 3'-UTR ends were designed and PCR analysis and sequencing performed. Three different transcripts were detected, suggesting the presence of three different *gf nestin* isoforms. The 3 cDNAs were fully sequenced resulting in transcripts of 2188, 2508 and 4040 nucleotides. Predicted proteins were estimated to be 344, 274 and 860 amino acids in length, respectively. To investigate the potential role of the nestin isoforms, extracts were made from brain regions and pituitary with differing neurogenic capacities. These included neuroendocrine tissues such as hypothalamus and telencephalon, and also optic tectum, midbrain, cerebellum, vagal lobes and brain stem. Differential expression of nestin isoforms was observed. In the female goldfish, the ~37kDa band was present in all brain regions and pituitary whereas the ~70 kDa band was present in all brain regions but not pituitary. Nestin appears to be influenced by both seasonality and sex in goldfish. In sexually regressed female goldfish, the nestin protein of ~30 kDa and ~90 kDa is absent in the telencephalon compared to sexually mature female fish. Moreover, abnormalities in the functioning and signalling pathways of dopaminergic (DA) neurons can lead to reproductive and motor defects. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that leads to severe dopamine loss in teleost brain. MPTP injection decreased nestin protein expression in the pituitary, hypothalamus, telencephalon and brain stem. This suggests the occurrence of neuronal regeneration to alleviate the DA loss. Studying the differential roles and regulation of these nestins will lead to a better understanding of seasonal neuronal plasticity and regeneration following injury in vertebrates.

Larval exposure to fluoxetine suppresses the stress response in adult zebrafish
[P47]

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The increase use of pharmaceuticals by humans and the endocrine disruptive properties of at least some of these drugs have a potentially detrimental effect on the aquatic ecosystem. The active ingredient of the antidepressant Prozac™, fluoxetine (FLX), is detected in aquatic environments at concentrations as high as 540 ng/L. Fluoxetine is a selective serotonin reuptake inhibitor that increases the extracellular brain levels of serotonin. Since serotoninergic systems are highly conserved, the presence of FLX in aquatic environments may result in unintended effects to aquatic vertebrates. We investigated the effects of environmental FLX concentrations on the stress and reproductive axes of zebrafish (ZF) *Danio rerio*. Embryos/larvae were exposed to either control or two concentrations of FLX: 0.54 μg/L and 54 μg/L from 3 hpf (hours post fertilization) to 6 dpf (days). The stress response was assessed by examining the physiological and behavioural effects following standardized net and novel tank stressors, respectively, at adulthood (6 months). Fluoxetine exposure decreased basal and stress-induced whole-body cortisol levels at 6 months in a concentration-dependent manner ($p<0.05$). Additionally, these fish did not respond to an i.p. of 0.0625 IU ACTH injection ($p<0.05$). Furthermore, the FLX-treated fish displayed more anxiety-like behaviours (novel tank response) compared to controls, with more severe effects observed at the lower FLX concentration ($p<0.05$). Aromatase activities decreased in ovaries of females exposed to the highest FLX concentration ($p<0.05$). The offspring of the FLX-treated parents exhibited a white-body phenotype, which was not observed in the controls ($p<0.05$). These results demonstrate that early life exposure to FLX disrupts the ZF stress and reproductive systems. FLX also disrupts development in the offspring of exposed parents. Further studies will determine if the effects observed in the FLX-treated embryos/larvae persist across generations.
Supported by grants from NSERC.

Ontogeny of the hypothalamus-pituitary-interrenal axis functioning in zebrafish
[S5, invited]

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Zebrafish (*Danio rerio*) is an excellent model for developmental studies and is being increasingly used for biomedical research. We have been studying the onset of stress response, and the role of stress steroid signaling in regulating early development using this species as a model. The cortisol stress axis is a highly coordinated and integrated endocrine system, involving multiple tissues that functions to allow animal to cope with stressors. Although, the developing oocytes and embryos have cortisol, we have shown that *de novo* cortisol synthesis in response to a stressor commences only after hatch in zebrafish. Therefore, cortisol content in the zygote and during embryogenesis is of maternal origin. Consequently, a working hypothesis in my laboratory has been that maternal stress and the associated elevation in cortisol levels affect developmental programming events. Specifically, we have shown that maternal cortisol deposition in the oocytes is essential for early zebrafish development, including the onset of the hypothalamus-pituitary-interrenal axis (HPI) activation. Furthermore, I will discuss the role of glucocorticoid receptor (GR), a key cortisol signaling protein, activation in regulating zebrafish

development. Using morpholinos to knockdown GR, along with microarray to indicate transcriptome changes, we have identified several molecular mechanisms, critical for developmental programming, as cortisol-responsive in zebrafish. Our results suggest that maternal stress, and the associated abnormal cortisol deposition, leads to developmental dysfunction, including abnormal activation of the HPI axis in zebrafish. The insights gained from these studies have implications in biomedical research, especially in treating stress/cortisol-related developmental disorders.

An ancient modulator of stress-related metabolism: role of teneurin C-terminal associated peptide-1 (TCAP-1) and its receptor latrophilin (LPHN)
[P23]

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TCAP is an endogenous peptide hormone that protects against organismal stress. Evidence indicates that it originally evolved by a lateral gene transfer from prokaryotes to a single-celled ancestor of multicellular animals, where it became associated with the regulation of energy production in cells. In vertebrates, there are four isoforms of TCAP, each of which is associated with a proprotein called 'teneurin'. In the mammalian brain, TCAP-1 is expressed as a separate mRNA distinct from teneurin-1 and its mature peptide acts as a ligand to latrophilin (LPHN), a G-protein coupled receptor (GPCR). Using an in vitro approach with immortalized hippocampal and hypothalamic neurons we have established that TCAP-1 associates with LPHN to potentially inhibit downstream GPCR activity and may reduce diacylglycerol (DAG) and inositol triphosphate (IP3). Also, TCAP binding activates a neurotrophic-like signal cascade stimulating energy production via an insulin-independent pathway and regulates cytoskeletal elements underlying neuronal process development such as dendrites, spines, filopodia and neurites. Knock-down of the TCAP-teneurin gene results in reduction of cell growth and process outgrowth.

Primary radial glial cell culture as a model for dopaminergic regulation of neuroestrogen synthesis in the fish forebrain
[P71]

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Estrogens produced locally in the vertebrate brain play many fundamental roles in differentiation, neural regeneration, neuroendocrine functions, reproductive functions and socio-sexual behaviours. Radial glial cells (RGCs) are neuronal precursor cells that are very abundant in fish brains and are the exclusive site of aromatase B expression and estrogen synthesis from androgen precursors. Using in vivo approaches coupled to a novel in vitro cell culture

preparation we address the important questions of whether RGCs are capable of de novo steroid synthesis from cholesterol and what regulates neuroestrogen synthesis. We found a close anatomical relationship between RGCs and tyrosine hydroxylase positive catecholaminergic neurons along the telencephalon ventricular surface. Immunofluorescence analysis indicates that cultured RGCs from female goldfish retain their normal in vivo characteristics. More than 95% of the RGCs co-express glial fibrillary acidic protein and brain lipid binding protein. Gene cloning and sequencing revealed the presence of steroidogenic acute regulatory protein, and the key cytochrome P450 steroidogenic enzymes in cultured RGCs, indicting the potential of RGCs to produce numerous steroids in addition to estrogens. As determined using gene cloning and immunocytochemistry, RGCs express dopamine receptor (D1R). Pharmacological experiments established that activation of the D1R up-regulates aromatase B by a cAMP/PKA/CREB-dependent mechanism. These emerging neuroanatomical and gene expression data indicate that RGCs express steroidogenic enzymes and that neurotransmitters in neighboring neurons can regulate neuroestrogen synthesis. Other anatomical data suggest that RGCs may also communicate with catecholaminergic neurons. While the exact functional significance of dopaminergic regulation or aromatase remains firmly established, these data have major implications for our understanding neurogenesis given the known roles of RGCs and estrogens in this fundamental process.

Autocrine regulation of prolactin release from tilapia prolactin cells: modulation of hormonal responses by extracellular osmolality
[S13, contributed]

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Prolactin (Prl) is an adenohypophysial hormone known to trigger over 300 distinct physiological actions throughout vertebrates. In the euryhaline Mozambique tilapia, *Oreochromis mossambicus*, Prl plays a critical role in osmoregulation in fresh water. Consistent with its hyperosmoregulatory actions, Prl is released in direct response to a fall in extracellular osmolality. In addition to changes in extracellular osmolality, extra-pituitary hormones also regulate Prl release; less is known, however, on the actions of Prl on its own release. To investigate the autocrine regulation of Prl, dispersed Prl cells of Mozambique tilapia were perfused with hyper- (355 mOsm/kg) or hyposmotic (300 mOsm/kg) media containing ovine Prl (oPRL, 10 µg/mL) or either of two isoforms of tilapia Prl, $tPrl_{177}$ and $tPrl_{188}$ (1 µg/mL). All Prls tested increased the release of tilapia Prls from dispersed Prl cells, however, the magnitude of responses varied between Prl isoforms, and was dependent on extracelular osmolality. While oPrl increased $tPrl_{177}$ release regardless of extracellular osmolality and $tPrl_{188}$ release in hyposmotic medium, promotion of $tPRL_{188}$ in hyperosmotic medium was not evident as in hyposmotic medium. By contrast, $tPrl_{188}$ stimulated its own release in hyper- but not hyposmotic medium; $tPrl_{177}$ release was stimulated in both osmolalities. Overall, $tPrl_{177}$ served as a potent stimulator of both tilapia Prls, regardless of extracellular osmolality. The effects of oPrl or tilapia Prls appear within 5-10 min and last the entire course of exposure, outlasting the effects of

a hyposmotic stimulus alone. Taken together, our data clearly shows that tilapia Prls are regulated by a positive feedback system that is modulated by extracellular osmolality.

Multiplex detection of KRAS mutations in colorectal cancer FFPE samples using droplet digital PCR
[P116]

Yang, W., Shelton, D.N., Berman, J.R., Zhang, B., Cooper, S., Tzonev, S., Hefner, E., Regan, J.F. (Presented by Sean Taylor)
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Targeted therapies in many cancers have allowed unprecedented progress in the treatment of disease. However, routine implementation of genomic testing is limited due to: 1) limited amounts of sample (pg-ng range) per biological specimen, 2) diagnostic turnaround time and workflow, 3) cost, and 4) difficulties in detection of mutational loads below 5%. KRAS is mutated in approximately 40% of colorectal cancers. The majority of mutations affect codons 12, 13, and 61, and are indicative of a negative response to αEGFR therapy. To optimize therapy strategies for personalized care, it is critical to rapidly screen patient samples for the presence of multiple KRAS mutations. We have developed a multiplexing strategy to screen seven clinically-actionable KRAS mutations in cancer samples using digital PCR. This panel covers KRAS point mutations with individual frequencies higher than 1% and covering 98% of KRAS mutant colorectal cancers (Faulkner et al. 2010). No pre-amplification step is required. This KRAS screening assay was used to quantify KRAS mutational load in a panel of FFPE specimens from advanced metastatic colorectal cancer patients. KRAS mutations present at <1% fractional abundance were detected in multiple samples. This sensitive and inexpensive method reduces the risk of contamination and can be easily implemented in molecular diagnostic laboratories for rapid, routine screening of cancer patients.

Toxicogenomics analysis of liver responses in rats and mice exposed to the food contaminant furan: applications in risk assessment
[S10, invited]

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A change in paradigm in human health risk assessment is required, with emphasis on increased use of pathway perturbation data. We explored the use of toxicogenomics in risk assessment using the known rodent hepatocarcinogen furan, a contaminant in heated foods. We applied standard guideline histopathology/clinical chemistry tests in parallel with analysis of pathway perturbations (measured using DNA microarrays and RNA-seq). Mice (female B3C6F1) or rats (male or female Fisher F344) were sub-chronically exposed to carcinogenic and non-

carcinogenic doses of furan. Toxicogenomics provided mechanistic information to explain gender differences in rats, revealing differences in metabolism and oxidative stress response. Pathway analysis in both species supported that furan causes oxidative stress, cytotoxicity and liver regeneration, an established mode of action (MoA) in cancer. This was supported by meta-analysis against publicly available data, revealing that furan expression profiles correlated with other chemicals that operate via this MoA or that are substrates of Cyp2E1 (the enzyme that metabolizes furan). The high dose in mice clustered most closely with a mouse model of liver regeneration. Benchmark dose analysis (BMD: mathematical modeling to determine the dose at which a pre-determined increase above controls occurs) revealed consistency between the traditional endpoints and toxicogenomics in both species. Specifically, the BMDs for pathway perturbations (both RNA-seq and microarrays) were similar to associated apical endpoints in the same rodents. In addition, the pathway BMDs were similar to BMDs for hepatocellular adenomas and carcinomas in two-year cancer bioassay studies. Finally, increased serum T4 levels occurred in male rats following 90 day exposures to furan. However, liver transcriptional profiles were consistent with hypothyroidism. Subsequent study in rats demonstrated no effect on serum free-T4 and little effect on TH-regulated gene expression in liver or other tissues, with indications that this may result from an increase in serum T4 carrier protein that causes an increase in the bound hormone fraction only. Although blood serum data were indicative of hyperthyroidism, the data suggest that the liver is actually deficient in T3. Overall, the study provides strong evidence of the utility of toxicogenomic analysis of in vivo rodent studies in establishing MoAs and comparative BMDs to apical endpoints for chemical agents.

Effects of thyroid hormone and dexamethasone on thymocyte cell death and proliferation in *Xenopus laevis* tadpoles
[P79]

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Metamorphosis in anurans is accompanied by a dramatic loss of larval thymocytes as a new adult antibody repertoire is formed. Rising levels of glucocorticoids during metamorphosis have been shown to inhibit lymphocyte proliferation and induce thymus lymphocyte cell death. Here we show that treatment of tadpoles with exogenous thyroid hormone (T3, 5 nM) and the cortisol analog, dexamethasone (DEX, 2 uM), each separately induce thymus involution and apoptosis, and together exhibit a synergistic effect that accelerates these processes. To determine if each hormone affects the thymus directly, we cultured Nieuwkoop-Faber (NF) stage 54 thymus gland explants in the presence of either hormone. After 3 days of culture with DEX or T3, thymus gland surface areas decreased by 22% and 12%, respectively (controls did not change significantly), indicating that each hormone affects the thymus directly. Using antibodies against epithelial-cadherin, we measured changes in total thymus size, as well as changes in thymus cortex and medulla surface area. Treatments with either T3 or DEX produced a disproportionate regression of thymus cortex compared with the medulla. Taken together, our findings suggest that both glucocorticoids and thyroid hormones each contribute to thymus remodeling during metamorphosis.

Neuropeptidergic control of feeding in starfish: characterization of the SALMFamide signaling system
[P24]

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Neuropeptides and their cognate G protein-coupled receptors (GPCRs) are signaling molecules that regulate many vital processes, including feeding, reproduction and development. Several neuropeptidergic signaling systems are found across the Bilateria and where peptide sequences are highly conserved, homologs have been identified in both protostomes and deuterostomes (e.g. vasopressin/oxytocin-type neuropeptides). In other cases, it is difficult to infer evolutionary relationships by simply comparing the neuropeptide sequences due to a lack of sequence conservation. In such cases, evolutionary relationships can sometimes be established by comparing the receptor sequences as these tend to be more conserved. The SALMFamides are a family of neuropeptides that occur in species belonging to the phylum Echinodermata (Deuterostomia) but homologs have, as yet, not been identified in other phyla. Recent advances in next-generation sequencing have enabled us to obtain transcript sequences encoding SALMFamides in several echinoderm species. In starfish there are two SALMFamide genes. On the one hand, the L-type gene encodes a precursor protein that gives rise to peptides with a LxFamide C-terminal motif. On the other hand, the F-type gene encodes a precursor protein that largely gives rise to peptides with a FxFamide C-terminal motif. Here, we have identified the mRNA sequences encoding the L-type and F-type SALMFamide precursors in the starfish Asterias rubens. The A. rubens L-type and F-type SALMFamide precursors are predicted to produce seven and eight SALMFamides, respectively, some of which have been identified and sequenced using mass spectrometry. We have also localized the transcripts encoding L-type and F-type SALMFamide precursors in A. rubens using mRNA in situ hybridization (ISH). Our results reveal that these genes are widely expressed, with labeled cells detected in the radial nerve cord, circumoral nerve ring, tube feet and cardiac stomach. Previous work from our laboratory has revealed that L-type SALMFamides cause relaxation of cardiac stomach, tube foot and apical muscle preparations in A. rubens. Furthermore, L-type SALMFamides cause eversion of the cardiac stomach, a process that occurs naturally when starfish feed extra-orally. Hence SALMFamides may mediate the neural control of feeding behaviour in A. rubens. On-going studies are investigating the physiological significance of the "cocktails" of SALMFamide neuropeptides that occur in the starfish and other echinoderms. Furthermore, we have identified a candidate SALMFamide receptor in A. rubens and pharmacological characterization of this receptor may reveal how echinoderm SALMFamides are evolutionarily related to neuropeptides in other phyla. This the first comprehensive study to characterize a neuropeptidergic signaling system in echinoderms, advancing our understanding of the evolution and comparative physiology of neuropeptides that regulate feeding in animals.
This work was supported by a grant from the Leverhulme Trust.

Identification and characterization of the adipokinetic hormone/corazonin-related peptide (ACP) signalling system in *Rhodnius prolixus*: a novel role for truncated receptor variants [S2, contributed]

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Neuropeptides and their G protein-coupled receptors (GPCRs) are widespread throughout Metazoa and in several cases, clear homologs can be identified in both protostomes and deuterostomes. One such neuropeptide is the mammalian gonadotropin-releasing hormone (GnRH) which is related to the arthropod adipokinetic hormone (AKH) and the recently discovered AKH/corazonin-related peptide (ACP). ACP and AKH are two independent signalling systems and as such have distinct roles. Recently, we characterized the AKH signalling system in the kissing bug, *Rhodnius prolixus*, and showed that it is involved in lipid mobilization. In the present study, we identify and characterize the ACP signalling system in *R. prolixus*. We isolated the cDNA sequence encoding *R. prolixus ACP* (*Rhopr-ACP*) and examined its spatial expression pattern using quantitative PCR (qPCR). *Rhopr-ACP* is predominantly expressed in the central nervous system (CNS). However, unlike *Rhopr-AKH*, which is only found in the corpus cardiacum (CC), *Rhopr-ACP* is found in both the brain and CC. The function of the ACP signalling system in arthropods is currently unknown. Hence, in order to gain an insight into the role of this signalling system in *R. prolixus*, we also isolated cDNA sequences of three splice variants (A, B and C) encoding *R. prolixus* ACP receptor (Rhopr-ACPR) and functionally characterized the receptors using a heterologous assay. *Rhopr-ACPR-A* encodes a truncated receptor that lacks two of the characteristic seven transmembrane domains whereas Rhopr-ACPR-B and C have all the characteristic features of a GPCR. In order to pharmacologically characterize these receptors, we expressed them in Chinese hamster ovary (CHO) cells stably expressing the human G-protein G16 (CHO/G16). Interestingly, Rhopr-ACPR-A, B and C were all activated by Rhopr-ACP, albeit at different sensitivities. To our knowledge, this is the first study to show that a truncated receptor from an invertebrate can elicit a secondary messenger response. Moreover, Rhopr-ACPR-B and C but not Rhopr-ACPR-A can couple with Gq alpha subunits and cause an increase in intracellular calcium concentration when expressed in CHO cells stably expressing aequorin (CHOK1-aeq). This indicates that the absence of two transmembrane domains in Rhopr-ACPR-A prevents it from coupling with Gq alpha subunit but not G16. Expression analysis using qPCR indicates that *Rhopr-ACPR* is highly expressed in the CNS. The receptor is expressed in both the brain and CC, suggesting that it may control the release of other hormones found in the CC in a manner analogous to GnRH. Lastly, we investigated the possible involvement of this signalling system in ecdysis by determining the expression of *Rhopr-ACP* and *Rhopr-ACPR* in CNS at various time points around ecdysis. Transcript levels of both *Rhopr-ACP* and *Rhopr-ACPR* are upregulated immediately after ecdysis and remain high for a few days indicating that this neuropeptide may be involved in processes associated with post-ecdysis.

Differential hepatic gene expression profile of male fathead minnows exposed to environmental pollutants individually and in mixture

[P48]

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Environmental contaminants are known to impair reproduction, metabolism and development in wild life and humans. Efficient, specific, and robust biomarkers will be needed to develop effective screening tools. A number of environmental contaminants are known to disrupt endocrine system, and interfere with hormonal control of reproduction, growth and metabolism. To understand the health impact of endocrine disruptive chemicals, it would be essential to investigate the mechanisms by which contaminants affect normal physiological and endocrine axis. The aim of present study was to investigate the effects of exposure to a number of endocrine disruptive chemicals present in the Alberta Rivers [Nonylphenol (NP), BPA, DEHP and mixture of the three chemicals] on liver transcriptional response in fathead minnow following OECD 21-day fish screening assay protocol. We used well-characterized Agilent (EcoArray, Inc., Gainesville, FL) microarray system to investigate gene expression profiles. Pathway analysis revealed a distinct mode of action for the individual chemicals and their mixture. The results demonstrate differential changes in over 980 genes in response to exposure to contaminants individually and in mixture. IPA core and toxicity analysis, and gene ontology were used to investigate the biological processes, pathways and the top regulators affected by these compounds. A number of canonical pathways were significantly affected, including cell cycle & proliferation, inflammatory, innate immune response, stress response, and drug metabolism. It was possible to narrow down to 18 genes that were affected very significantly by all treatment groups, which can be used as suitable biomarkers for initial screening of environmental water samples. The microarray data were verified using quantitative Real-time PCR. We were also able to identify specific genes that were affected by NP, BPA and DEHP individually, and demonstrate that the gene expression pattern change significantly when fish were exposed to mixture of the contaminants.

Overall the results of this study provide novel information on mechanisms of health impact of contaminants tested based on pathway analysis, and identify a number of specific new biomarkers that can be used for screening environmental water samples. Acknowledgement: Study was funded by NSERC grants.

Dissecting oxygen-dependent and -independent actions of hypoxia-inducible factors in early development using CRISPR/Cas9 gene editing
[S11, invited]

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Hypoxia-inducible factor1-3 (HIFs) are oxygen-sensing transcription factors conserved in all metazoans. HIFs up-regulate many target genes to alter hematopoiesis, angiogenesis, glucose metabolism, and the cell cycle in response to hypoxia. HIFs also have oxygen-independent functions in early development with poorly understood mechanisms. We have discovered that in

addition to the full-length Hif-3α, there is a spliced hif-3 transcript in zebrafish termed as Hif-3α2. This truncated Hif-3α isoform lacks the oxygen-dependent degradation domain. Western blot and immunocytochemistry analysis results suggest that Hif-3α2 protein is expressed in all embryonic stages and most adult tissues and its level is not regulated by oxygen tension. When introduced into zebrafish embryos or cultured cells, Hif-3α2 is localized in the nucleus and has transcriptional activity. While overexpression of full-length Hif-3α resulted in global growth retardation, Hif-3α2 overexpressing embryos often lack somites in one side of the body. Likewise, the heart is often located in the middle or even the right side of the body, suggesting a body symmetry defect. Further analyses revealed that overexpression of Hif-3α2 impairs the development of kupffer's vesicle, a ciliated and transient embryonic 'organ of asymmetry' that directs LR development. Co-expression experiments suggested that Hif-3α2 inhibits Wnt or β-Catenin-induced gene expression and dorsalization. Co-IP and GST pull-down assays showed that H3-513 binds to β-Catenin and induces its degradation. Next, we used a CRISPR/Cas9-based strategy to genetically delete Hif-3α2 or the full-length Hif-3α, respectively. Preliminary results indicate that knockout of Hif-3α2 but not the full-length Hif-3α led to increased Wnt/β-Catenin signaling activity. These findings not only have provided novel insights into oxygen sensing and HIF actions in early development, but will also provide a new tool to study alternatively spliced isoforms in vivo.

Disruption of zebrafish FSH receptor (*fshr*) but not LH receptor (*lhcgr*) gene by TALEN leads to failed follicle activation in females followed by sexual reversal to males
[S11, contributed]

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Gonadotropins are primary hormones that control vertebrate reproduction. In a recent study, we analyzed the impacts of FSH and LH on zebrafish reproduction by disrupting FSH and LH genes (*fshb* and *lhb*) using TALEN technology. Using the same approach, we successfully deleted FSH and LH receptor genes (*fshr* and *lhcgr*) in the present study. In contrast to the deficiency of its cognate ligand FSH, the *fshr*-deficient females showed a complete failure of follicle activation with all ovarian follicles arrested at the PG-PV transition, which is the marker for puberty onset in females. Interestingly, after blockade at the PG stage for varying times, all females reversed to males, and all theses males were fertile. In *fshr*-deficient males, spermatogenesis was normal in adults, but the initiation of spermatogenesis in juveniles was retarded. In contrast to *fshr*, the deletion of *lhcgr* gene alone caused no obvious phenotypes in both males and females; however, double mutation of *fshr* and *lhcgr* resulted in infertile males. In summary, our results in the present study showed that Fshr was indispensable to folliculogenesis and the disruption of *fshr* gene resulted in a complete failure of follicle activation followed by masculinization into males. In contrast, *lhcgr* does not seem to be essential to zebrafish reproduction in both males and females. 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.

Genetic analysis of gonadotropin functions in the zebrafish by TALEN-mediated targeted disruption of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) subunit genes (*fshb* and *lhb*)
[P98]

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Vertebrate reproduction is controlled by two gonadotropins (FSH and LH) from the pituitary. Despite numerous studies on FSH and LH in fish species, their functions in reproduction still remain poorly defined. This is partly due to the lack of powerful genetic approaches for functional studies in adult fish. This situation is now changing with the emergence of genome-editing technologies, especially TALEN and CRISPR/Cas9. In this study, we deleted the hormone-specific genes of both FSH and LH in the zebrafish using TALEN. This was followed by phenotype analysis for key reproductive events, including gonadal differentiation, puberty onset, gametogenesis, final maturation, and fertility. FSH-deficient zebrafish (*fshb*$^{-/-}$) were surprisingly fertile in both sexes; however, the development of both ovary and testis was significantly delayed. In contrast, LH-deficient zebrafish (*lhb*$^{-/-}$) showed normal gonadal growth, but the females failed to spawn and were therefore infertile. Using previtellogenic (PV) follicles as the marker, we observed a significant delay of puberty onset in *fshb* mutant but not *lhb* mutant females. Interestingly, FSH seemed to play a role in maintaining female status as we repeatedly observed sexual reversal in *fshb* mutant. Neither *fshb* nor *lhb* mutation alone seemed to affect gonadal differentiation; however, double mutation of the two genes led to all males although the development of the testis was significantly delayed. In summary, our data confirmed some well-known functions of FSH and LH in fish while also providing evidence for novel functions, which would be difficult to reveal using traditional biochemical and physiological approaches.

Efficient targeted gene disruption in Xenopus embryos using TALEN and CRISPR/Cas9
[S11, invited]

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Specific gene disruption is essential for studying gene function in biomedical research. Transactivator like effector nucleases (TALENs) have been proven to be effective for specific gene disruption across animal models. We also reported that TALENs induced somatic mutations in *X. tropicalis* embryos with reliably high efficiency. We modified the Golden Gate method for TALEN assembly to make the constructs suitable for microinjection into *X. tropicalis* embryos. Eight pairs of TALENs were constructed to target eight *X. tropicalis* gene loci, and all of them induced indel mutations successfully with high efficiencies of up to 95.7% at the

targeted loci. Given the fact of high gene disruption efficiency, TALEN could be used to evaluate the phenotype at G0 generation. The RNA guided Cas9 nuclease, derived from type II of *Clustered Regularly Interspaced Short Palindromic Repeats* (CRISPR/Cas9) system, emerges as another effective tool for gene disruption in various animal models including *X. tropicalis*. Here we utilized CRISPR/Cas9 to disrupt genes that are involved in neural crest formation in *X. tropicalis* embryos. Fifteen genes were disrupted with efficiencies in a range up to 65% at the targeted loci. Furthermore, duplex mutations of *pax3* and *zic1*, and *snail1*and *snail 2* were induced by Cas9 and corresponding gRNAs. We also generated segmental deletions and inversion at the *pax3* and *snail1* gene loci by using Cas9 and a pair of gRNAs. We found that Cas9 induced on-target DNA cleavage with high specificity at most tested gene loci, however cautions needs to be taken as it can induce off-target cleavages at some loci. The D10A Cas9 carried a mutation that can convert the Cas9 endonuclease into a nicknase. In line with the observation with mammalian cells, D10A coupled with a pair of gRNA can induce indel mutations in *X. tropicalis* embryos. We also showed evidence that D10A nicknase can reduce the off-target mutagenesis rate in *X. tropicalis* embryos. Taken together, both TALEN and CRISPR/Cas9 are efficient and robust tools for genome editing in *X. tropicalis*. The gene disruption efficiencies induced by CRISPR/Cas9 were lower than those by TALENs in *X. tropicalis*, but CRISPR/Cas9 system is easier to handle and good for a large scale screening.

Reproductive, histological and transcriptional effects of synthetic progestins medroxyprogesterone acetate and dydrogesterone and their binary mixtures in zebrafish (*Danio rerio*)
[S3, invited]

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Medroxyprogesterone acetate (MPA) and dydrogesterone (DDG) are synthetic progestins widely used in human medicine and lifestock. Although aquatic organisms are exposed to them through wastewater and animal farm run-off, very little is known about their effects in the environment. Here we provide a comprehensive analysis of the responses of zebrafish (Danio rerio) to MPA, DDG and their binary mixtures at measured concentrations between 4.5 and 1663 ng/L. DDG and both mixtures impaired reproductive capacities (egg production, gonadosomatic index) of breeding pairs and led to histological alterations in gonads. Transcriptional analysis of up to 28 genes belonging to different pathways demonstrated alterations in steroid hormone receptors and steroidogenesis enzymes, both in different organs of adult zebrafish and eleuthero-embryos. Alterations occurred at environmentally realistic levels of 43 ng/L MPA, 89 ng/L DDG, and 39 ng/L MPA + 145 ng/L DDG. Among others, the strong transcriptional alterations of circadian rhythm genes indicate responses beyond endocrine related activities. The mixtures displayed additive effects in most but not all parameters in adults and eleuthero-embryos, supporting the concentration addition concept. Our data suggest that MPA and DDG and their mixtures induce

multiple endocrine responses at environmentally relevant concentrations and adverse effects on zebrafish reproduction at higher levels.

Generating and characterizing nuclear progestin receptor (Pgr) knockouts in zebrafish
[S11, contributed]

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Final oocyte maturation (FOM) and ovulation are two sequential and important physiological processes for advancing fully-grown immature oocytes to become fertilizable eggs in animals. Progestins, progesterone derivatives, are the most critical signaling steroid for initiating these two physiological processes in basal vertebrates. It is well-established that progestin induces FOM via an elusive membrane receptor and a nongenomic steroid signaling process, which precedes progestin triggered ovulation that is mediated through a nuclear progestin receptor (Pgr) and genomic signaling pathway. To determine whether Pgr plays a role in a nongenomic signaling mechanism during FOM, we knocked out Pgr in zebrafish using transcription activator-like effector nucleases (TALENs) and studied the oocyte maturation phenotypes of Pgr knockouts (Pgr-KOs). Two distinct pairs of TALEN artificial nucleases targeting two separate loci in the first exon of the zebrafish Pgr coding region were synthesized, and the transcripts of each TALEN pair were microinjected into zygotes. Three TALENs-induced mutant lines with different frame shift mutations were selected and crossed to generate homozygous offspring. Homozygous Pgr-KO female fish were all infertile while no fertility effects were evident in homozygous Pgr-KO males. Examination of the follicles from homozygous Pgr-KO female revealed that oocytes developed and underwent FOM normally compared to the wild-type controls, but these mature oocytes were trapped within the follicular cells and failed to ovulate from the ovaries. Further examination of fully-grown immature oocytes from homozygous Pgr-KO females *in vitro* revealed that these oocytes underwent normal germinal vesicle breakdown (GVBD) and FOM, but failed to ovulate even after treatment with human chronic gonadotropin (HCG) or progestin (17α,20β-dihydroxyprogesterone or DHP), which typically induce FOM and ovulation in wildtype follicles. The results indicate that anovulation and infertility in homozygous Pgr-KO female fish was, at least in part, due to a lack of functional Pgr-mediated genomic progestin signaling in the follicular cells adjacent to the oocytes. Our study of Pgr-KO supports previous results that demonstrate a role for in steroid-dependent genomic pathways leading to FOM, and the first convincing evidence that Pgr is not essential for initiating nongenomic progestin signaling and triggering meiosis resumption.

The effects of 11-deoxycortisol on the molecular physiology of the tight junction complex in an extant agnathan
[P111]

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Corticosteroids play an important role in the regulation of salt and water balance in aquatic vertebrates. This role is probably best characterized in teleost fishes where cortisol is the dominant corticosteroid hormone. In contrast, comparatively little is known about the role of corticosteroids in the maintenance of ionoregulatory homeostasis in less derived vertebrates such as extant agnathans. This is primarily because the identity of corticosteroid hormones in these organisms has been hard to pin down. But recent studies strongly support the idea that 11-deoxycortisol is the functional corticosteroid in the lamprey *Petromyzon marinus* (e.g. Close et al. 2010). In vertebrates, including teleost fishes, corticosteroids have been established to play a significant role in modulating paracellular permeability across ionoregulatory epithelia, primarily by acting on tight junction (TJ) proteins of the TJ complex. In particular, cortisol reduces the paracellular permeability of the teleost gill epithelium which in freshwater (FW) fishes will limit passive ion loss to the surroundings. Nothing is known about the molecular physiology of the TJ complex in agnathans or whether the TJ complex may respond to corticosteroids in a manner that is functionally similar to more derived vertebrates. Therefore we have characterized the molecular elements of the lamprey TJ complex and in this study report on the effects of 11-deoxycortisol on the molecular physiology of the lamprey TJ complex.
Close et al. (2010) 11-Deoxycortisol is a corticosteroid hormone in the lamprey. PNAS 107: 13942-13947.

A closer look into the roles played by Kiss1, Kiss2 and NKB in the network controlling reproduction in the striped bass, *Morone saxatilis*
[S7, contributed]

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Several important neuropeptides, acting upstream in the neuroendocrine control axis of vertebrate reproduction, have recently been identified. Their study in lower vertebrates, however, is still lagging behind that of mammals due to differences in distribution and functions among species and the abundance of multiple neuropeptide isoforms. Among them, the most studied are the kisspeptins, followed by neurokinin B (NKB), which have been shown to act at the level of the hypothalamus (GnRH neurons) and the pituitary (gonadotropes). We used the teleost *Morone saxatilis* to study the roles of the kisspeptins (Kiss1&2) and NKB at different reproductive stages and to examine their neuronal interactions and mutual effects. Two peptide antagonists, one that inhibits the activation of Kiss1 receptor (pep 234) and another that attenuates Kiss2 function (pep 359) were identified and used to better define the roles of the kisspeptin systems. Only Kiss2 upregulated GnRH1 expression in brain slices *in vitro*, and pep 359 reversed this effect. Kiss2 enhanced secretion of LH while both kisspeptins stimulated the expression and secretion of FSH

in pituitary primary cell culture. The antagonists revealed that, in addition to a hypothalamic effect, endogenous kisspeptin may also act in an autocrine/paracrine fashion in the pituitary. Treatment of spermiating males with the antagonists decreased sperm production. This effect was associated with decreased GnRH1 mRNA levels in the brain and lower GnRH1/higher LH content in the pituitaries. The treatment also dramatically reduced the levels of arginine-vasotocin (AVT) transcript levels. The preoptic AVT (known for its role in male spawning behaviour) neurons express Kiss2 receptor. These results underscore the importance of kisspeptin in the regulation of spawning and indicate that kisspeptin may simultaneously act on multiple physiological and behavioural pathways.

Neuroanatomical studies of NKB and kisspeptin in the brain of mature males revealed that NKB and its receptor are predominantly expressed in the habenula, preoptic area and the hypothalamus. Like in the mammalian arcuate nucleus, we detected co-expression of NKB and NKB receptor in Kiss2 neurons in the hypothalamic nucleus recessus lateralis. Together with the lack of kisspeptin effect on NKB, these results suggest that NKB may modulate Kiss2 in this region. Altogether, our results begin to unravel a network of neuropeptides that coordinate to control reproduction.