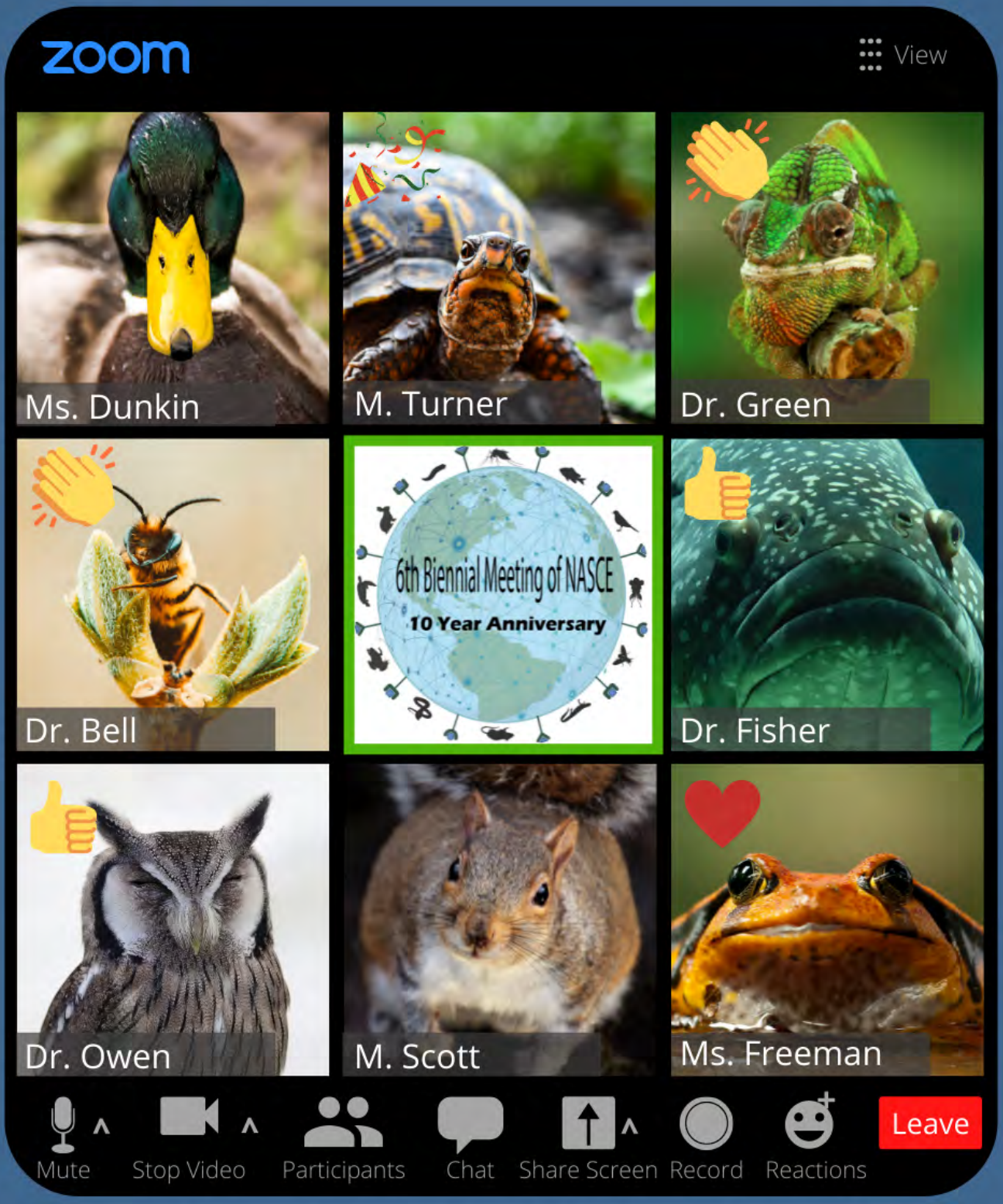
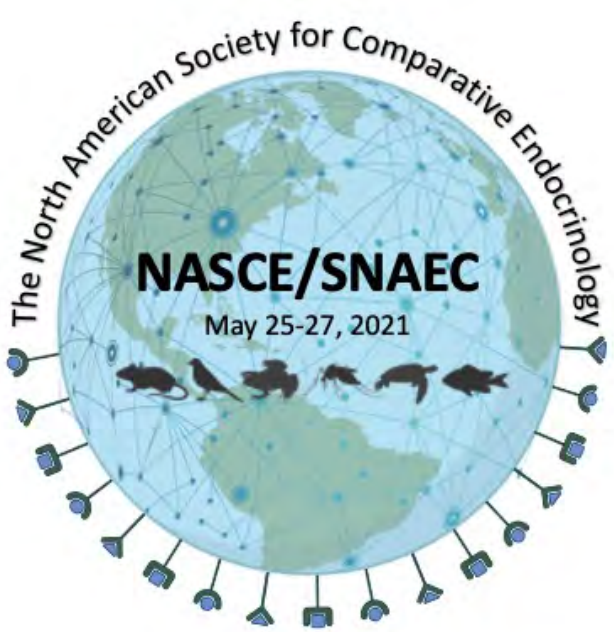


6th Biennial North American Society for Comparative Endocrinology

Virtual Meeting on Zoom

May 25-27, 2021



Designed by NASCE 2021 logo contest winners: Farwa Sajadi and Britney Picinic

VIRTUAL ORGANIZING COMMITTEE
CO-CHAIRS

CHRISTOPHER J. MARTYNIUK
(UNIVERSITY OF FLORIDA)

JEAN-PAUL V. PALUZZI
(YORK UNIVERSITY)

MARICELA LUNA
(UNAM Juriquilla)

VIRTUAL ORGANIZING COMMITTEE
MEMBERS

VALERIE LANGLOIS
(INRS)

MARTA ROMANO
(CINVESTAV del I. P.N., MEXICO CITY)

DAN BUCHHOLZ
(UNIVERSITY OF CINCINNATI)

Program-at-a-glance

***Note: All times shown in Eastern daylight time (GMT-4).
Here is a link to help sort out timezones: http://time-time.net/times/time-zones/usa-canada/current-eastern-time-est.php

Tuesday, May 25, 2021				
10:30-11:00	**Welcome + Announcements**			
11:00-11:45	**Plenary #1: Dr. Nancy Denslow** Threats to the fish endocrine system from poly- and perfluorinated chemicals in the environment			
11:45-12:15	**Morning Break & NASCE Trainees Strategies for Success**			
12:15-13:30 **Session** **Chairs**	**Session 1: Thyroid hormone action on organ maturation and tissue regeneration (Part I)** **Liezhen Fu**	**Session 2: New Frontiers in Endocrine Disrupting Chemicals: From Novel Mechanisms of Action to Monitoring (Part I)** **Valerie Langlois & Nancy Denslow**	**Session 3: Neuropeptides involved in invertebrate nutritional regulation and reproduction (Part I)** **Angela Lange & Jimena Leyria**	**Session 4: Growth, Metabolism, Hormones and Behavior (Part I)** **Maricela Luna & Aurea Orozco**
13:30-14:00	**Lunch Break**			
14:00-14:45	**Comparative Endocrinology Welcome Mixer – Networking**			
14:45-15:15	**Afternoon Health Break – NASCE Yoga/Fitness**			
15:15-16:30 **Session** **Chairs**	**Session 5: Thyroid hormone action on organ maturation and tissue regeneration (Part II)** **Liezhen Fu**	**Session 6: New Frontiers in Endocrine Disrupting Chemicals: From Novel Mechanisms of Action to Monitoring (Part II)** **Valerie Langlois & Nancy Denslow**	**Session 7: Neuropeptides involved in invertebrate nutritional regulation and reproduction (Part II)** **Angela Lange & Jimena Leyria**	**Session 8: Growth, Metabolism, Hormones and Behavior (Part II)** **Maricela Luna & Aurea Orozco**
16:30-17:30	**NASCE Trainee Mixer**			

Wednesday, May 26, 2021				
11:00-11:45	**Plenary #2: Dr. Vance Trudeau** Peptide identity crisis resolved: secretoneurin is a new reproductive hormone			
11:45-12:15	**Morning Break & EDI Panel Session #1**			
12:15-13:30 **Session** **Chairs**	**Session 9: Non-invasive methods to measure corticoids and sex steroids in domestic animals and wild fauna** **Marta Romano**	**Session 10: Neuroendocrine regulation of ionic, osmotic, and acid-base balance in vertebrates** **Jason P. Breves & Stephen D. McCormick**	**Session 11: Developmental roles of corticosteroids and their receptors** **Dan Buchholz**	**Session 12: Avian Endocrine and Metabolic Responses to Urbanization** **Pierre Deviche & Karen Sweazea**
13:30-14:00	**Lunch Break**			
14:00-14:45	**Dr. Angela Lange - Gorbman-Bern Lecture**			
14:45-15:15	**Afternoon Health Break – NASCE Yoga/Fitness**			
15:15-17:00 **Session** **Chairs**	**Session 13: Lightning Round** **Maricela Luna**	**Session 14: Lightning Round** **Santiago M Pech-Pool**	**Session 15: Lightning Round** **Eugene Cheung**	**Session 16: Lightning Round** **Farwa Sajadi**

Thursday, May 27, 2021				
11:00-11:45	**Plenary #3: Dr. Maurice Elphick** Evolution and comparative physiology of neuropeptide signaling systems: new insights from echinoderms			
11:45-12:15	**Morning Break & EDI Panel Session #2**			
12:15-13:30 **Session** **Chairs**	**Session 17: The relevance of neurosteroids and steroidogenic enzymes in comparative endocrinology** **Marta Romano**	**Session 18: Hormone mediated control of ion and fluid homeostasis in invertebrates** **Jean-Paul Paluzzi**	**Session 19: Novel neuropeptides: what can the comparison of invertebrate and vertebrate systems teach us?** **Vance Trudeau**	**Session 20: Recent Topics in Comparative Endocrinology** **Christopher J. Martyniuk**
13:30-14:00	**Lunch Break**			
14:00-14:45	**Dr. Jan Mennigen - Gorbman-Bern New Investigator**			
14:45-15:30	**Comparative Endocrinology Closing Mixer - Networking**			
15:30-16:15	**Closing Ceremony and Trainee Awards**			

Code of conduct

It is expected that all conference attendees will communicate openly with respect and consideration for others, valuing a diversity of views and opinions. By attending the NASCE 2021 conference, all attendees must agree voluntarily to abide by the following code of conduct:

All authors associated with an NASCE 2021 presentation must agree on all information contained in the presentation. Attendees are expected to not fabricate, falsify, or suppress results, deliberately misrepresent research findings, or otherwise commit scientific fraud.

Presenters and attendees cannot screenshot, capture, or otherwise share images or presentation data without a presenter's expressed written permission. Presentations should be considered privileged for NASCE 2021 registered attendees and should not be shared and/or reposted outside the virtual platform.

NASCE is dedicated to providing a safe, hospitable, professional and productive environment for everyone attending the annual conference, regardless of age, gender identity or expression, race, ethnicity, colour, cultural background, religion, place of origin, pregnancy or parental role, sexual orientation, ability, or socio-economic situation. It is important to remember that a community where people feel uncomfortable or threatened is neither healthy nor productive. Intimidating, threatening, or harassing conduct during the conference is prohibited. Conference attendees in violation of these rules may be sanctioned or expelled from the conference, at the discretion of NASCE executive and/or the local organizing committee.

Harassment of conference participants will not be tolerated in any form. Harassment includes offensive gestures or verbal comments related to ethnicity, religion, disability, physical appearance, gender, or sexual orientation; deliberate intimidation; sustained disruption of talks or other events; unwelcome attention; and spamming online discussion boards, chats, question section, or social media by saying or reposting the same word/content repeatedly in order to disrupt the conversation. Participants asked to stop any harassing behavior are expected to comply immediately.

If a participant engages in harassing behavior, NASCE executive and/or the local organizing committee may take appropriate action, which could range from a simple warning to expulsion from this and future conferences. If you are being harassed, notice that someone else is being harassed, or have any other concerns, please contact a member of the NASCE Executive (http://nasce-snaec.com/contacts/) or the local organizing committee co-chairs Jean-Paul Paluzzi (paluzzi@yorku.ca), Christopher Martyniuk (cmartyn@ufl.edu) or Maricela Luna (lunam@unam.mx), who will work to resolve the situation.

Confidentiality will be maintained to the extent that it does not compromise the rights of others. The NASCE executive and/or the local organizing committee may revoke meeting credentials to anyone engaged in online harassment and may seek the assistance of law enforcement if necessary. We value your attendance and want to make your experience as productive and professionally stimulating as possible.

Presenter Guidelines

1 - Zoom basics:

A. Download Zoom app onto your desktop and be familiar with Zoom functions, namely mute/unmute and share screen.
B. Reach out to your Session Chair or the Organizing Committee if needed.

2. Before the meeting:

A. Respond to an email from your Session Chair about pre-recording your talk and making your talk available to NASCE 2021 attendees for 3 days after the meeting.
B. Pre-record your talk to the Zoom cloud, provide link to Session Chair.

3. Pre-recording your talk in Zoom:

A. In Zoom, you'll need a license through your University or some other paid entity. Basic Zoom (free account) is not sufficient.
B. Start a Zoom meeting with "save recording to cloud" selected, share your screen, make sure it is recording, give talk, stop recording.
C. Shortly afterwards, you will receive an email from Zoom with a private link to your recording with which you can trim away the beginning and end portions of your talk if desired.
D. Send the public link to your recording (in the same email from Zoom) to your Session Chair.
E. If your Zoom access does not allow you to record a video to the cloud, please contact Dan Buchholz (buchhodr@ucmail.uc.edu) who will provide you with a Zoom meeting link for pre-recording your talk. Please be aware that if you choose this option, Dan Buchholz will receive an email from Zoom with the Zoom cloud recording link instead of you. Please indicate at the beginning of your email request who is your Session Chair so Dan will know who to forward your pre-recording to.

4. During the meeting:

A. Enter the meeting ~ 5 min. prior to your scheduled presentation time and make your presence known to the Session Chair.
B. A **reminder to all speakers**: **invited/SOTA talks** are **20 min**, **contributed talks** are **10 min** and **lightning round talks** are **5 min**. Each presentation will be followed by up to 5 min of questions from the audience.
C. Give an awesome talk when invited to by your session chair.
In case of poor internet connectivity or you prefer your pre-recorded talk, the Session Chair will play your talk during your time.
D. Answer questions during the 5 min. allotted for questions.
E. At the end, breath deeply, be proud of a job well done, enjoy the rest of the meeting!

Guidance for Asking Questions

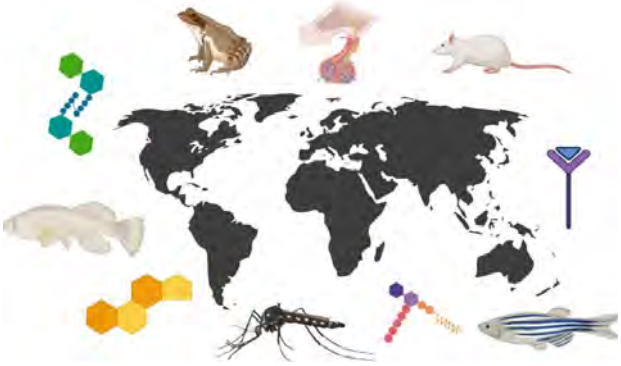
The session chairs and local organizing committee have decided that the preferred method of asking questions following the Plenary and Gorbman-Bern lectures as well as the scientific sessions will be to use the "Raise Hand" feature. This feature is located in the "Reactions" tab at the base of the Zoom window and will allow the Chair to recognize you and allow a question to be asked timer permitting. Participants will be displayed in the Chair's participant list in the order that they raised their hands. The large number of people present will make it challenging for the Chair to monitor Chat or to manage Participants unmuting themselves simultaneously.

General and Comparative Endocrinology:Special Issue

General and Comparative Endocrinology
Supports *open access*

4.5
CiteScore

2.426
Impact Factor



Special issue of the sixth biennial meeting of the North American Society for Comparative Endocrinology (Sociedad Norteamericana de Endocrinología Comparada; Societé Nord-Americaine d'Endocrinologie Comparée).

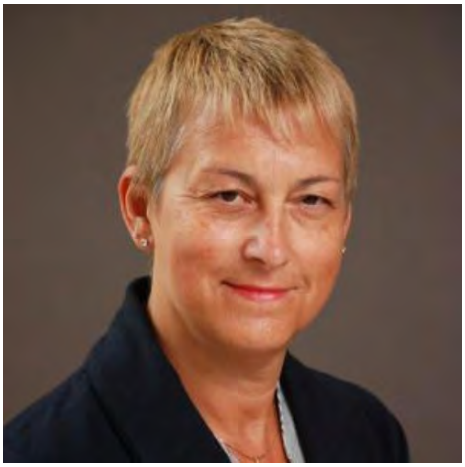
Delegates of the NASCE2021 meeting will have the opportunity to submit manuscripts for the "Special issue of the sixth biennial meeting of the North American Society for Comparative Endocrinology (Sociedad Norteamericana de Endocrinología Comparada; Societé Nord-Americaine d'Endocrinologie Comparée) in General and Comparative Endocrinology.

Participants interested in submitting a paper to the SI should send a letter of intent via email to Chris Martyniuk (cmartyn@ufl.edu) by **July 1, 2021**. Profs. Chris Martyniuk (USA), Jean-Paul Paluzzi (Canada), and Maricela Luna (Mexico) will act as Guest Editors for the special issue.

We invite all plenary and State-of-the-art (invited) speakers to submit review articles (15 to 20 pages, double spaced with no limit on the number of figures or references). All symposium speakers are invited to submit a mini-review or an original article (up to 12 pages double spaced and up to 4 figures with no limit on the number of references). The guest editors will also select some lightning round session speakers and invite the authors to submit manuscripts (mini review or original article) to the SI.

The **submission deadline will be December 31, 2021**, and the SI site on the **GCE editorial platform will be active on July 15, 2021**. You will find the instructions for preparation of the manuscripts and also the submission process by accessing the GCE web site (http://www.journals.elsevier.com/general-and-comparative-endocrinology). The project will be designated "**Proceedings of the 6th NASCE**". All questions regarding the special issue and General and Comparative Endocrinology should be directed to the Editor-in-Chief, Professor Mark A. Sheridan (gce@elsevier.com).

Plenary Speakers



DR. ANGELA LANGE
GORBMAN-BERN LECTURE

Angela B. Lange is a Professor of Biology. She obtained her B.Sc. and Ph.D. from York University, Toronto, Canada, prior to taking up an NSERC Post-doctoral Fellowship in the Department of Zoology, University of Toronto. In 1988, Professor Lange received a prestigious NSERC University Research Fellowship bridging to a tenure-stream faculty position in the Department of Biology, University of Toronto Mississauga. Professor Lange was promoted to full professor in 1996. At the University of Toronto, Professor Lange has held a variety of key administrative positions, including Chair of the Department of Biology, Vice-Dean, Undergraduate, Teaching and Learning, Vice-Dean, Faculty and Acting Vice-Principal Academic & Dean, UTM.

Professor Lange is an internationally renowned scholar in the field of comparative insect endocrinology and physiology. She has examined hormonal, synaptic and neuromodulatory mechanisms that regulate reproductive, metabolic and cardiac physiology in insect model organisms. Professor Lange was one of the pioneering researchers in understanding the role of octopamine and adipokinetic hormones as metabolic hormones in the regulation of lipid mobilization during locust flight. In addition, she has identified, localized and characterized a variety of insect hormones and their receptors, which have mammalian orthologs; these include insulin-like peptides (insulin orthologs), adipokinetic hormone (GnRH), allatostatin-A (galanin), crustacean cardioactive peptide (neuropeptide S), SIFamide (GnIH), sulfakinin (cholecystokinin) and tachykinin-like peptides (substance P), amongst others. In addition, she has worked extensively on the serotonergic and octopaminergic / tyraminergic (related to mammalian noradrenaline) signaling systems in various insects. As such, her pioneering comparative research has improved our understanding of the mechanisms by which these neurohormones and neuromodulators regulate diverse physiology and behavior in insects, and facilitates our understanding of their vertebrate counterparts. She has published over 160 scientific articles and 34 invited reviews and chapters in prestigious journals including Proceedings of the National Academy of Sciences, Annual Review of Entomology, General and Comparative Endocrinology and Journal of Comparative Neurology. She has over 6400 citations, an h-index of 40 and i10-index of 149. She has presented 234 papers at scientific meetings and symposia with 38 invited papers to such conferences.

Professor Lange was a member of the Natural Sciences and Engineering Research Council of Canada Grant Selection Committee for Biological Systems and Functions and also a member of the selection committee for the NSERC Herzberg Canada Gold Medal Selection Committee, the Council's highest research honor. She is currently an associate editor for Frontiers in Invertebrate Physiology. She has been a member of the International Federation of Comparative Endocrine Societies Council since 2005 and currently is Secretary of the North American Society for Comparative Endocrinology.

NEUROENDOCRINE CONTROL OF REPRODUCTION IN TWO INSECT MODELS: THE KISSING BUG, RHODNIUS PROLIXUS, AND THE LOCUST, LOCUSTA MIGRATORIA

Angela B Lange

Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

Successful oogenesis (growth of eggs) and oviposition (egg-laying) of fertilized eggs in a suitable substrate, requires the integration and coordination of various tissues of the reproductive system, as well as accompanying behaviours (e.g. feeding, digestion, digging). This integration is coordinated by signals from both the nervous and the endocrine systems.

The medically-important kissing bug, Rhodnius prolixus, a major vector for Chagas disease, is an obligate blood-feeder and takes a very large blood meal once in each instar. This blood meal triggers endocrinological events associated with growth, development and reproduction. In the adult female, neurohormones control short-term changes in physiology associated with nutrient storage and distribution, and long-term changes that influence oogenesis and oviposition, i.e. the number of eggs produced and deposited. Insulin-like peptides play a major role in these activities.

Also critical for deposition of viable eggs is the coordination of the muscles of the various structures of the reproductive system. This coordination involves a variety of neurons, including motor and sensory neurons, and their neuroactive chemicals. We have studies and described these events in detail in the locust, Locusta migratoria, and shown that female reproductive tissues are under central control from neurons releasing neuropeptides and amines. Central pattern generators (CPGs) are involved in coordinating oviposition, with CPGs controlling digging of the ovipositional hole, egg retention in the lateral oviducts during digging, sperm release onto the eggs, and egg-laying.

These two models will be discussed, illustrating commonalities in the neurochemical architecture used for coordinating the parts.

Acknowledgements: Research supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

DR. JAN MENNIGEN
GORBMAN-BERN NEW INVESTIGATOR

Dr. Jan Mennigen obtained his PhD in 2011 from the University of Ottawa, Canada under the co-supervision of Dr. Vance Trudeau and Dr. Thomas W. Moon. Following post-doctoral studies as Marie-Curie fellow at the Institut National de la Recherche Agronomique (INRAe) in St. Pée-sur-Nivelle, France and at the University of Texas at Austin, USA, he took up his current position as Assistant Professor at the University of Ottawa in the Department of Biology in 2016 and was promoted to Associate Professor in 2021. Dr. Mennigen's research focuses on the comparative endocrinology of metabolism reproduction in fish models and the investigation of effects of endocrine disrupting chemicals (EDCs) in these systems. He received a EU Marie-Curie postdoctoral fellowship award (2011), the president's poster award of the Endocrine Society (2014), and is the recipient of the 2021 NASCE Gorbman-Bern New Investigator award.

Jan is an Associate Professor in the Biology Department at the University of Ottawa (ON, Canada). He completed his dissertation with Drs. Vance Trudeau and Thomas Moon (University of Ottawa), where he investigated the endocrine disrupting effects of the pharmaceutical and emerging aquatic pollutant fluoxetine in goldfish with particular emphasis on metabolic and reproductive physiology. He then joined the Institut National de la Recherche Agronomique (INRAe) as Marie-Curie fellow, where he studied the endocrine and nutritional regulation and metabolic function of hepatic microRNAs in rainbow trout. He subsequently joined the lab of Dr. Andrea Gore at the University of Texas at Austin, where he investigated multigenerational reproductive consequences of embryonic estrogenic PCB exposures at the physiological and gene expression level in the rat. Jan's current research program employs a variety of fish models (zebrafish, goldfish, rainbow trout) to comparatively investigate the endocrine regulation of metabolism and reproduction as well as their disruption within and across generations as consequence of developmental exposure to endocrine disrupting chemicals (EDCs). As a comparative physiologist, Jan's work aims to integrate observations at the molecular and cellular level with organismal level approaches. Jan's laboratory is currently supported by the National Science and Engineering Research Council of Canada (NSERC).

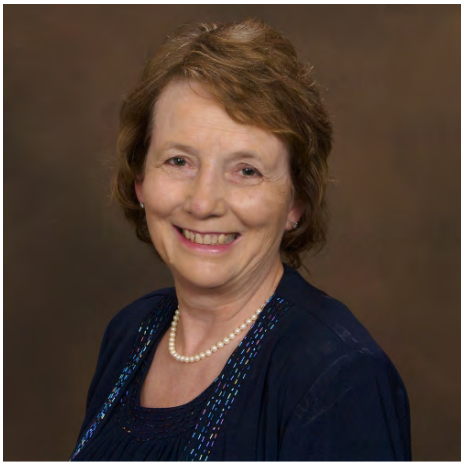
HORMONAL CONTROL OF GLUCOREGULATION IN CARNIVOROUS RAINBOW TROUT

Jan A Mennigen

Department of Biology, University of Ottawa, ON, Canada

Rainbow trout are carnivorous fish which exhibit prolonged postprandial hyperglycemia when fed carbohydrate rich diets (>20%) or administered exogenous glucose. This 'glucose-intolerant' phenotype has been recognized since the 1940s, and has been a focus of research in the aquaculture sector in an effort to identify bottlenecks towards improved utilization of ecologically and economically sustainable diets rich in carbohydrates. Nonetheless, a detailed understanding of the endocrine regulation to the rainbow trout's 'glucose-intolerant' phenotype is largely lacking. This presentation will describe how integrative experimental approaches (using organismal, tissue, cellular and molecular level analyses) were used to define how the action of insulin contributes to this metabolic phenotype in rainbow trout. Specifically, rainbow trout, in contrast to mammals, exhibit limited acute capacity for insulin-dependent suppression of glucose production and an inhibitory effect on glucose clearance at the organismal level. In the liver, the principal site of de novo gluconeogenesis, evidence suggests that differential transcriptional and/or posttranscriptional regulation of teleost- and salmonid- specific paralogues of key gluconeogenic enzymes Pck and G6pase may contribute to the incomplete suppression of this pathway in response to insulin and nutritional stimuli. Together, these comparative investigations of endocrine regulation of glucose-metabolism provide novel integrative insight into the 'glucose intolerant' phenotype in rainbow trout.

Acknowledgements: Supported by the National Science Foundation (NSERC-DG #147476).



DR. NANCY DENSLOW

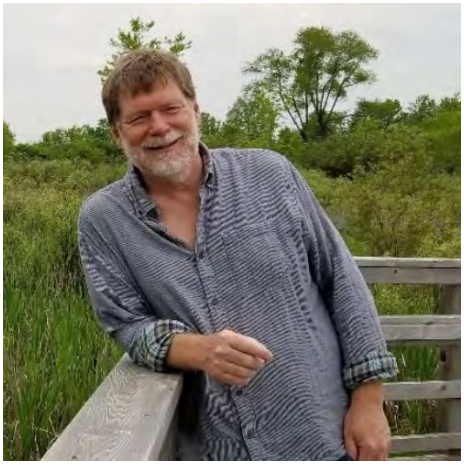
Nancy Denslow is a professor in the Department of Physiological Sciences and in the Center for Environmental and Human Toxicology at the University of Florida. She received her Ph.D. from the University of Florida in Biochemistry and Molecular Biology. Nancy has pioneered the use of molecular technologies for environmental toxicology especially focusing on high throughput in vitro assays, biomarker development and toxicogenomics approaches for evaluating contaminants of emerging concern. She has specialized in assessing the effects of organochlorine pesticides and endocrine disruptors that are found at relatively high levels in the environment. Nancy has over 250 peer-reviewed publications. She has received several awards for her research including the Founders Award from the Society of Environmental Toxicology and Chemistry. In addition to this society, she is also a member of the Society of Toxicology, and the Association of Biomolecular Research Facilities, a society devoted to the "OMICS" technologies. Nancy's research has received funding from EPA, NSF, USGS and NIH.

THREATS TO THE FISH ENDOCRINE SYSTEM FROM POLY- AND PERFLUORINATED CHEMICALS IN THE ENVIRONMENT

Nancy Denslow

University of Florida

Per- and polyfluoroalkyl substances (PFAS) have been released into the environment through the use of aqueous film-forming foams and a multitude of industrial and consumer-based products. PFAS have become an important emerging concern worldwide, as these substances are found in biota, water and sediments and constitute potential hazards to environmental and human health. They are known endocrine disruptors, causing infertility and hormone disruption in addition to immunotoxicity, hepatotoxicity and cancer, but their modes of action are still not well-delineated. While it is generally thought that PFAS activate the peroxisome proliferator-activated receptors, the concentrations required for transactivation of these receptors are several orders higher than concentrations found in humans or biota, suggesting alternative mechanisms may be more sensitive. While most studies concentrate on <10 PFAS, this group of chemicals comprises more than 7,000 different molecules with different functional groups. From in vitro cell based assays, it appears that PFAS primarily target PPARs, but in addition can target estrogen receptors, and alter the expression of thyroid hormone receptors, and perhaps this multitude of targets may be the underlying mechanisms for the varied adverse outcomes of exposure. To begin to understand the toxicity mechanisms in vivo, several fish exposure experiments were undertaken. In fathead minnows, early life exposure alters proper heart development, heartbeat and lipid profiles. In RNAseq experiments with adults, main pathways targeted include lipid related pathways, hormone biosynthesis, glucose metabolism and respiratory chain, among others. These studies point to a main effect of PFAS on the less studied but fundamental biochemical pathways, including mitochondrial function, respiration, and metabolism. PFAS are persistent environmental hazards and how they perturb the endocrine system of aquatic organisms continues to be an area where more research is necessary.



DR. VANCE TRUDEAU

Dr. Vance Trudeau is the University of Ottawa Research Chair in Neuroendocrinology and is also the Director of the Graduate Program in Biology. He received his Ph.D. from the University of Alberta and was a post-doctoral fellow in France and Canada. Vance is the founding Vice-President of NASCE and was the second President of NASCE. He is part of the "Three Amigos" of Comparative Endocrinology (along with Bob Denver and Carlos Arámburo) that established the meeting we are virtually attending ☺ Vance is also the president of the International Society for Fish Endocrinology (2021-25). Professor Trudeau is the 2017 recipient of the Outstanding Contribution to Canadian Ecotoxicology from the Canadian Ecotoxicology Workshop. His main research interests include the neuroendocrine control of the reproduction and stress in fish. Trudeau is a major proponent of the neuroendocrine disruption hypothesis for EDC actions. He is also particularly interested in applying basic principles of evolutionary endocrinology to ecotoxicology and EDC research, and for captive breeding of endangered amphibians - helping zoos and conservation groups breed amphibians. Trudeau is most proud of the successes of his graduate students and what they have accomplished in their own careers after leaving the lab. He and his students and collaborators have published >280 papers. He has extensive collaborators around the world, including China, Australia, Argentina, USA, among others. In the plenary, he will provide the historical context and new evidence that the neuropeptide secretoneurin is a new sex hormone.

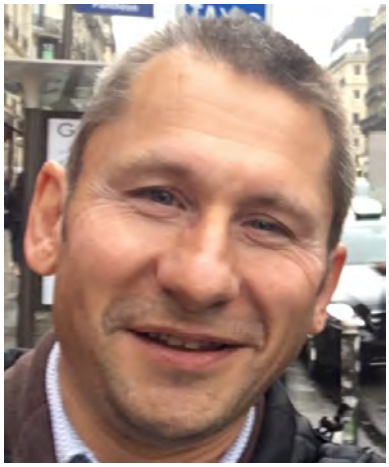
PEPTIDE IDENTITY CRISIS RESOLVED: SECRETONEURIN IS A NEW REPRODUCTIVE HORMONE

Trudeau VL* (1), Peng D (1), Lu C (1), Erandani WCKU (1), Chen L (2), Mitchell K (1), Tao BB (2), Hu W (2), Tan Y (3), Zhang D (3)

(1) Department of Biology, University of Ottawa, Ottawa, Ontario, Canada
(2) State Key Laboratory of Freshwater Ecology and Biotechnology,
Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China
(3) Department of Biology, Saint Louis University, Saint Louis, Missouri, USA

At the inaugural NASCE2011 meeting, we suggested that the secretogranin-2 (Scg2)-derived peptide secretoneurin (SN) was a hormone-like candidate. We reported then that SN stimulated luteinizing hormone (Lh) release from pituitary cells of in vitro in the absence of Gnrh. Most vertebrates have one Scg2 precursor and produce one SN form. In contrast, in teleosts there are the paralogous scg2a and scg2b genes, producing distinct SNa and SNb peptides. High sequence conservation suggests important roles for the Scg2/SN system. Zebrafish harbouring frameshift mutations in scg2 exhibit impaired reproduction. Double scg2a/scg2b mutant females are poor ovulators and spawning success for mutant couples is <10%. This defect is partially rescued by injection of SNa but not SNb. SNa increased hypothalamic gnrh3 and pituitary lhb mRNA in wildtype zebrafish. Conversely, there is very low expression of hypothalamic gnrh3 and pituitary lhb mRNA in mutants compared to wildtype fish. These data indicate that Scg2/SN controls both Gnrh and Lh. Double transgenesis experiments have revealed that Gnrh3 and Scg2a neurons are in the olfactory bulbs. The number of these Gnrh3 neurons is decreased significantly in scg2a mutants: incubations with SNa rescues this defect. Both scg2a and scg2b mRNAs are also found in multiple hypophysiotropic brain regions and the pituitary. In the preoptic-neurohypophysial tract, isotocin in magnocellular cells colocalizes with SNa, further implicating it in the control of reproduction. In the zebrafish pituitary gland, SNa immunoreactivity is found primarily in the cells of the pars intermedia and other regions but not in gonadotrophs of the pars distalis. Nano-liquid chromatography coupled to mass spectroscopy measurements of SNa and SNb in brain reveal variations across the spawning cycle in relation to time of ovulation. Together, these observations provide compelling evidence that the Scg2/SN system is critical for fish reproduction.

Acknowledgements: Supported by NSERC (VLT), University of Ottawa Research Chair in Neuroendocrinology (VLT), Chinese Academy of Sciences (WH) and Saint Louis University Startup Funds (DZ). The presenting author acknowledges that his research at uOttawa takes place on unceded territory of the Algonquin Anishinaabeg.



DR. MAURICE ELPHICK

Maurice Elphick grew up in the town of Eastbourne in Sussex, England. He trained in Biology (B.Sc. 1985-1988) at Royal Holloway, University of London, and it was here as an undergraduate that he developed an interest in comparative animal physiology. He proceeded onto a Ph.D. at Royal Holloway (1988-1991), working under the supervision of Mike Thorndyke for a thesis titled 'Neuropeptide structure and function in echinoderms'. A key finding was the discovery of SALMFamides – the first echinoderm neuropeptides to be purified and sequenced – work that was done during a research placement in the laboratory of Mike Greenberg and David Price at the Whitney Laboratory, University of Florida. In 1992 Maurice proceeded onto a postdoctoral position in Mick O'Shea's group at the University of Sussex and here he initiated a programme of research on nitric oxide signalling invertebrates, using the insect Schistocerca gregaria and the mollusc Lymnaea stagnalis as experimental systems. In 1995 Maurice was appointed as Lecturer in Animal Physiology at Queen Mary University of London and he was promoted to Senior Lecturer in 1999, Reader in 2001 and Professor in 2004. At Queen Mary he continued his research on nitric oxide signalling (supported by grants from BBSRC) and established a new programme of research on the evolution and neurobiology of endocannabinoid signalling (supported by grants from Leverhulme Trust, Wellcome Trust and BBSRC). His key contributions to endocannabinoid research included i). mapping of the expression in rodent brain of proteins that mediate or regulate endocannabinoid signalling, providing a basis for his hypothesis that endocannabinoids mediate retrograde signalling at synapses in the brain (1998), which was then proven in 2001. ii). investigation of the evolution of endocannabinoid signalling and discovery of cannabinoid receptors in invertebrate chordates. In 2012 Maurice switched the focus of his research back to neuropeptide signalling, primarily using the common European starfish Asterias rubens as an experimental system (supported by grants from Leverhulme Trust and BBSRC). This was facilitated by advances in sequencing technology that enabled sequencing of the A. rubens neural transcriptome and then more recently the A. rubens genome, which was sequenced as part of the Sanger Institute's 25 Genomes Project. Maurice's research on echinoderms has provided important insights into neuropeptide evolution, revealing 'missing links' between the more widely studied protostome invertebrates (e.g. Drosophila, C. elegans) and vertebrates. Furthermore, his research has provided fascinating insights into neuropeptide function in the context of the unique pentaradial body plan of adult echinoderms. To date, Maurice has published ~130 research papers/reviews that have attracted ~10,000 citations. Maurice has also served previously or currently on the editorial board of the journals Invertebrate Neuroscience, Comparative Biochemistry & Physiology and Journal of the Marine Biological Association and on the councils of the Society for Experimental Biology and the European Society for Comparative Endocrinology. Maurice is strongly committed to training and teaching in comparative physiology and neuroscience and since 1995 has supervised twenty-one PhD students and taught physiology and neuroscience to several thousand undergraduate students at Queen Mary.

THE EVOLUTION AND COMPARATIVE PHYSIOLOGY OF NEUROPEPTIDE SIGNALING SYSTEMS: NEW INSIGHTS FROM ECHINODERMS

Maurice R. Elphick

School of Biological & Chemical Sciences, Queen Mary University of London, UK

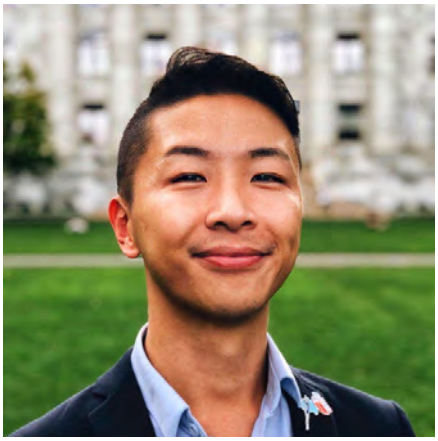
The physiology and behavior of animals is controlled or regulated by a huge variety of neuropeptide signaling systems, typically by acting locally in the nervous system as neuromodulators or systemically as neurohormones. Reconstruction of the evolutionary history of neuropeptide signaling systems has been facilitated recently by transcriptome/genome sequencing and experimental identification of G-protein coupled receptors that mediate effects of neuropeptides. Here I will discuss how research on neuropeptide signaling in echinoderms, and in particular the starfish Asterias rubens, is providing important insights into neuropeptide evolution and function. The primary rationale for investigating neuropeptide signaling in echinoderms is from an evolutionary perspective because as invertebrate deuterostomes they provide 'missing links' between neuropeptides in protostome invertebrates (e.g. Drosophila, C. elegans) and neuropeptides in vertebrates.

Thus, molecular characterization of neuropeptide-receptor pathways in A. rubens has, for example, provided key insights into the evolution of the NPS/CCAP-, GnRH/Corazonin-, luqin- and PrRP/sNPF- type signaling systems. Furthermore, as bilaterian animals that exhibit a derived pentaradial body plan as adult animals, echinoderms also provide a unique context for exploration of neuropeptide function. For example, neuropeptides that regulate the unusual extra-oral feeding behaviour (e.g. oxytocin-type and CCK-type neuropeptides) and reproductive biology (e.g. relaxin-type neuropeptides) in starfish have been identified, revealing evolutionary conservation/diversification of neuropeptide function in the Bilateria. In addition, analysis of the anatomy of neuropeptide systems in A. rubens is providing new insights into the neuroarchitecture of echinoderm nervous systems; for example, a CRH-type neuropeptide has been identified as a molecular marker for motoneurons that innervate the muscle layer of locomotory organs (tube feet) in starfish. Looking ahead, reconstructing the evolution of neuropeptide function in the phylum Echinodermata and in the Bilateria more generally will be facilitated by experimental characterization of neuropeptide signaling in the ever-growing list of taxa for which genome sequences are being obtained.

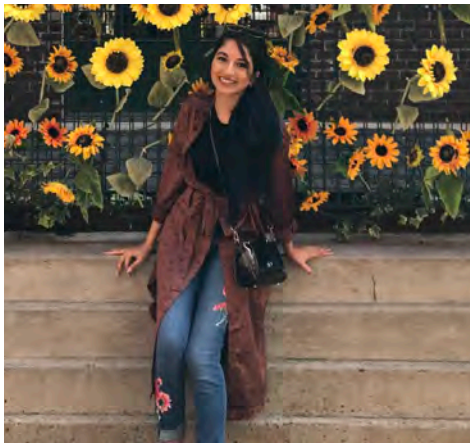
Acknowledgements: I am grateful to past and present members of my research group and many collaborators for their superb contributions to our research on neuropeptide signaling in echinoderms. I am also grateful to BBSRC, Leverhulme Trust, China Scholarship Council and CONACyT for financial support for our research.

Trainee Advisory Committee

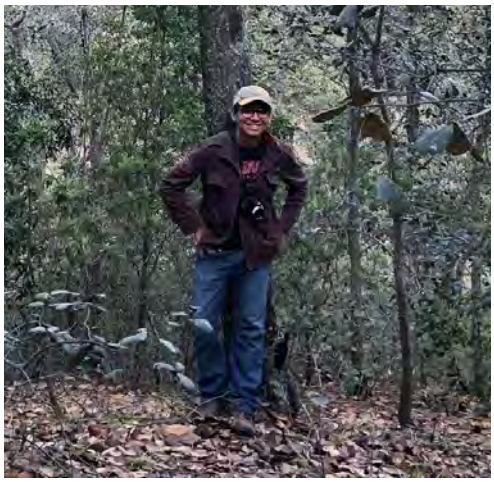
The North American Society for Comparative Endocrinology (NASCE; Societé Nord-Americaine d'Endocrinologie Comparée (SNAEC); Sociedad Norteamericana de Endocrinologìa Comparada (SNAEC)) Trainee Advisory Committee (TAC) includes graduate students or post-doctoral fellows representing trainee members of NASCE. Our current TAC members are:



Eugene Cheung, Ph.D., is a research fellow at Harvard Medical School and Brigham & Women's Hospital in Boston. He obtained his Ph.D. at North Carolina State University at Raleigh, his M.Sc. at the University of Windsor, and his H.B.Sc. at the University of Toronto. Eugene's research interests focus on the neuroendocrine regulation of metabolism and reproduction.



Farwa Sajadi is a PhD candidate working in the lab of Dr. Jean-Paul Paluzzi, in the Department of Biology, York University. Her work focuses on examining the cellular mechanism and cross-talk between the hormonal regulators in Aedes mosquitoes. As a recipient of several awards and academic achievements, Farwa is continuing her research in mosquito physiology, which will hopefully have a huge influence on the damaging role mosquitoes play in disease epidemiology.



Santiago M. Pech Pool, recently obtained his PhD in chemical-biological sciences, and is interested in the study of neuro-immuno-endocrinology. For that reason, Santiago completed his doctoral thesis in the laboratory of hormone biochemistry of Dr. Carlos Arámburo, in Querétaro, Mexico. Currently he is coordinator of a science program in a private institution, likes photography and I also to do science communication.

Program

Tuesday, May 25, 2021				
10:30-11:00	**Welcome + Announcements**			
11:00-11:45	**Plenary #1: Dr. Nancy Denslow** Threats to the fish endocrine system from poly- and perfluorinated chemicals in the environment			
11:45-12:15	**Morning Break & NASCE Trainees Strategies for Success**			
12:15-13:30 **Session** **Chairs**	**Session 1: Thyroid hormone action on organ maturation and tissue regeneration (Part I)** **Liezhen Fu**	**Session 2: New Frontiers in Endocrine Disrupting Chemicals: From Novel Mechanisms of Action to Monitoring (Part I)** **Valerie Langlois & Nancy Denslow**	**Session 3: Neuropeptides involved in invertebrate nutritional regulation and reproduction (Part I)** **Angela Lange & Jimena Leyria**	**Session 4: Growth, Metabolism, Hormones and Behavior (Part I)** **Maricela Luna & Aurea Orozco**
12:15-12:20	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIRS**	**SHORT INTRODUCTION BY SESSION CHAIRS**	**SHORT INTRODUCTION BY SESSION CHAIRS**
12:20-12:40	**Buisine, N** QUESTIONING WHETHER A LACK THYROID HORMONE MEDIATED METAMORPHOSIS IN AMPHIBIANS CORRESPONDS TO A LACK OF POST-EMBRYONIC TRANSITION? *Tribondea A, Sachs LM, Buisine N*	**Mehinto, Alvine C.** BIOANALYTICAL TOOLS FOR WATER QUALITY MONITORING: ASSESSING THE OCCURRENCE AND POTENTIAL TOXICITY OF ENDOCRINE DISRUPTING CHEMICALS IN CALIFORNIA WATERS *Alvine C. Mehinto*	**Leyria, J** NUTRITION-DEPENDENT CONTROL OF INSECT REPRODUCTION: THE INVOLVEMENT OF INSULIN/TOR SIGNALLING PATHWAY IN REPRODUCTIVE PERFORMANCE OF RHODNIUS PROLIXUS, A VECTOR OF CHAGAS DISEASE *Leyria J, Orchard I, Lange AB*	**Riesgo-Escovar JR** MORE THAN MEETS THE EYE: SEX-SPECIFIC DIFFERENCES IN METABOLISM AND OXIDATIVE STRESS RESPONSE IN INSULIN SIGNALING COMPROMISED AND CONTROL FLIES *Álvarez-Rendón JP, Riesgo-Escovar JR*

12:45-12:55	**Tanizaki, Yuta** A ROLE OF ENDOGENOUS HISTONE ACETYLTRANSFERASE STEROID HORMONE RECEPTOR COACTIVATOR (SRC) 3 IN THYROID HORMONE SIGNALING DURING XENOPUS INTESTINAL METAMORPHOSIS *Yuta Tanizaki, Lingyu Bao, Yun-Bo Shi*	**Robitaille, Julie** EVALUATION OF A TWO-TIER BIOASSAY-BASED APPROACH TO ASSESS ENDOCRINE DISRUPTING CHEMICALS IN WASTEWATER IN QUEBEC *Robitaille J, Desrosiers M, Guay I, Métivier M, Veilleux E, Langlois VS*	**Ling, Lin** CROSS-TALK OF INSULIN-LIKE PEPTIDES, JUVENILE HORMONE, AND 20-HYDROXYECDYSONE COORDINATING METABOLISM IN REPRODUCING MOSQUITO AEDES AEGYPTI *Lin Ling, Alexander S. Raikhel*	**Corona, R** OLFACTION AND REPRODUCTION, ROLE OF PROLACTIN *Corona R, Morales T*
13:00-13:10	**Craver, JJ** ADAMTS9 IN PRIMORDIAL GERM CELL MIGRATION AND GONADAL DEVELOPMENT IN ZEBRAFISH *Carver JJ, He Y, Zhu Y*	**Miller, JGP** THE IN VITRO OOCYTE MATURATION ASSAY IS PREDICTIVE OF EX VIVO OOCYTE MATURATION INHIBITION IN ZEBRAFISH (DANIO RERIO) EXPOSED TO ORGANOPHOSPHATE INSECTICIDES *Miller JGP, Van Essen D, Brinkmann M, Raza Y, Dubiel J, Doering JA, Wiseman SB*	**Feng, YL** IDENTIFICATION OF SOURCES OF RELAXIN-LIKE GONAD-STIMULATING PEPTIDE AS A REGULATOR OF SPAWNING IN STARFISH *Feng YL, Piñon Gonzalez VM, Egertová M, Mita M, Elphick MR*	**Souders II, Christopher L** BEHAVIORAL RESPONSES OF A ZEBRAFISH ADRENERGIC RECEPTOR BETA2 RECEPTOR KNOCKOUT MODEL TO ADRENORECEPTOR AGONISTS *Christopher L Souders II, Christine Larrea, Hunter Davis, David Kim, Jordan T. Schmidt, Jasenka Zubcevic, Christopher J. Martyniuk*
13:15-13:25	**Evans, EP** TEMPERATURE-SENSITIVE COMPONENTS OF THE MOLECULAR MEMORY IN THE AMERICAN BULLFROG, RANA CATESBEIANA *Evans EP, Koide EM, Helbing CC*	**Corrie, LM** DIOCTYL SODIUM SULFOSUCCINATE AS A POTENTIAL ENDOCRINE DISRUPTOR OF THYROID HORMONE ACTIVITY IN AMERICAN BULLFROG, RANA (LITHOBATES) CATESBEIANA, TADPOLES *Corrie LM, Kempe MN, Blajkevitch O, Shang D, Helbing CC*	**Afifi, S** IDENTIFYING KEY RESIDUES CONFERRING LIGAND SPECIFICITY AND EFFORTS TO ELUCIDATE THE FUNCTION OF THE ADIPOKINETIC HORMONE/CORAZON IN-RELATED PEPTIDE AND ITS RECEPTOR IN THE MOSQUITO AEDES AEGYPTI *Afifi S, Paluzzi JP*	**Olivares-Hernández, JD** GROWTH HORMONE (GH) ENHANCES ENDOGENOUS MECHANISMS OF NEUROPROTECTION AND NEUROPLASTICITY AFTER OXYGEN AND GLUCOSE DEPRIVATION INJURY (OGD) AND RE-OXYGENATION (OGD/R) IN CHICKEN HIPPOCAMPAL CELL CULTURES *Olivares-Hernández JD, Balderas-Márquez JE, Carranza M, Luna M, Martínez-Moreno CG, Arámburo C*
13:30-14:00	**Lunch Break**			
14:00-14:45	**Comparative Endocrinology Welcome Mixer – Networking**			
14:45-15:15	**Afternoon Health Break – NASCE Yoga/Fitness**			

15:15-16:30 Session Chairs	**Session 5: Thyroid hormone action on organ maturation and tissue regeneration (Part II)** **Liezhen Fu**	**Session 6: New Frontiers in Endocrine Disrupting Chemicals: From Novel Mechanisms of Action to Monitoring (Part II)** **Valerie Langlois & Nancy Denslow**	**Session 7: Neuropeptides involved in invertebrate nutritional regulation and reproduction (Part II)** **Angela Lange & Jimena Leyria**	**Session 8: Growth, Metabolism, Hormones and Behavior (Part II)** **Maricela Luna & Aurea Orozco**
15:15-15:20	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIRS**	**SHORT INTRODUCTION BY SESSION CHAIRS**	**SHORT INTRODUCTION BY SESSION CHAIRS**
15:20-15:40	**Hasebe, T** SIGNALING PATHWAYS ESSENTIAL FOR THYROID HORMONE-INDUCED ADULT STEM CELL DEVELOPMENT DURING INTESTINAL REMODELING OF XENOPUS LAEVIS *Hasebe T, Fujimoto K, Buchholz DR, Ishizuya-Oka A*	**Bottalico, LN** MULTI-GENERATIONAL METABOLOMICS OF BISPHENOL-A EXPOSURE IN C57BL/6J MICE: GENDER AND LIFE-STAGE SPECIFIC EXPOSURE EFFECTS AND CONNECTIONS WITH THE CIRCADIAN CLOCK *Bottalico LN, Bansal A, Briskin A, Malik D, Sengupta A, Garai S, Grant G, Simmons R, and Weljie AM*	**El-Gendy, A** CROSS-TALKS BETWEEN NUTRIENTS, NEUROENDOCRINE SYSTEM AND BIOLOGICAL CLOCKS IN THE REGULATION OF DIGESTION, FORAGING, CELL PROLIFERATION AND REPRODUCTION IN THE COCKROACH, PERIPLANETA AMERICANA *El-Gendy A, Mikani A, Kamrzzaman ASM, Yoshida Y, Mohamed AA, Takeda M*	**Ávila-Mendoza J** BRAIN-DERIVED NEUROTROPHIC FACTOR INDUCES PRODUCTIVE OPTIC NERVE REGENERATION IN MICE DEFICIENT FOR KRÜPPEL-LIKE FACTORS 9 AND 13 *Ávila-Mendoza J, Jessy D. Martinez, Sara J. Aton, Robert J. Denver*
15:45-15:55	**Koide, EM** THE CHILLING TAIL OF THYROID HORMONE INDUCED METAMORPHOSIS *Koide EM, Abbott EA, Helbing CC*	**Bhandari, RK** HERITABLE EFFECTS OF ENDOCRINE-DISRUPTING CHEMICALS IN MEDAKA *Wang X, Feng Y, Chakraborty S, Bhandari RK*	**Tsai, PS** FUNCTIONAL AUTHENTICATION AND KNOCKDOWN OF A GASTROPOD ADIPOKINETIC HORMONE RECEPTOR IN THE SEA HARE *Tsai PS, Laphyai P, Zhang H, Sanders KN*	**Lazcano, Ivàn** T3 EXERTS EARLY EFFECTS UPON MYELINATION IN THE ZEBRAFISH *Iván Lazcano, Yasmin Hernández Linares, Patricia Villalobos, Aurea Orozco*
16:00-16:10	**Wang, Shouhoung** EVOLUTIONARY DIVERGENCE IN TAIL REGENERATION BETWEEN XENOPUS LAEVIS AND XENOPUS TROPICALIS *Shouhong Wang, Yun-Bo Shi*	**Feng, Y** EFFECTS OF EXPOSURE TO DELTA-9 TETRAHYDROCANNA BINOL (THC) ON PATERNAL REPRODUCTIVE TISSUE AND OVERALL HEALTH OF OFFSPRING IN MEDAKA, ORYZIAS LATIPES *Feng Y, Wang X, Bhandari RK*	**Haddad, A** IDENTIFICATION OF A TACHYKININ RECEPTOR AND ITS IMPLICATION IN METABOLISM IN RHODNIUS PROLIXUS, A CHAGAS DISEASE VECTOR *Haddad A, Leyria J, Lange AB*	**Deal, CK** EFFECTS OF THYROID HORMONE ON FEEDING BEHAVIOR AND THE EXPRESSION OF APPETITE REGULATING NEUROPEPTIDES *Deal CK, Volkoff H*

16:15-16:25	**Mayasich, S** DEVELOPMENT OF TYPE 2 AND TYPE 3 IODOTHYRONINE DEIODINASE KNOCKOUT XENOPUS TROPICALIS USING CRISPR/CAS12A GENE EDITING *Mayasich S, Degoey P, Haselman J, Degitz S*	**Reh, B** EFFECT OF POTASSIUM PERCHLORATE ON DEVELOPING MEDAKA PRIMORDIAL GERM CELLS *Reh B, Feng Y, Wang X, Bhandari RK*	**Lavine, Laura** HOST QUALITY INDUCES PHENOTYPIC PLASTICITY IN THE BROWN RICE PLANTHOPPER Xinda Lin, Yili Xu, *Jianru Jiang, Mark Lavine, Laura Corley Lavine* CANCELLED	**Ladisa, C** METABOLOMICS INVESTIGATION OF SEASONALLY RELATED CHANGES IN MALE GOLDFISH (CARASSIUS AURATUS) LIVER METABOLISM *Ladisa C, Ma Y, Habibi HR*
16:30-17:30	**NASCE Trainee Mixer**			

Wednesday, May 26, 2021				
11:00-11:45	**Plenary #2: Dr. Vance Trudeau** Peptide identity crisis resolved: secretoneurin is a new reproductive hormone			
11:45-12:15	**Morning Break & EDI Panel Session #1**			
12:15-13:30 **Session** **Chairs**	**Session 9: Non-invasive methods to measure corticoids and sex steroids in domestic animals and wild fauna** **Marta Romano**	**Session 10: Neuroendocrine regulation of ionic, osmotic, and acid-base balance in vertebrates** **Jason P. Breves & Stephen D. McCormick**	**Session 11: Developmental roles of corticosteroids and their receptors** **Dan Buchholz**	**Session 12: Avian Endocrine and Metabolic Responses to Urbanization** **Pierre Deviche & Karen Sweazea**
12:15-12:20	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIRS**	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIRS**
12:20-12:40	**Lopez-Bejar, Manuel** NON-INVASIVE HORMONE MONITORING TO STUDY THE ENDOCRINOLOGY OF DOMESTIC, ZOO AND WILDLIFE SPECIES *Manel Lopez-Bejar*	**Yamaguchi, Y** IDENTIFICATION AND CHARACTERIZATION OF NEUROHYPOPHYSIAL HORMONE RECEPTORS IN THE HAGFISH, EPTATRETUS BURGERI *Yamaguchi Y, Takagi W, Kaiya H, Yoshida M*	**Faught, E** COORDINATION OF STRESS-RELATED BEHAVIOUR IN LARVAL ZEBRAFISH: THE ROLE OF CRHR1, MR AND GR *Faught E, Vijayan MM*	**Vitousek, MN** URBANIZATION AND AVIAN ENDOCRINE RESPONSES *Vitousek MN*
12:45-12:55	**Brammer-Robbins, E** NOVEL INSIGHT INTO FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS) REPRODUCTIVE PHYSIOLOGY: IMPLICATIONS FOR CONSERVATION BIOLOGY *Brammer-Robbins E, Nouri MZ, Denslow N, Larkin I, Martyniuk CJ*	**Woo, DW** THERMALLY-INDUCED CHANGES IN CELL VOLUME AND HORMONE RELEASE IN PROLACTIN CELLS OF MOZAMBIQUE TILAPIA *Woo DW, Malintha GHT, Celino-Brady FT, Breves JP, Yamaguchi Y, Seale AP*	**Shaughnessy, CA** THE ROLES OF CORTICOSTEROIDS DURING THE SEA LAMPREY METAMORPHOSIS: OSMOREGULATORY AND GLUCONEOGENIC ACTIONS *Shaughnessy CA, McCormick SD*	**Basile, AJ** A Four-Week Urban Diet Impairs Vasodilation Bot Not Nutritional or Metabolic Physiology in Wild-caught Mourning Doves (Zenaida macroura) *Basile AJ, Renner MW, Kayata L, Mohr AE, Deviche P, Sweazea KL*
13:00-13:10	**Legacki, E** THE MEASUREMENT OF STEROID HORMONES IN ATLANTIC SALMON MUCUS *Legacki E, Peterson B, Boggs A*	**Culbert, BM** THE ROLE OF THE CORTICOTROPIN-RELEASING FACTOR SYSTEM DURING SMOLTIFICATION IN ATLANTIC SALMON *Culbert BM, Regish AM, Hall DJ, McCormick SD, Bernier NJ*	**Nozari, Amin** BRIEF DEVELOPMENTAL EXPOSURE TO FLUOXETINE CAUSES LIFE-LONG DISRUPTION OF THE STRESS RESPONSE IN ZEBRAFISH *Amin Nozari, Vance L. Trudeau, Carole Yauk, Remi Gagné*	**Angelier, F** ARE CORTICOSTERONE LEVELS A PROXY OF ENVIRONMENTAL CONSTRAINTS IN A DECLINING URBAN EXPLOITER, THE HOUSE SPARROW *Angelier F, Grace JK, Dupont S, Beaugeard E, Meillère A, Brischoux F*

13:15-13:25	**González-de-la-Vara, MR** DEAD NEWBORN CALVES AFFECTS CORTISOL, BEHAVIOR AND MILK PRODUCTION IN PRIMIPAROUS DAIRY COWS *González-de-la-Vara M R, De Anda FJ, Romano MC*	**Seale, AP** EFFECTS OF FRESHWATER ACCLIMATION ON THYROID HORMONES AND BRANCHIAL DEIODINASES IN MOZAMBIQUE TILAPIA *Seale AP, Seale LA, Gilman CL, Zavacki AM, Larsen PR, Inokuchi M, Breves JP*	**Sterner, Z** GLUCOCORTICOID RECEPTOR MEDIATES CORTICOSTERONE AND THYROID HORMONE SYNERGY DURING FROG METAMORPHOSIS *Sterner Z, Buchholz, D*	**Deviche, P** PAST AND FUTURE: THE IMPACT OF URBANIZATION ON AVIAN PHYSIOLOGY *Deviche P, Sweazea K*
13:30-14:00	**Lunch Break**			
14:00-14:45	**Dr. Angela Lange - Gorbman-Bern Lecture**			
14:45-15:15	**Afternoon Health Break – NASCE Yoga/Fitness**			
15:15-17:00 **Session Chairs**	**Session 13: Lightning Round** **Maricela Luna**	**Session 14: Lightning Round** **Santiago M Pech-Pool**	**Session 15: Lightning Round** **Eugene Cheung**	**Session 16: Lightning Round** **Farwa Sajadi**
15:15-15:20	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIR**
15:20-15:25	**Alonso-Gómez, A** COULD THE IGF-1 ACT AS AN ENDOCRINE MEDIATOR BETWEEN CIRCADIAN OSCILLATORS IN GOLDFISH? *Alonso-Gómez A, Madera D, Alonso-Gómez AL, Delgado MJ, Valenciano AI*	**Jewell, K** FIBROBLAST GROWTH FACTOR 8-DEFICIENT MICE EXHIBIT NORMAL NEONATAL GONADAL MORPHOLOGY AND FUNCTION *Jewell K, Tsai PS*	**Paul, B** CORTICOSTEROID DEFICIENT 21-HYDROXYLASE (CYP21A2) KNOCKOUT TADPOLES SURVIVE THROUGH METAMORPHOSIS *Paul B, Shewade LH, Buchholz DR*	**Wang, C** THE INFLUENCE OF CRF ON EVOKED CALCIUM INFLUX IN THE OPTIC TECTUM OF THE SOUTH AFRICAN CLAWED FROG XENOPUS LAEVIS. *Wang C, Keyel PA, Carr JA*
15:30-15:35	**Epardo D** EVALUATION OF THE NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE NEURAL RETINA *Epardo D, Balderas-Márquez JE, Fleming T, Carranza M, Luna M, Harvey S, Ávila-Mendoza J, Arámburo C, Martínez-Moreno CG*	**Gilmour KM** THE ROLE OF THE SOMATOTROPIC AXIS IN SOCIAL STATUS-DEPENDENT GROWTH AND METABOLISM IN RAINBOW TROUT *Gilmour KM, Best C, Magnan J, Jennings K, Touma K, Bernier NJ, Mennigen JA*	**Cabrera-Busto, J** CORTICOSTEROIDS MEDIATE GLUCOCORTICOID ACTIONS IN THE LESSER SPOTTED CATSHARK (SCYLIORHINUS CANICULA) *Cabrera-Busto J, Mancera JM, Ruiz-Jarabo I*	**Malintha, GHT** OSMOTIC REGULATION OF TRANSCRIPTION FACTOR MRNA LEVELS IN PROLACTIN CELLS OF MOZAMBIQUE TILAPIA *Malintha GHT, Celino-Brady FT, Seale AP*

15:40-15:45	**Madera, D** IS NOCTURNIN EXPRESSION MODIFIED BY DIFFERENT THERMAL CONDITIONS IN GOLDFISH? *Madera D, Alonso-Gómez A, Valenciano AI, Delgado MJ, Alonso-Gómez AL*	**Menon, NM** CAN FROGS GET ANXIOUS? THE INFLUENCE OF SIMULTANEOUS EXPOSURE TO A PREDATOR AND FOOD ON FORAGING-AVOIDANCE BEHAVIOR AND GENE EXPRESSION IN THE OPTIC TECTUM OF AFRICAN-CLAWED FROGS (XENOPUS LAEVIS) *Menon NM, Carr JA*	**Wonho, Na** DIRECT ACTIVATION OF TRNA METHYLTRANSFERASE-LIKE 1 (METTL1) GENE BY THYROID HORMONE RECEPTOR IMPLICATES A ROLE IN ADULT INTESTINAL STEM CELL DEVELOPMENT AND PROLIFERATION DURING XENOPUS TROPICALIS METAMORPHOSIS *Wonho Na, Liezhen Fu, Nga Luu, Yun-Bo Shi*	**Tan, J** MOLECULAR CHARACTERIZATION OF CCHAMIDE2 AND DEORPHANIZATION OF ITS RECEPTOR IN THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI *Tan J, Paluzzi JP*
15:50-15:55	**Celino-Brady, FT** SEX-DEPENDENT MODULATION OF GENES INVOLVED IN GROWTH AND REPRODUCTION BY GROWTH HORMONE AND LUTEINIZING HORMONE IN TILAPIA *Celino-Brady FT, Breves JP, Seale AP*	**Gong, N** CHARACTERIZATION OF A LEPTIN RECEPTOR ORTHOLOG IN A JAWLESS VERTEBRATE (AGNATHAN) *Gong N, McCormick SD, Sheridan MA*	**Kuecks-Winger, HN** TISSUE-SPECIFIC HISTONE VARIATIONS IN PREMETAMORPHIC RANA (LITHOBATES) CATESBEIANA. *Kuecks-Winger HN, Thambirajah AA, Helbing CC*	**Piñon Gonzalez, V** MECHANISMS OF NEUROPEPTIDERGIC REGULATION OF REPRODUCTIVE PHYSIOLOGY IN STARFISH *Piñon Gonzalez V, Elphick MR*
16:00-16:05	**Curtis, GH** LEPTIN SIGNALING STIMULATES PERIPHERAL ANGIOGENESIS DURING XENOPUS LARVAL DEVELOPMENT *Curtis GH, Reeve RE, Whitfield K, Crespi EJ*	**Saiz, N** A REV-ERBA AGONIST ELICITS STRONG ANORECTIC RESPONSES IN FISH *Saiz N, Herrera-Castillo L, Cebrián A, Villar V, Isorna E, Delgado MJ, de Pedro N*	**Mathewson, AS** MITIGATION OF GLYPHOSATE EXPOSURE-INDUCED DEVELOPMENTAL DEFECTS BY VITAMIN-C CO-TREATMENT Weidman D, Mathewson AS, Killian D, Bhandari RK	**Jing, QQ** ALTERNATION OF HEPATIC GLYCOLYSIS, LIPOGENESIS, AND BLOOD BIOCHEMISTRY IN TIGER PUFFER (TAKIFUGU RUBRIPES) UNDER TWO DIFFERENT CULTURE SYSTEMS *Jia YD, Wang ZY, Li MY, Jing QQ, Zhai JM, Guan CT*
16:10-16:15	**Imbery, JJ** CHARACTERIZING THE SKIN MICROBIOTA IN RANA CATESBEIANA ACROSS NATURAL METAMORPHOSIS AND HORMONE EXPOSURE *Imbery JJ, Jia B, Van Rossum T, Lo R, Brinkman FSL, Helbing CC*	**Borski, Russell J** INTERACTIONS OF GLUCOREGULATORY HORMONES AND LEPTIN IN THE CONTROL OF GLUCOSE HOMEOSTASIS IN THE MOZAMBIQUE TILAPIA *Russell J. Borski, Courtney A. Deck, Jamie Mankiewicz*	**Weidman, D** MITIGATION OF GLYPHOSATE-INDUCED DEVELOPMENTAL DEFECTS IN MEDAKA FISH *Bhandari R, Weidman D, Matthewson A* CANCELLED	**Nasri, A** NESFATIN-1 AND NESFATIN-1-LIKE PEPTIDE STIMULATE PROOPIOMELANOCORTIN SYNTHESIS IN MURINE ATT-20 CORTICOTROPHS THROUGH THE AMP/PKA/CREB SIGNALING PATHWAY *Nasri A, Unniappan S*

16:20-16:25	**Balderas-Márquez JE** NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE CHICKEN RETINA DURING INFLAMMATION *Balderas-Márquez, Epardo D, Carranza M, Luna M, Arámburo C, Martínez-Moreno CG*	**Benrabaa, Samiha** IDENTIFICATION AND CHARACTERIZATION OF THE HALLOWEEN AND ECDYSONE-RESPONSIVE GENES IN THE OVARIES OF RHODNIUS PROLIXUS *Samiha Benrabaa, Ian Orchard, Angela B. Lange*	**Guzman, E** TRANSGENERATIONAL DIFFERENCES IN MEDAKA GUT MICROBIOTA POPULATION INDUCED BY ANCESTRAL BPA EXPOSURE *Guzman E, Wang X, Feng Y, Bhandari RK*	**Khalid, E** LIGAND-BIAS IN GNRH TRANSDUCTION NETWORKS: ROLES OF RECEPTOR-INTERACTING EFFECTORS IN THE CONTROL OF GOLDFISH PITUITARY HORMONE SECRETION *Khalid E, Chang JP*
16:30-16:35	**Alvarez-Rendón, JP** CHARACTERIZATION OF METABOLIC DEFECTS IN INSULIN-SIGNALING IMPAIRMENT DROSOPHILA MELANOGASTER *Alvarez-Rendón JP, Riesgo-Escovar JR*	**Urban-Sosa, VA** THE SYNTHESIS AND RELEASE OF PITUITARY GROWTH HORMONE IS DIFFERENTIALLY REGULATED BY SEVERAL NEUROPEPTIDES AMONG VERTEBRATES *Urban-Sosa VA, Ávila-Mendoza J, Carranza M, Martínez-Moreno CG, Luna M, Arámburo C*	**Godwin, J** EFFECTS OF GRANDPARENTAL EMBRYONIC BISPHENOL A EXPOSURE ON THE LIVER OF GRANDCHILDREN OF MEDAKA FISH *Godwin J, Chakraborty S, Bhandari RK*	**Whitfield, Kourtnie** DOES LEPTIN SIGNALING REGULATE MUCUS SECRETION IN XENOPUS LAEVIS EMBRYONIC EPIDERMIS, A MODEL FOR RESPIRATORY EPITHELIUM? *Kourtnie Whitfield, Robyn Reeve, Grace Curtis, Erica Crespi*
16:40-16:45	**Rajeswari JJ** PHOENIXIN-20 (PNX-20) SUPPRESSES FOOD INTAKE AND PROMOTES GLYCOLYSIS IN ZEBRAFISH *Rajeswari JJ, Blanco AM, Unniappan S*	**Jia, YD** COMPARISON OF THE PITUITARY AND GONADOTROPINS CELL LOCALIZATION IN TURBOT AND MOUSE *Jia YD, Gao YH, Hong L, Lin JX*	**Andersen, ND** INCREASED DE-DIFFERENTIATION OF GONADOTROPIN-RELEASING HORMONE NEURONS IN A FGF SIGNALING-DEFICIENT MOUSE *Andersen ND, Tsai PS*	**Fuse, M** STARVATION INFLUENCES NOCICEPTION AND SENSITIZATION IN AN INSECT MODEL *Crawford N, Fuse M*
16:50-16:55		**Pech-Pool, Santiago M** THYROTROPIN-RELEASING HORMONE (TRH) AND SOMATOSTATIN (SST), BUT NOT GROWTH HORMONE-RELEASING HORMONE (GHRH) NOR GHRELIN (GHRL), REGULATE EXPRESSION AND RELEASE OF IMMUNE GROWTH HORMONE (GH) IN CHICK BURSAL B-LYMPHOCYTE CULTURES *Santiago M Pech-Pool, Laura C Berumen, Carlos G Martínez-Moreno, Guadalupe García-Alcocer, Martha Carranza, Carlos Arámburo, Maricela Luna*		

Thursday, May 27, 2021				
11:00-11:45	**Plenary #3: Dr. Maurice Elphick** Evolution and comparative physiology of neuropeptide signaling systems: new insights from echinoderms			
11:45-12:15	**Morning Break & EDI Panel Session #2**			
12:15-13:30 **Session** **Chairs**	**Session 17: The relevance of neurosteroids and steroidogenic enzymes in comparative endocrinology** **Marta Romano**	**Session 18: Hormone mediated control of ion and fluid homeostasis in invertebrates** **Jean-Paul Paluzzi**	**Session 19: Novel neuropeptides: what can the comparison of invertebrate and vertebrate systems teach us?** **Vance Trudeau**	**Session 20: Recent Topics in Comparative Endocrinology** **Christopher J. Martyniuk**
12:15-12:20	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIR**	**SHORT INTRODUCTION BY SESSION CHAIR**
12:20-12:40	**Shaw, K** THE ROLE OF AROMATASE IN THE GATEWAYS OF SOCIAL PERCEPTION IN TELEOSTS *Shaw K , Trudeau VL*	**Sajadi, F** EXAMINING THE ROLE OF THE V-TYPE H+-ATPASE IN CAPA-MEDIATED INHIBITION IN AEDES AEGYPTI MALPIGHIAN TUBULES *Sajadi F, Paluzzi JP*	**Thorat, L** THYROSTIMULIN AND ITS INVERTEBRATE HOMOLOG, GPA2/GPB5: A SPECIAL FOCUS ON THE GPA2/GPB5 HORMONE RECEPTOR IN DROSOPHILA MELANOGASTER *Thorat L, Patel S, Paluzzi JP*	**McDonald, M Danielle** SEROTONIN: A UBIQUITOUS YET OBSCURE HORMONE IN TELEOST FISH *McDonald, M. Danielle*
12:45-12:55	**Patricio-Gómez, J.M** STEROIDS AND NEUROSTEROIDS-LIKE HORMONES SYNTHESIS IN TAENIA CRASSICEPS WFU CYSTICERCI *Patricio-Gómez JM, Valdez R, Figueroa M, Romano MC*	**Picinic, BN** THE REGULATION OF AQUAPORIN ABUNDANCE BY DIURETIC HORMONES IN THE MALPIGHIAN TUBULES OF THE LARVAL DISEASE VECTOR MOSQUITO, AEDES AEGYPTI *Picinic BN, Donini A, Paluzzi JP*	**Tinoco, AB** FUNCTIONAL CHARACTERIZATION OF CHOLECYSTOKININ/SULFAKININ TYPE NEUROPEPTIDE SIGNALLING IN AN ECHINODERM REVEALS EVOLUTIONARILY ANCIENT ROLE IN INHIBITORY REGULATION OF FEEDING PROCESSES *Tinoco AB, Barreiro-Iglesias A, Yañez-Guerra LA, Delroisse J, Zhang Y, Gunner EF, Zampronio C, Jones LM, Egertová M, Elphick MR*	**Reeve, Robyn E** EVOLUTIONARY CONSERVED ACTIONS OF LEPTIN ON IMMUNE FUNCTION ACROSS DEVELOPMENTAL STAGES IN XENOPUS LAEVIS *Robyn E. Reeve, Erica J. Crespi*
13:00-13:10	**Bernier, NJ** USING SCALE CORTISOL CONTENT AS A BIOINDICATOR OF SOCIAL STATUS AND LONG-TERM HEALTH IN WILD FISH *Bernier NJ , Culbert BM, Yin-Liao I, Balshine S, Laberge F*	**Al-Dailami, A** EXPLORING THE ROLE OF GLYCOPROTEIN HORMONES GPA2/GPB5 ON THE DIURETIC PROCESS IN THE MEDICALLY IMPORTANT INSECT, RHODNIUS PROLIXUS *Al-Dailami A, Leyria J, Orchard I, Lange AB*	**Lu, C** A COMPLETE PACKAGE OF METHODS TO SIMULTANEOUSLY EXTRACT AND QUANTIFY SECRETONEURIN, OTHER NEUROPEPTIDES AND STEROID HORMONES FROM SMALL TISSUE SAMPLES USING LC-MS *Lu C, Peng D, Hu W, Trudeau VL*	**Breton, T** CHARACTERIZING THE G PROTEIN-COUPLED RECEPTOR FAMILY SREB (GPR27, GPR85, GPR173), AND A NEW MEMBER (SREB4), ACROSS FISH EVOLUTION *Breton T, Sampson W, Clifford B, Phaneuf A, Smidt I, True T, Wilcox A Lipscomb T, Murray C, DiMaggio M*

13:15-13:25	**Greville, LJS** AGE, SEX, AND SEASONAL COMPARISONS OF URINARY ESTRADIOL IN THE BIG BROWN BAT *Greville LJS, Bueno LM, Pollock T, Faure PA*	**Nguyen, T** CHARACTERIZATION OF RYAMIDE NEUROPEPTIDE AND ITS RECEPTOR IN THE HUMAN DISEASE-VECTOR, AEDES AEGYPTI *Nguyen T, Paluzzi JP*	**Peng, D** CHARACTERIZATION OF THE SECRETOGRANIN-II/SECRETONEURIN SYSTEM IN THE BRAIN-PITUITARY-GONADAL AXIS OF ZEBRAFISH *Peng D, Lu C, Levavi-Sivan B, Hu W, Trudeau VL*	**Baltazar-Lara, R** NEUROPROTECTIVE EFFECTS OF GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR TYPE 1 (IGF-1) AFTER HYPOXIC-ISCHEMIC INJURY IN CHICKEN CEREBELLAR CELL CULTURES *Baltazar-Lara R, Ávila-Mendoza J, Martínez-Moreno CG, Carranza M, Pech-Pool S, Vázquez-Martínez O, Díaz-Muñoz M, Arámburo C, Luna M*
13:30-14:00	**Lunch Break**			
14:00-14:45	**Dr. Jan Mennigen - Gorbman-Bern New Investigator**			
14:45-15:30	**Comparative Endocrinology Closing Mixer - Networking**			
15:30-16:15	**Closing Ceremony and Trainee Awards**			

Lightning Rounds

#13-1 COULD THE IGF-1 ACT AS AN ENDOCRINE MEDIATOR BETWEEN CIRCADIAN OSCILLATORS IN GOLDFISH?
Alonso-Gómez A, Madera D, Alonso-Gómez AL, Delgado MJ, Valenciano AI

#13-2 EVALUATION OF THE NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE NEURAL RETINA
Epardo D, Balderas-Márquez JE, Fleming, Carranza M, Luna M, Harvey S, Ávila-Mendoza J, Arámburo C, Martínez-Moreno CG

#13-3 IS NOCTURNIN EXPRESSION MODIFIED BY DIFFERENT THERMAL CONDITIONS IN GOLDFISH?
Madera D, Alonso-Gómez A, Valenciano AI, Delgado MJ, Alonso-Gómez AL

#13-4 SEX-DEPENDENT MODULATION OF GENES INVOLVED IN GROWTH AND REPRODUCTION BY GROWTH HORMONE AND LUTEINIZING HORMONE IN TILAPIA
Celino-Brady FT, Breves JP, Seale AP

#13-5 LEPTIN SIGNALING STIMULATES PERIPHERAL ANGIOGENESIS DURING XENOPUS LARVAL DEVELOPMENT.
Curtis GH, Reeve RE, Whitfield K, Crespi EJ

#13-6 CHARACTERIZING THE SKIN MICROBIOTA IN RANA CATESBEIANA ACROSS NATURAL METAMORPHOSIS AND HORMONE EXPOSURE
Imbery JJ, Jia B, Van Rossum T, Lo R, Brinkman FSL, Helbing CC

#13-7 NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE CHICKEN RETINA DURING INFLAMMATION
Balderas-Márquez JE, Epardo D, Carranza M, Luna M, Arámburo C, Martínez-Moreno CG

#13-8 CHARACTERIZATION OF METABOLIC DEFECTS IN INSULIN-SIGNALING IMPAIRMENT DROSOPHILA MELANOGASTER
Alvarez-Rendón JP, Riesgo-Escovar JR

#13-9 PHOENIXIN-20 (PNX-20) SUPPRESSES FOOD INTAKE AND PROMOTES GLYCOLYSIS IN ZEBRAFISH
Rajeswari JJ, Blanco AM, Unniappan S

#14-1 FIBROBLAST GROWTH FACTOR 8-DEFICIENT MICE EXHIBIT NORMAL NEONATAL GONADAL MORPHOLOGY AND FUNCTION

Jewell K, Tsai PS

#14-2 THE ROLE OF THE SOMATOTROPIC AXIS IN SOCIAL STATUS-DEPENDENT GROWTH AND METABOLISM IN RAINBOW TROUT

Gilmour KM, Best C, Magnan J, Jennings K, Touma K, Bernier NJ, Mennigen JA

#14-3 CAN FROGS GET ANXIOUS? THE INFLUENCE OF SIMULTANEOUS EXPOSURE TO A PREDATOR AND FOOD ON FORAGING-AVOIDANCE BEHAVIOR AND GENE EXPRESSION IN THE OPTIC TECTUM OF AFRICAN-CLAWED FROGS (XENOPUS LAEVIS)

Menon NM, Carr JA

#14-4 CHARACTERIZATION OF A LEPTIN RECEPTOR ORTHOLOG IN A JAWLESS VERTEBRATE (AGNATHAN)

Gong N, McCormick SD, Sheridan MA

#14-5 A REV-ERBA AGONIST ELICITS STRONG ANORECTIC RESPONSES IN FISH

Saiz N, Herrera-Castillo L, Cebrián A, Villar V, Isorna E, Delgado MJ, de Pedro N

#14-6 INTERACTIONS OF GLUCOREGULATORY HORMONES AND LEPTIN IN THE CONTROL OF GLUCOSE HOMEOSTASIS IN THE MOZAMBIQUE TILAPIA

Russell J. Borski, Courtney A. Deck, and Jamie Mankiewicz

#14-7 IDENTIFICATION AND CHARACTERIZATION OF THE HALLOWEEN AND ECDYSONE-RESPONSIVE GENES IN THE OVARIES OF RHODNIUS PROLIXUS.

Samiha Benrabaa, Ian Orchard, Angela B. Lange

#14-8 THE SYNTHESIS AND RELEASE OF PITUITARY GROWTH HORMONE IS DIFFERENTIALLY REGULATED BY SEVERAL NEUROPEPTIDES AMONG VERTEBRATES

Urban-Sosa VA, Ávila-Mendoza J, Carranza M, Martínez-Moreno CG, Luna M, Arámburo C

#14-9 COMPARISON OF THE PITUITARY AND GONADOTROPINS CELL LOCALIZATION IN TURBOT AND MOUSE

Jia YD, Gao YH, Hong L, Lin JX

#14-10 THYROTROPIN-RELEASING HORMONE (TRH) AND SOMATOSTATIN (SST), BUT NOT GROWTH HORMONE-RELEASING HORMONE (GHRH) NOR GHRELIN (GHRL), REGULATE EXPRESSION AND RELEASE OF IMMUNE GROWTH HORMONE (GH) IN CHICK BURSAL B-LYMPHOCYTE CULTURES

Santiago M Pech-Pool, Laura C Berumen, Carlos G Martínez-Moreno, Guadalupe García-Alcocer, Martha Carranza, Carlos Arámburo, Maricela Luna

#15-1 CORTICOSTEROID DEFICIENT 21-HYDROXYLASE (CYP21A2) KNOCKOUT TADPOLES SURVIVE THROUGH METAMORPHOSIS

Paul B, Shewade LH, Buchholz DR

#15-2 CORTICOSTEROIDS MEDIATE GLUCOCORTICOID ACTIONS IN THE LESSER SPOTTED CATSHARK (SCYLIORHINUS CANICULA)

Cabrera-Busto J, Mancera JM and Ruiz-Jarabo I

#15-3 DIRECT ACTIVATION OF TRNA METHYLTRANSFERASE-LIKE 1 (METTL1) GENE BY THYROID HORMONE RECEPTOR IMPLICATES A ROLE IN ADULT INTESTINAL STEM CELL DEVELOPMENT AND PROLIFERATION DURING XENOPUS TROPICALIS METAMORPHOSIS

Wonho Na, Liezhen Fu, Nga Luu, Yun-Bo Shi

#15-4 TISSUE-SPECIFIC HISTONE VARIATIONS IN PREMETAMORPHIC RANA (LITHOBATES) CATESBEIANA

Kuecks-Winger HN, Thambirajah AA, Helbing CC

#15-5 MITIGATION OF GLYPHOSATE EXPOSURE-INDUCED DEVELOPMENTAL DEFECTS BY VITAMIN-C CO-TREATMENT

Weidman D, Mathewson AS, Killian D, Bhandari RK

#15-6 MITIGATION OF GLYPHOSATE-INDUCED DEVELOPMENTAL DEFECTS IN MEDAKA FISH

Bhandari R, Weidman D, Matthewson A

#15-7 TRANSGENERATIONAL DIFFERENCES IN MEDAKA GUT MICROBIOTA POPULATION INDUCED BY ANCESTRAL BPA EXPOSURE

Guzman E, Wang X, Feng Y, Bhandari RK

#15-8 EFFECTS OF GRANDPARENTAL EMBRYONIC BISPHENOL A EXPOSURE ON THE LIVER OF GRANDCHILDREN OF MEDAKA FISH

Godwin J, Chakraborty S, Bhandari RK

#15-9 INCREASED DE-DIFFERENTIATION OF GONADOTROPIN-RELEASING HORMONE NEURONS IN A FGF SIGNALING-DEFICIENT MOUSE

Andersen ND, Tsai P-S

#16-1 THE INFLUENCE OF CRF ON EVOKED CALCIUM INFLUX IN THE OPTIC TECTUM OF THE SOUTH AFRICAN CLAWED FROG XENOPUS LAEVIS

Wang C, Keyel PA, Carr JA

#16-2 OSMOTIC REGULATION OF TRANSCRIPTION FACTOR MRNA LEVELS IN PROLACTIN CELLS OF MOZAMBIQUE TILAPIA

Malintha GHT, Celino-Brady FT, and Seale AP

#16-3 MOLECULAR CHARACTERIZATION OF CCHAMIDE2 AND DEORPHANIZATION OF ITS RECEPTOR IN THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI

Tan J, Paluzzi JP

#16-4 MECHANISMS OF NEUROPEPTIDERGIC REGULATION OF REPRODUCTIVE PHYSIOLOGY IN STARFISH

Piñon Gonzalez V, Elphick MR

#16-5 ALTERNATION OF HEPATIC GLYCOLYSIS, LIPOGENESIS, AND BLOOD BIOCHEMISTRY IN TIGER PUFFER (TAKIFUGU RUBRIPES) UNDER TWO DIFFERENT CULTURE SYSTEMS

Jia YD, Wang ZY, Li MY, Jing QQ, Zhai JM, Guan CT

#16-6 NESFATIN-1 AND NESFATIN-1-LIKE PEPTIDE STIMULATE PROOPIOMELANOCORTIN SYNTHESIS IN MURINE ATT-20 CORTICOTROPHS THROUGH THE AMP/PKA/CREB SIGNALING PATHWAY

Nasri A and Unniappan S

#16-7 LIGAND-BIAS IN GNRH TRANSDUCTION NETWORKS: ROLES OF RECEPTOR-INTERACTING EFFECTORS IN THE CONTROL OF GOLDFISH PITUITARY HORMONE SECRETION.

Khalid E, Chang JP

#16-8 DOES LEPTIN SIGNALING REGULATE MUCUS SECRETION IN XENOPUS LAEVIS EMBRYONIC EPIDERMIS, A MODEL FOR RESPIRATORY EPITHELIUM?

Kourtnie Whitfield, Robyn Reeve, Grace Curtis, Erica Crespi

#16-9 STARVATION INFLUENCES NOCICEPTION AND SENSITIZATION IN AN INSECT MODEL

Crawford N, Fuse M

Abstracts

Tuesday, May 25, 2021

Session 1: Thyroid hormone action on organ maturation and tissue regeneration (Part 1)

QUESTIONING WHETHER A LACK THYROID HORMONE MEDIATED METAMORPHOSIS IN AMPHIBIANS CORRESPONDS TO A LACK OF POST-EMBRYONIC TRANSITION?

Tribondea A, Sachs LM, Buisine N

Molecular Physiology and Adaptation, Muséum National d'Histoire Naturelle, Paris, France

Amphibian metamorphosis is the striking and spectacular developmental process marking the end of larval development, and by which which an aquatic larva (tadpole) transforms into a terrestrial air breathing animal. This transition has been referred to as the post-embryonic transition. The deep cellular and molecular switch underlie profound anatomical and physiological changes. This also marks an important shift of ecological niche, which illustrate the intricate connection between the endocrine control of gene expression with thyroid hormone signaling and ecology. Most of our current understanding on the endocrine control of metamorphosis and the cascade of molecular regulatory events derive from the anuran Xenopus species. Although all anurans undergo metamorphosis, many urodeles do not, which has been attributed to some sort of defect of thyroid hormone signaling. Does it mean that non-metamorphosing urodeles do not undergo post-embryonic transition? Comparative approaches combining functional genomics and molecular endocrinology showed that a larval tissue of non-metamorphosing species (Axolotl and palmate newt) display the characteristic features of transcriptional response to thyroid hormones (eg. induction of klf9), together with ample tissue remodeling. Strickingly, although the lists of differentialy regulated genes is vastly different between species, they all belong to the same biological pathways. This shows that the lack or delay of metamorphosis does not imply a defect of thyroid hormone signaling. We would like to propose that metamorphosis and post-embryonic development may be uncoupled and thus may correspond to two developmental transitions.

Acknowledgements: Supported by the Muséum scientific program ATM (NB, LS), CNRS PEPs ExoMOD (LS), Muséum pre-doctorate studentship (NB) Muséum post-doctorate fellowship (LS)

A ROLE OF ENDOGENOUS HISTONE ACETYLTRANSFERASE STEROID HORMONE RECEPTOR COACTIVATOR (SRC) 3 IN THYROID HORMONE SIGNALING DURING XENOPUS INTESTINAL METAMORPHOSIS

Yuta Tanizaki, Lingyu Bao, Yun-Bo Shi

Section on Molecular Morphogenesis, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, Maryland, MD, USA

Thyroid hormone (T3) plays an important role in regulating vertebrate developmental, cellular and metabolic processes via T3 receptor (TR). Liganded TR recruit coactivator complexes that include steroid receptor coactivators (SRC1, SRC2 or SRC3), which are histone acetyltransferases, to T3 responsive promoters. The functions of endogenous coactivators during T3-dependent mammalian adult organ development remain largely unclear in part due to the difficulty to access and manipulate late stage embryos and neonates. We use Xenopus metamorphosis as a model for postembryonic development in vertebrates. This process is controlled by T3 and involves drastic changes in every organ/tissue and can be easily manipulated. We have previously found that SRC3 was upregulated in the intestine during amphibian metamorphosis. To determine the function of endogenous SRC3 during intestinal remodeling, we have generated Xenopus tropicalis animals lacking a functional SRC3 gene and analyzed the resulting phenotype. While removing SRC3 had no apparent effect on

external development and animal gross morphology, the SRC3 (-/-) tadpoles displayed a reduction in the acetylation of histone H4 in the intestine comparing to that in wild type animals. Furthermore, the expression of TR target genes was also reduced in SRC3 (-/-) tadpoles during intestinal remodeling. Importantly, SRC3 (-/-) tadpoles had inhibited/delayed intestinal remodeling during natural and T3-induced metamorphosis, including reduced adult intestinal stem cell proliferation and apoptosis of larval epithelial cells. Our results thus demonstrate that SRC3 is a critical component of the TR-signaling pathway in vivo during intestinal remodeling.

Acknowledgements: This work was supported by the intramural Research Program of NICHD, NIH. Y. Tanizaki was supported in part by Japan Society for the Promotion of Science (NIH) Fellowship.

ADAMTS9 IN PRIMORDIAL GERM CELL MIGRATION AND GONADAL DEVELOPMENT IN ZEBRAFISH

Carver JJ (1), He Y (1, 2), Zhu Y (1)

(1) Department of Biology, Howell Science Complex, East Carolina University, 1000 E. 5th Street, Greenville, NC 27858, USA
(2) College of Fisheries, Guangdong Ocean University, Zhanjiang, Guangdong, China

Development of a functional gonad is a complex process that is critical for fertility and reproductive health. Metalloproteinases facilitate organogenesis via enzymatic modification of membrane located signaling molecules and the extracellular matrix (ECM). Adamts9 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 9) is a secreted metalloproteinase that is widely expressed during development. Its sequence and structure are highly conserved from C. elegans to humans. Knocking out orthologs of Adamts9 caused unmigrated and mis-migrated primordial germ cells (PGCs) in the invertebrates C. elegans and Drosophila, respectively. However, the roles of Adamts9 in germ cell migration and gonad development in vertebrates is still unknown partly due to the embryonic lethality of Adamts9 knockout (Adamtst9 KO) mice. Zebrafish Adamts9 KO can survive to adulthood and provide an opportunity for studying Adamts9's role in gonadal development. Our previous study found heavily male biased sex ratios in adult fish, only a few infertile female Adamts9 KO zebrafish could be found at 6-12 months post fertilization(mpf). In the present study, we try to identify early defects in gonad development caused by Adamts9 KO that lead to male sex bias in adult fish. We found the expression of adamts9 begins around the germ ring stage, approximately 7.5 hours post fertilization(hpf), and reached peak levels at 2-3 days post fertilization(dpf). Typically, all PGCs completed their migrations and clustered tightly together in the gonadal ridge by 24hpf in wildtype embryos; however, the migration was delayed at both 15hpf and 24hpf in Adamts9 KO zebrafish embryos. In contrast to invertebrate models, all PGCs were able to reach the gonadal ridge at 48hpf in Adamts9 KO. At 2 weeks post fertilization(wpf), significantly less germ cells were found in Adamts9 KO. By 3wpf, Adamts9 KO had significantly less developing stage I ooctyes. Gonads were significantly smaller in Adamts9 KO at 4 and 5wpf. Our results suggest Adamts9 plays important roles in PGC migration, germ cell growth, gonadal development and sex differentiation in zebrafish.

Acknowledgements: This work is supported by NIH 2R15GM1100461-02 awarded to Y. Zhu.

TEMPERATURE-SENSITIVE COMPONENTS OF THE MOLECULAR MEMORY IN THE AMERICAN BULLFROG, RANA CATESBEIANA

Evans EP, Koide EM, Helbing CC

Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

Thyroid hormones are essential to the development of all vertebrates. In amphibians, rising levels of thyroid hormones (THs) prompt the postembryonic metamorphosis from tadpole to frog. THs are necessary and sufficient for metamorphic development, and complex cascades of gene expression downstream later result in a diverse array of mature tissues. The American bullfrog Rana catesbeiana's metamorphosis is temperature sensitive, as they undergo metamorphosis at 24˚C but not at 4˚C, even in the presence of exogenous THs that would otherwise trigger it. Tadpoles exposed to THs in cold temperatures undergo accelerated metamorphosis when returned to warm temperatures, even when hormones are no longer present. R. catesbeiana retain a "molecular memory" of their exposure to THs that allows them to initiate gene expression programs associated with metamorphosis without executing them until temperatures rise. The objectives of this study are to identify how the transcriptomic response varies between the tailfin and back skin of tadpoles during early metamorphosis, and how this contributes to establishing the organ autonomous molecular memory. Organ culture captures the tissue-specific response to temperature when metamorphosis is induced with T3. RNA was extracted from cultured biopsies and gene expression was quantified with qPCR. Preliminary data shows differential gene expression in tadpoles exposed to different temperature regimes, in a repeated measures design that allows direct comparison between the tailfin and back skin of individuals. It also highlights the importance of thrb, thibz, and klfx and six1 (two novel genes with DNA binding elements) in the TH response under varied temperatures. These genes are important in preserving molecular

memory and prompting downstream changes including apoptosis, remodeling and proliferation. Molecular memory is useful in investigating the effect of temperature on the diverse transcriptional regulators that control metamorphosis.

Acknowledgements: Supported by NSERC Discovery Grant, University of Victoria Master's Fellowship, British Columbia Graduate Scholarship, University of Victoria Graduate Award, Chair in Transgender Studies Master's Degree Research Scholarship.

Session 2: New Frontiers in Endocrine Disrupting Chemicals: From Novel Mechanisms of Action to Monitoring (Part 1)

BIOANALYTICAL TOOLS FOR WATER QUALITY MONITORING: ASSESSING THE OCCURRENCE AND POTENTIAL TOXICITY OF ENDOCRINE DISRUPTING CHEMICALS IN CALIFORNIA WATERS

Alvine C. Mehinto

Southern California Coastal Water Research Project, 3535 Harbor Blvd, suite 110, Costa Mesa, CA 92626

The occurrence of emerging contaminants (also known as CECs) for which little relevant toxicity data is available, continues to pose a challenge for utilities and regulators. In recent years, bioanalytical tools have been proposed to assess complex environmental mixtures and their potential risks to public and ecological health. These cell-based assays are designed to respond to known and unknown chemicals acting via a common mode of action. As such, they offer an integrative approach to complement targeted chemical analyses and toxicity testing. In recent years, the state of California has invested in the optimization and application of these tools to supplement existing monitoring tools. Cell assays, including the estrogen receptor alpha (ERa) and glucocorticoid receptor (GR) assays, have been applied to screen various aqueous matrices across California (e.g. freshwater streams rivers, effluents, recycled water). These studies have shown that cell assays can be used to identify contaminated samples and prioritize sites requiring further chemical and toxicity analyses. More specifically wastewater effluents typically had the highest levels of endocrine activity followed by samples from effluent-dominated rivers, while most streams analyzed showed little to no bioactivity. Cell assay results compared favorably to the targeted chemistry data. Additional research established the link between cell-based and animal/community responses to determine the risks associated with observed bioactivity. Altogether, our findings showed that cell assays can be useful monitoring tools for assessing chemical mixtures and infer their toxicity potential.

EVALUATION OF A TWO-TIER BIOASSAY-BASED APPROACH TO ASSESS ENDOCRINE DISRUPTING CHEMICALS IN WASTEWATER IN QUEBEC

Robitaille J (1), Desrosiers M (2), Guay I (2), Métivier M (2), Veilleux E (2), Langlois VS (1)

(1) Institut national de la recherche scientifique (INRS), Centre Eau Terre Environnement, Quebec City, QC

(2) Ministère de l'Environnement et de la Lutte contre les changements climatiques (MELCC), Quebec City, QC

Endocrine disrupting chemicals (EDCs) are contaminants which can alter the normal function of hormones and can lead to problems of development and reproduction in aquatic wildlife. However, EDCs are not yet regulated in municipal and industrial wastewater in any countries, including in Canada. As an initiative of the MELCC, this project aims to develop a two-tier approach using bioassays to assess EDC effect levels in wastewater focusing on reproductive endpoints. Based on an exhaustive literature review, we selected three in vitro bioassays for the Tier 1: the transactivation assay of the human estrogen and androgen receptors, and the assay of steroidogenesis in H295R cells. For the Tier 2, the fish short term reproduction assay (FSTRA) in fathead minnow was selected to validate any positive scores obtained in the Tier 1. The optimization of each method is being finalized. For the Tier 1, preliminary results will be presented showing the comparison between various methods of preparation of wastewater samples to expose cells in vitro. To decrease the cost and time associated with the FSTRA, we compared a 7-day exposure to the standard 21-day exposure using one concentration of pure chemicals: 17α-ethinylestradiol, 17β-trenbolone, and propiconazole. The impact of each compound on fecundity and vitellogenin levels in plasma was determined and data obtained were similar between both exposure times. This suggests a 7-day exposure would be suitable to identify endocrine disruption activity (EDA) in water samples.

To validate all of this, we are currently comparing side-by-side, the effects of a municipal effluent on the in vitro bioassays (aforementioned) with the 7-day and 21-day FSTRA. Chemical analysis will be done on the effluent daily. This study will determine if the in vitro bioassays can predict the EDA of the effluent on fish and their efficiency in comparison to

analytical chemistry data. This will be the first step in demonstrating the usefulness of those in vitro bioassays to survey EDCs in wastewater in the province of Quebec. When fully operational, those in vitro bioassays could be used to conduct survey of EDCs in wastewater in the province. Data could then inform the different actors on steps to take to manage and mitigate the quality of wastewater being released into Quebec's ecosystems.

Acknowledgements: Supported by the MELCC (VSL), NSERC Discovery Grant (VSL), Canada Research Chairs (VSL), FRQNT scholarship (JR), NSERC-CREATE-PURE scholarship (JR), EcotoQ scholarship (JR).

THE IN VITRO OOCYTE MATURATION ASSAY IS PREDICTIVE OF EX VIVO OOCYTE MATURATION INHIBITION IN ZEBRAFISH (DANIO RERIO) EXPOSED TO ORGANOPHOSPHATE INSECTICIDES

Miller JGP (1), Van Essen D (1), Brinkmann M (2), Raza Y (1), Dubiel J (1), Doering JA (1), Wiseman SB (1,3)

(1) Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada

(2) School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, Saskatoon, Canada

(3) Water Institute for Sustainable Environments (WISE), University of Lethbridge, Lethbridge, Alberta, Canada

Oogenesis is the process by which a primary oocyte develops into a fertilizable oocyte after oocyte maturation occurs, making it critical to successful reproduction in fishes. During successful oocyte maturation, 17α,20β-dihydroxy-4-pregnen-3-one (MIH) is synthesized in follicular cells, released to activate the membrane progestin receptor (mPR), which thereby induces maturation by germinal vesicle breakdown. In vitro, anthropogenic chemicals like pesticides and phthalates can interact with the mPR to inhibit MIH induced oocyte maturation. Using zebrafish as a model organism, the objective of this research was to establish whether assays of in vitro of oocyte maturation are predictive of reproductive performance. Malathion, an organophosphate insecticide known to inhibit MIH induced oocyte maturation in vitro was used as a model chemical. It was established that the magnitude of MIH stimulated oocyte maturation inhibition after in vitro exposure was highly similar to the magnitude of inhibition of ex vivo MIH stimulated maturation of oocytes extracted from fish exposed to waterborne malathion. This identical trend was observed in response to the structurally related organophosphate, dimethoate. To determine whether the in vitro assay is predictive of reproductive performance, female zebrafish were exposed for 21 days to 0, 0.5, 5, or 50 µg/L of malathion or dimethoate to evaluate daily fecundity and fertility. After exposure, oocytes were excised and induced to mature ex vivo. A significant decrease in ex vivo MIH stimulated oocyte maturation was observed after 21-day exposure in all fish exposed to malathion in comparison to control groups. However, exposure to malathion did not impact fecundity or fertilization success. This study increases understanding of oogenesis as a target of chemical stressors, but the link between impairment of oocyte maturation and reproductive performance requires additional research.

Acknowledgements: This study was supported by Discovery Grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to SBW (#704454). Support was also provided by a grant from the Canadian Foundation for Innovation (CFI) and a Canada Research Chair in Aquatic and Mechanistic Toxicology, to SBW (#35224). JAD received support from the NSERC Postdoctoral Fellowships Program. JM received support from Alberta Innovates Technology Futures grant.

DIOCTYL SODIUM SULFOSUCCINATE AS A POTENTIAL ENDOCRINE DISRUPTOR OF THYROID HORMONE ACTIVITY IN AMERICAN BULLFROG, RANA (LITHOBATES) CATESBEIANA, TADPOLES

Corrie LM (1), Kempe MN (1), Blajkevitch O (2), Shang D (2), Helbing CC (1)

(1) Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

(2) Pacific Environmental Science Centre, Science And Technology Branch, Environment and Climate Change Canada, North Vancouver, British Columbia, Canada

Thyroid hormones thyroxine (T4) and triiodothyronine (T3) are required for regulating complex developmental processes in vertebrates, and are highly sensitive to endocrine disrupting compounds. Previous studies demonstrate that dioctyl sodium sulfosuccinate (DOSS), a common constituent of pharmaceuticals, cosmetics, and food products, disrupts canonical signaling of adipocyte differentiation by binding a nuclear hormone receptor in the same superfamily as thyroid hormone (TH) receptors. The present study aims to determine if DOSS is capable of disrupting TH signaling using the American bullfrog, Rana (Lithobates) catesbeiana, a cosmopolitan frog species that undergoes TH-dependent metamorphosis to transition from an aquatic tadpole to a terrestrial juvenile frog. Premetamorphic R. catesbeiana tadpoles were injected with 2 pmol/g body weight T3 or 10 pmol/g body weight T4 to induce precocious metamorphosis, then exposed for 48 hours to environmentally- or clinically-relevant DOSS concentrations (0.5, 5, and 50 mg/L). Gene expression of three

classical TH-responsive targets (thra, thrb, and thibz) was measured in tadpole liver and tail fin tissue through reverse transcription quantitative polymerase chain reaction (RT-qPCR). DOSS disrupted gene expression in liver and tail fin tissue at all three concentrations tested but the patterns of expression differed by tissue, gene transcript, and TH treatment status. To our knowledge, this is the first demonstration that DOSS can alter TH signaling. Further exploration into DOSS disruption of TH signaling is warranted as exposure may affect other TH-dependent processes such as salmon smoltification and perinatal human development.

Acknowledgements: Supported by a NSERC Discovery Grant (CCH).

Session 3: Neuropeptides involved in invertebrate nutritional regulation and reproduction (Part 1)

NUTRITION-DEPENDENT CONTROL OF INSECT REPRODUCTION: THE INVOLVEMENT OF INSULIN/TOR SIGNALLING PATHWAY IN REPRODUCTIVE PERFORMANCE OF RHODNIUS PROLIXUS, A VECTOR OF CHAGAS DISEASE

Leyria J, Orchard I, Lange AB

Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

The blood-sucking Rhodnius prolixus is a vector of Chagas disease, one of the most neglected tropical diseases affecting several million people, mostly in Latin America. The blood meal is an event with a high epidemiological impact since adult mated females feed several times, with each meal resulting in a bout of egg laying, and thereby the production of hundreds of offspring. In insects, insulin-like peptide (ILP) signalling along with the target of rapamycin (ToR) are involved in detecting and interpreting nutrient levels. By means of RNA-Sequencing we have examined how a blood meal influences mRNA expression in key tissues involved in reproduction (central nervous system, fat body and ovaries). Although there is an up-regulation of the genes involved in ILP/ToR signalling in unfed insects, western blot analysis reveals that this signalling is only activated in tissues of fed insects, i.e. phosphorylation of proteins. Immunofluorescence and RNA interference (RNAi) studies suggest that during the unfed condition FoxO signalling may be responsible for the up-regulation of transcripts involved in the ILP/ToR signalling cascade. Moreover, insulin stimulates protein phosphorylation in the fat body and ovaries, suggesting that unfed females are in a sensitized state and respond to food by rapidly activating ILP signalling. Also, by RNAi we show that ILP/ToR pathway is involved in the coordination of the synthesis of yolk protein precursors by the fat body and the storage of carbohydrate by oocytes, thus influencing the numbers of eggs laid. Our study reveals a network of regulatory pathways implicated in reproductive performance. These analyses serve as a starting point for new investigations that increase the chances of developing novel strategies for vector population control by translational research, with less impact on the environment and more specificity for a particular organism.

Acknowledgements: Supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants to A. B. Lange and I. Orchard.

CROSS-TALK OF INSULIN-LIKE PEPTIDES, JUVENILE HORMONE, AND 20-HYDROXYECDYSONE COORDINATING METABOLISM IN REPRODUCING MOSQUITO AEDES AEGYPTI

Lin Ling, Alexander S. Raikhel

Department of Entomology, University of California, Riverside, CA 92521

Female mosquitoes feed sequentially on carbohydrates (nectar) and proteins (blood) during each gonadotrophic cycle to become reproductively competent and effective disease vectors. Aedes aegypti mosquito encodes eight insulin-like peptides (ILPs) that are critical for controlling metabolism. However, a species-specific and stage-dependent outcome with the control of the whole set of ILPs remains to be investigated. Here, we studied interaction between two major developmental regulatory hormones, juvenile hormone (JH) and 20-hydroxyecdysone (20E), and eight mosquito ILPs to decipher regulation of metabolism in reproducing female mosquitoes. Chromatin immunoprecipitation assays showed direct physical interactions between ilp genes and the JH receptor, methoprene-tolerant, a transcription factor, Krüppel homolog 1 (Kr-h1), and two isoforms of the ecdysone response early gene, E74. The luciferase reporter assays showed that Kr-h1 activates ilps 2, 6, and 7, but represses ilps 4 and 5. The 20E pathway displayed the opposite effect in the regulation of ilps. E74B repressed ilps 2 and 6, while E74A activated ilps 4 and 5. Combining RNA interference, CRISPR-Cas9 epitope-tagging and enzyme-linked immunosorbent assay, we have shown that the JH and 20E regulate protein and circulating levels of all eight Ae. aegypti ILPs. Thus, we established a previously unidentified regulatory axis between ILPs, JH and 20E coordinating metabolism during gonadotrophic cycles of the disease vector, Ae. aegypti.

Acknowledgements: Supported by NIH Grant R01AI036959 (to A.S.R.).

IDENTIFICATION OF SOURCES OF RELAXIN-LIKE GONAD-STIMULATING PEPTIDE AS A REGULATOR OF SPAWNING IN STARFISH

Feng YL (1), Piñon Gonzalez VM (1), Egertová M (1) Mita M (2) Elphick MR (1)

(1) School of Biological & Chemical Sciences, Queen Mary University of London, UK

(2) Department of Biochemistry, Showa University School of Medicine, Japan

Oocyte maturation and gamete release (spawning) in starfish is triggered by relaxin-like gonad-stimulating peptide (RGP), a neuropeptide that was first isolated from the radial nerve cords of starfish. However, it is not known if the radial nerve cords are the source of RGP that triggers spawning physiologically. To address this issue, RGP expression in starfish has been investigated using a variety of techniques, including mRNA in situ hybridization, radioimmunoassay and enzyme-linked immunoassays. This has revealed that RGP expression can be detected in the radial nerve cords, circumoral nerve ring, arm tips, tube feet and stomach but not in the pyloric caeca or gonads. To determine more specifically where RGP is expressed in starfish at a cellular/subcellular level, here we used immunohistochemistry to analyse and compare RGP expression in two starfish species: Asterias rubens (order Forcipulatida) and Patiria pectinifera (order Valvatida). In both species, RGP-immunoreactive cells/fibres were revealed in the ectoneural region of the radial nerve cords and circumoral nerve ring, basiepithelial nerve plexus of the tube feet, the terminal tentacle and other arm tip-associated structures and the stomach. Furthermore, RGP expression was also revealed in the gonoducts of both male and female specimens of A. rubens and on-going experiments are investigating if RGP is also expressed in the gonoducts of P. pectinifera. The expression of RGP in the central nervous system, tube feet, arm tips and stomach indicate that this neuropeptide may be involved in regulation of a variety of physiological/behavioural processes in starfish. Furthermore, our discovery that RGP is expressed in the gonoducts of A. rubens proximal to its gonadotropic site of action in the gonads provides an important new insight into the mechanisms by which RGP may act as a regulator of spawning in starfish.

Acknowledgements: Supported by Queen Mary University of London and Chinese Scholarship Council (CSC) joint scholarship, Daiwa Anglo-Japanese Foundation.

IDENTIFYING KEY RESIDUES CONFERRING LIGAND SPECIFICITY AND EFFORTS TO ELUCIDATE THE FUNCTION OF THE ADIPOKINETIC HORMONE/CORAZONIN-RELATED PEPTIDE AND ITS RECEPTOR IN THE MOSQUITO AEDES AEGYPTI

Afifi S, Paluzzi JP

Department of Biology, York University, Toronto, ON, Canada

Neuropeptides regulate an array of physiological processes in insects, including feeding, reproduction, metamorphosis and diapause. The adipokinetic hormone/corazonin-related peptide (ACP) is an insect neuropeptide structurally intermediate between corazonin (CRZ) and adipokinetic hormone (AKH). Despite the structural similarity and the close evolutionary relationship between the ACP and the AKH, their signalling systems function independently. Several studies have characterized the AKH and CRZ signalling systems within diverse insect species, and the most notable functions include energy substrate mobilization and cardio-acceleratory activity, respectively. In contrast, the function of the ACP signalling system remains unclear. In this study, we aimed to localize the distribution of ACP in the Aedes aegypti nervous system using immunohistochemistry. In adult mosquitoes, ACP immunostaining is localized in two pairs of lateral neurosecretory cells in the brain and 2-3 cells in the thoracic ganglia. Extensive ACP-immunoreactive axonal projections emanating within each abdominal ganglia were also observed. These results suggest that ACP might act as a neuromodulator and/or neurotransmitter. Further, we also aimed to determine the specific regions of the ACP receptor (ACPr) most critical for ligand fidelity and specificity by creating ACPr chimera by singly replacing the complete or select highly conserved residues with the ACPr extracellular loops (ECL1, ECL2, and ECL3) and incorporating those from the AKH receptor. To date, heterologous functional assays have determined that three ACPr-ECL mutants receptors with complete replacement showed no response to either ACP or AKH. These results suggest the complete replacement of each extracellular loop is detrimental to ligand recognition. Lastly, while some data from other studies suggest ACP may play a similar role to AKH in the regulation of carbohydrate and lipid levels in the hemolymph, our results show that, unlike AKH, ACP does not influence energy substrate mobilization in the A. aegypti mosquito.

Session 4: Growth, Metabolism, Hormones and Behavior (Part 1)

MORE THAN MEETS THE EYE: SEX-SPECIFIC DIFFERENCES IN METABOLISM AND OXIDATIVE STRESS RESPONSE IN INSULIN SIGNALING COMPROMISED AND CONTROL FLIES

Álvarez-Rendón JP, Riesgo-Escovar JR

Department of Developmental Neurobiology and Neurophysiology, Instituto de Neurobiologia, Universidad Nacional Autónoma de México, Querétaro, México

The insulin intercellular pathway is an evolutionarily conserved signaling pathway, present in the model organism Drosophila melanogaster. Insulin signals availability of glucose in the blood (or hemolymph), product of digestion, but also growth (cell size and cell number) in a general sense. It is a fundamental anabolic signaling pathway. We have characterized several hippomorphic viable mutant combinations of genes coding for proteins required at different levels of the insulin pathway, and we have made use of these different flies to study both metabolic and behavioral phenotypes. We have generated stocks with the same genetic background. The use of a common genetic background minimizes genetic background effects, for both control and mutant strains, allowing direct comparison between different genotypes and experimental conditions.

In particular, we have studied the differences inherent between males and mated or virgin females, and in response to oxidative stress challenges, using both paraquat and H2O2 in the food as stressors. Our results show that significant differences arise between the sexes: whereas females are more sensitive to reduced or abnormally low insulin signaling, presenting lipid and carbohydrate accumulation, mutant males are less affected by the same degree of hypoactivity of the insulin pathway in terms of metabolic differences, but are more sensitive in these compromised insulin signaling conditions to oxidative stress via H2O2 than are females. Paraquat was found not to have a significant effect. This points of the complexity of the response, not only with respect to the sexes, but also with respect to the nature of the oxidative stress applied, and to the fact that these significant differences should be taken into account when analyzing responses and phenotypes.

Acknowledgements: Funding was provided by PAPIIT, #IN207719 and lab budget to J. R. Riesgo-Escovar.

OLFACTION AND REPRODUCTION, ROLE OF PROLACTIN

Corona R, Morales T

Department of Cellular and Molecular Neurobiology, Neurobiology Institute, National Autonomous University of Mexico, Querétaro, Mexico

Chemical communication is determinant for reproduction. Prolactin (PRL) has an important role in the regulation of this communication through its effects on the olfactory bulb (OB). Since embryonic stages, PRL receptor has been found mainly in the mitral cells of the OB, however it is not known whether this hormone can modulate its function. The objective of this work was to evaluate the participation of PRL in 1) reproductive maturation and behaviors and 2) its role in the modulation of OB activity. Female mice that received treatment (Tx) with PRL (5mg / kg) or vehicle during a juvenile period or in the adult stage were evaluated, either to determine the onset of puberty, or in adulthood, the estrous cyclicity and the display of olfactory behaviors. After receiving the Tx described before and prior to sacrifice, adult female mice were exposed to male odors to verify the activation (expression of cFos) of BO cells, additionally, some of these females were used for in vitro field recordings to evaluate the spontaneous electrical activity of the mitral cell layer of the accessory OB. Results: 1) Tx with PRL delayed the onset of puberty, altered the estrous cycle and some behavioral alterations were observed. 2) Tx with PRL changed the activation of granule cells of accessory OB, increasing basal activity. Also, the mitral cell layer activity of the accessory OB showed alterations with PRL Tx selectively in the posterior region. Overall, our results suggest that PRL participates in the establishment of puberty, the maintenance of reproductive responses and modulate the OB function.

Acknowledgements: Supported by National Autonomous University of Mexico DGAPA-PAPIIT IA202218 and IA200820 to RC and IN204718 to TM.

BEHAVIORAL RESPONSES OF A ZEBRAFISH ADRENERGIC RECEPTOR BETA2 RECEPTOR KNOCKOUT MODEL TO ADRENORECEPTOR AGONISTS

Christopher L. Souders II, Christine Larrea, Hunter Davis, David Kim, Jordan T. Schmidt, Jasenka Zubcevic, Christopher J. Martyniuk

Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL United States

Epinephrine and norepinephrine are catecholamines that regulate several physiological processes in animals. In fish, these hormones are released from chromaffin cells of the interrenal gland during stress and bind to adrenergic hormone receptors to increase heart rate and blood pressure, often referred to as the “fight-or-flight” response. Zebrafish (Danio rerio) have one β1AR gene and two β2AR genes (β2aAR and β2bAR) that mediate the effects of epinephrine. The

objectives of this study were to validate a CRISPR/Cas9 knockout model for adrenergic receptor β2a to learn more about this specific isoform and to develop a screen for chemicals and pharmaceuticals that disrupt the sympathetic nervous system. High-resolution melt curve analysis was used to genotype the F2 zebrafish for gene disruption and knockout. To test for potential behavioral differences among genotypes, norepinephrine (non-selective) and procaterol (a selective β2 adrenoreceptor agonist) were administered to larval fish in the water. Heterozygote pairs were first bred, and individual embryos were reared in 96 well plates and exposed to each chemical. Visual Motor Response at 6 dpf immediately following the addition of agonist for 3 hours was performed to test for differences in adrenergic receptor activation among genotypes. Wild type fish exposed to norepinephrine showed elevated dark zone activity compared to untreated fish, however ADRB2-/- individuals exposed to norepinephrine did not differ in activity compared to the other genotypes. Procaterol treatment also did not affect the genotypes differentially, suggesting loss of β2aAR may not negatively affect response to the adrenoreceptor agonists tested. This model once validated is expected to (1) offer new insight into adrenoreceptor activation and tissue responses in fish; (2) act as a high-throughput chemical screen for adrenoreceptor disruption and sympathetic nervous system impairment due to environmental chemicals and pharmaceuticals.

Acknowledgements: Supported by the University of Florida (CJM) and UF Graduate Student Fellowship (CLS)

GROWTH HORMONE (GH) ENHANCES ENDOGENOUS MECHANISMS OF NEUROPROTECTION AND NEUROPLASTICITY AFTER OXYGEN AND GLUCOSE DEPRIVATION INJURY (OGD) AND REOXYGENATION (OGD/R) IN CHICKEN HIPPOCAMPAL CELL CULTURES

Olivares-Hernández JD, Balderas-Márquez JE, Carranza M, Luna M, Martínez-Moreno CG, Arámburo C

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, Querétaro, México

Besides its classical functions as growth promoter and metabolic regulator, growth hormone (GH) also acts as a neurotrophin, being able to induce neuroprotection, neurite growth and synaptogenesis during the repair processes that occur in response to neural injury. After hypoxia-ischemia (HI) insult, the neural tissue activates endogenous neuroprotective mechanisms regulated by local neurotrophins that promote tissue recovery. In this work, we investigated the neuroprotective effects of GH in cultured embryonic chicken hippocampal neurons exposed to HI injury and re-oxygenation. Hippocampal cultures were incubated under oxygen-glucose deprivation (OGD, <5% O2, 1 g/L glucose) conditions for 24 h, and simultaneously treated with GH. Then, cells were either collected for analysis or submitted to re-oxygenation and normal glucose incubation conditions (OGD/R, 95% air, 5% CO2, 4.5 g/L glucose) for another 24 h, in the presence of GH. Results showed that OGD injury significantly reduced cell survival, the number of cells, dendritic length and number of neurites, whereas OGD/R stage restored most of those adverse effects. Also, OGD/R increased the mRNA expression of several synaptogenic markers (i.e. NRXN1, NRXN3, NLG1, and GAP43), as well as the growth hormone receptor (GHR). The expression of BDNF, IGF-1, and BMP4 mRNAs expression was augmented in response to OGD injury, and exposure to OGD/R returned it to normoxic control levels, while expression of NT-3 increased in both conditions. The addition of GH (10 nM) to hippocampal cultures during OGD reduced apoptosis and induced a significant increase in cell survival, number of cells, and doublecortin immunoreactivity (DCX-IR), above that observed in the OGD/R stage. GH treatment also protected dendrites and neurites during OGD, inducing an increase of their outgrowth during OGD/R. Furthermore, GH increased the expression of NRXN1, NRXN3, NLG1, and GAP43 after OGD injury. GH also increased BDNF expression after OGD, but reduced it after OGD/R. Conversely, BMP4 was upregulated by GH after OGD/R. These results indicate that GH protective actions in neural tissue may be explained by a synergic combination between its own effect and that of other local neurotrophins, which together accelerate the recovery of tissue damaged by HI.

Acknowledgements: The authors thank Pilgrim's Pride, Querétaro, México, who donated the fertilized broiler eggs used in this work. Partially supported by PAPIIT-DGAPA-UNAM (IN201817, IN208419, IN227020), and CONACYT (285004, 214971). JDOH and JEBM received a Posdoctoral (261533) and a M.Sc. (921788) fellowships, respectively.

Session 5: Thyroid hormone action on organ maturation and tissue regeneration (Part 2)

SIGNALING PATHWAYS ESSENTIAL FOR THYROID HORMONE-INDUCED ADULT STEM CELL DEVELOPMENT DURING INTESTINAL REMODELING OF XENOPUS LAEVIS

Hasebe T (1), Fujimoto K (1), Buchholz DR (2), Ishizuya-Oka A (1)

(1) Department of Biology, Nippon Medical School, Tokyo, Japan

(2) Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, USA

During amphibian metamorphosis, many organs are extensively remodeled from the larval to adult form. In the Xenopus laevis small intestine, most of the larval epithelial cells are removed by apoptosis, whereas a small number of them dedifferentiate into adult stem cells, which newly generate the adult epithelium analogous to the mammalian one. Since this larval-to-adult remodeling is totally dependent on thyroid hormone (TH), it is worth to analyze the expression and function of TH-response genes to clarify the molecular mechanisms of this process including stem cell development and formation of the stem cell niche. By using the organ culture technique, we have experimentally demonstrated that TH-activated Sonic hedgehog (Shh), bone morphogenetic protein 4 (BMP4), canonical and non-canonical Wnt, and Notch signaling pathways play important roles in the stem cell development. In addition, we have recently shown that Shh pathway activates the expression of the winged-helix transcription factor forkhead box1 (Foxl1) in the connective tissue cells just beneath the adult epithelial stem cells, suggesting the involvement of Foxl1-expressing cells, which have been shown to a critical niche component of the adult mammalian intestine, in the formation of the stem cell niche. In this symposium, we summarize the function of these signaling pathways and discuss the molecular mechanisms of TH-induced adult stem cell development during the vertebrate postembryonic development.

Acknowledgements: Supported in part by JSPS KAKENHI (Grant number 17K07475 to T. H. and 18K06320 to A. I.-O.).

THE CHILLING TAIL OF THYROID HORMONE INDUCED METAMORPHOSIS

Koide EM, Abbott EA, Helbing CC

Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

Throughout phyla, species have evolved to use temperature as a developmental cue. This is true of the bullfrog, Rana catesbeiana, that pauses tadpole metamorphosis into a frog at unfavourable temperatures. In warm temperatures (24°C), thyroid hormone (TH) induces metamorphosis. At 5°C however, this response is completely ablated. Interestingly, when tadpoles exposed to TH at 5°C are shifted to 24°C, an accelerated metamorphosis ensues, even when no more endogenous TH signal remains. This indicates that there is a memory where TH response is initiated but not executed until more permissive temperatures occur. Given that TH acts genomically to induce the metamorphic program, previous targeted analyses have queried whether this phenomenon can be seen on the transcriptomic level. Of the known TH response genes, the transcription factor (TF), TH induced bzip (thibz), was induced by TH at 5°C, where as other early response genes were not affected until transferred to 24°C. Herein we use RNA-seq analysis of cultured R. catesbeiana tail fin to reveal that the molecular memory is more extensive than previously thought, with over 80 genes being differentially affected by TH at 5°C. Gene Ontology analysis shows that an enriched proportion of these regulated genes are TFs including a previously undefined krueppel-like factor with an early but transient regulation. Time course experiments at 5°C and exposure to transcription and translation inhibitors indicate that the regulation of these TFs is not homogenous across transcripts alluding to the complexities of TH early transcriptomic response. We also demonstrate that TFs have an augmented regulation once brought into 24°C after a TH exposure period at 5°C, even without further TH exposure, indicating that there may be a priming at cold temperatures for a more accelerated transcriptomic response when permissive temperatures occur. The findings that a diverse transcriptomic response to TH is established in the molecular memory which primes for an accelerated program when permissive temperature occur indicates that temperature control may be a powerful tool for the study of TH signal induction. Further elucidation of the earliest programs in TH signaling is pivotal given the crucial importance of TH in vertebrate development.

Acknowledgements: Supported by a NSERC Discovery Grant (CCH) and a NSERC CGS-M Graduate Student Award (EMK).

EVOLUTIONARY DIVERGENCE IN TAIL REGENERATION BETWEEN XENOPUS LAEVIS AND XENOPUS TROPICALIS

Shouhong Wang, Yun-Bo Shi

Section on Molecular Morphogenesis, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, Maryland, MD, USA

Tissue regeneration is of fast growing importance in the development of biomedicine, particularly organ replacement therapies. Unfortunately, many human organs cannot regenerate. Anuran Xenopus laevis has been used as a model to study regeneration as many tadpole organs can regenerate. In particular, the tail, which consists of many axial and paraxial tissues, such as spinal cord, dorsal aorta and muscle, commonly present in vertebrates, can fully regenerate when amputated at late embryonic stages and most of the tadpole stages. Interestingly, between stage 45 when feeding begins to stage 47, the Xenopus laevis tail cannot regenerate after amputation. This period, termed “refractory period”, has been known for about 20 years. The underlying molecular and genetic basis is unclear in part due to the difficult to carry out genetic studies in this pseudo-tetraploid species. Here we compared tail regeneration between Xenopus laevis and the

highly related diploid anuran Xenopus tropicalis and found surprisingly that Xenopus tropicalis lacks the refractory period. Further molecular and genetic studies, more feasible in this diploid species, should reveal the basis for this evolutionary divergence in tail regeneration between two related species and facilitate the understanding how tissue regenerative capacity is controlled, thus with important implications for human regenerative medicine.

Acknowledgements: Supported by the intramural Research Program of Eunice Kennedy Shriver National Institute Child Health and Human Development (NICHD), National Institutes of Health (NIH), United states.

DEVELOPMENT OF TYPE 2 AND TYPE 3 IODOTHYRONINE DEIODINASE KNOCKOUT XENOPUS TROPICALIS USING CRISPR/CAS12A GENE EDITING

Mayasich S (1, 2), Degoey P (2), Haselman J (2), Degitz S (2)

(1) ORISE Post-doctoral fellow

(2) U.S. Environmental Protection Agency, Office of Research and Development, Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division, Duluth, MN, USA

Thyroid-mediated metamorphosis of amphibian tissues and organs is regulated in part by the activity of three iodothyronine deiodinase enzymes (dio1, 2, and 3) that catalyze tissue-localized metabolic activation/deactivation of thyroid hormones (THs) by removal of iodine. To understand potential apical effects of specific deiodinase activity loss on metamorphosis, as might occur with chemical inhibitors, we utilized a CRISPR/Cas12a system to induce DNA cleavage in the dio genes at locations near where the catalytic sites would occur in the translated proteins. Experiments separately targeting the Xenopus tropicalis dio2 and dio3 genes were initiated by microinjecting zygotes with gene-specific guide-RNA/Cas12a ribonucleo-protein (RNP) complexes. A rapid, non-invasive swab DNA collection and PCR genotyping method was developed to screen >200 injected tadpoles to separate gene disrupted "crispants" from wildtype (WT) tadpoles. Putative mutations occurred at a rate of >60% in dio2-RNP-injected tadpoles, and 50% in dio3-RNP-injected tadpoles screened at 3 weeks post-injection. Dio2 crispants reached Nieuwkoop and Faber (NF) developmental stage 62 earlier than controls and abnormalities included delayed limb development and asynchronous metamorphosis. In dio3 crispants a high level of mortality (83%) was observed beginning as the tadpoles reached prometamorphic stages. Tadpoles evaluated shortly after death displayed precocious tail and gill resorption at early stages of prometamorphosis, which suggests suffocation was the likely cause of death. Surviving crispant founder (F0) animals were recently bred (F0×F0, and F0×WT) and spawns are currently being evaluated for germline transmission of mutant genes. Homozygous mutants will be utilized to characterize TH profiles and definitive apical outcomes.

Acknowledgements: This work was funded wholly by the U.S. Environmental Protection Agency.

Session 6: New Frontiers in Endocrine Disrupting Chemicals: From Novel Mechanisms of Action to Monitoring (Part 2)

MULTI-GENERATIONAL METABOLOMICS OF BISPHENOL-A EXPOSURE IN C57BL/6J MICE: GENDER AND LIFE-STAGE SPECIFIC EXPOSURE EFFECTS AND CONNECTIONS WITH THE CIRCADIAN CLOCK

Bottalico LN (1, 2), Bansal A (3), Briskin A (2), Malik D (2), Sengupta A (2), Garai S (2), Grant G (2), Simmons R (1, 4, 5), and Weljie AM (1, 2)

(1) Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

(2) Department of Systems Pharmacology and Translational Therapeutics, Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

(3) John Curtain School of Medical Research, Australian National University, Canberra, Australia

(4) Center for Research on Reproduction and Women's Health, University of Pennsylvania, Philadelphia, PA, USA

(5) Division of Neonatology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

Endocrine disrupting chemicals disrupt hormone action and are linked to development of metabolic disease. Bisphenol A (BPA) is a high production volume chemical used in manufacture of polycarbonate plastics and epoxy resins. While rodent

models demonstrate BPA-induced impacts on body weight, pancreatic function, glucose homeostasis and insulin signaling, specific molecular mechanisms of BPA-induced diabetes and obesity related outcomes remain to be elucidated. In parallel, the circadian clock is a critical regulator of metabolic homeostasis. While extensive crosstalk exists between circadian and endocrine systems, the potential for EDCs to disrupt circadian-driven cellular metabolism or physiology is not well characterized. A mass spectrometry-based metabolomics workflow was utilized to assess biochemical impacts induced by BPA in a multi-generational maternal exposure model in which pregnant dams were subject to dietary low-dose BPA exposure. 340 aqueous phase metabolites were profiled from liver and adipose tissue harvested from C57BL/6J mice. Multivariate modeling revealed generation, gender, and dose-specific metabolic fingerprints of BPA exposure in liver and adipose tissues, and an acute metabolic signature of gestational BPA exposure in male and female neonate liver. Across datasets, BPA perturbed a range of redox, nutrient and energy metabolism-related biochemical pathways and altered abundance of metabolites previously characterized as circadian. In particular, a life-stage dependent exposure impact was observed in gestationally-exposed male and female mice in which significant alteration of nucleic acid metabolism-related pathways was observed in early life, which shifted to a predominant impact on amino acid metabolism-related pathways in adult mice. BPA-induced biochemical pathway impacts warrant further investigation as these pathways are important for nutrient cycling and energy homeostasis and have a strong aspect of circadian regulation.

Acknowledgements: This work was supported by pilot grant NIEHS P30-ES013508 (AMW and RS), NIDDK R01-DK120757 (AMW) and fellowship funding NIEHS T32-ES019851 and NIGMS K12-GM081259 (LNB).

HERITABLE EFFECTS OF ENDOCRINE-DISRUPTING CHEMICALS IN MEDAKA

Wang X (1, 2), Feng Y (1), Chakraborty, S (1), Bhandari RK (1)

(1) Department of Biology, University of North Carolina at Greensboro, NC 27412, USA

(2) School of Life Sciences, South China Normal University, Guangzhou, China

Environmental chemical exposures can elicit heritable health effects, primarily when they occur during sensitive windows of embryonic development. Environmentally induced phenotypic traits have been found to be inherited by subsequent generations even after the exposure occurred several generations ago. The effects that develop in the absence of exposure but result from ancestral exposure are called transgenerational health effects. Transgenerational health effects in humans are not clearly understood; however, the studies from non-human studies suggest that chemicals can leave exposure-specific epigenetic marks on germline cells that are transmitted to subsequent generations skipping epigenetic reprogramming of the embryo resulting in adverse health outcomes. Multigenerational health effects, which are caused by direct contact with the chemical, seem to be different from transgenerational health effects. We are studying mechanisms underlying the developmental origins of adult and transgenerational adverse health outcomes using Japanese medaka (Oryzias latipes) as an animal model. Embryonic BPA (100 ug/L), EE2 (0.05 ug/L), and atrazine (5 ug/L) exposure did not induce phenotypic abnormalities in F0 males in adulthood, whereas a significant reduction in fertility was observed in F2 generation males. Subsequent epigenetic and transcriptomic analysis of the BPA exposed lineage revealed a significant increase in androgen receptor alpha (AR alpha) promoter methylation in primordial germ cells (PGCs) and reduction in AR alpha expression in testicular somatic cells of the F2 males. Epigenome-wide analysis of PGCs and sperm of the father and somatic cells of the offspring uncovered DNA methylation dynamics during epigenetic inheritance suggesting a germline to soma transfer of epimutations in the F1 and F2 generation. In this talk, I will discuss the importance of epigenetic reprogramming, present epigenetic marks of the ancestral BPA, EE2, and atrazine exposure that are transmitted to offspring across two subsequent generations, and transcriptional pathways linked to these epigenetic alterations which are potentially useful for the prediction of adverse health outcomes.

Acknowledgements: Supported by NIH (R21ES027123, R21HD098621), USGS G15AS00092, UNC Greensboro Internal Grant to RKB

EFFECTS OF EXPOSURE TO DELTA-9 TETRAHYDROCANNABINOL (THC) ON PATERNAL REPRODUCTIVE TISSUE AND OVERALL HEALTH OF OFFSPRING IN MEDAKA, ORYZIAS LATIPES

Feng Y (1), Wang X (1,2), Bhandari RK (1)

(1) Department of Biology, University of North Carolina at Greensboro, NC 27412, USA

(2) College of Life Sciences, South China Normal University, Guangzhou 510631, China

Marijuana is the most used illegal drug in the United States, and its use has been associated with many health issues. Epidemiological studies suggest that consumption of marijuana leads to neurological, respiratory, urogenital, and immune health abnormalities. After the legalization of marijuana for recreational and medicinal use in the United States, more and

more young adults of child-bearing age have legal access to it. Research suggests that THC and other cannabinoids may be useful for the treatment of pain, nausea, epilepsy, obesity, and several human health conditions; however, its harmful effects on reproductive health are not clearly understood. Cannabis or delta-9 tetrahydrocannabinol (THC, primary psychoactive constituent of cannabis) has been found to induce significant changes in sperm methylome; however, whether these alterations lead to abnormal health outcomes in the offspring are currently unknown. To determine paternal THC exposure effects on offspring reproductive health, we exposed reproductively active male medaka (d-rR) fish to 0, 30, 120, and 600 ug/L THC for 21 days which overlaps with their complete spermatogenic cycle. The lower two concentrations are within the human-relevant concentrations. Paternal THC exposure led to delayed hatching in the derived offspring. THC exposure altered the expression patterns of genes related to metabolism, neuron-specific RNA splicing mechanism, and myocyte formation in the gonads of the exposed father. Fertilization efficiency of the offspring born with the 30 µg/L exposed father showed a significant decrease compared to control and other offspring born with exposed father. Analysis of transcriptome and epigenome data from derived offspring is still in progress, the current data shows a significant impact of THC exposure on the somatic tissues of the fathers and fertility of the male offspring.

EFFECT OF POTASSIUM PERCHLORATE ON DEVELOPING MEDAKA PRIMORDIAL GERM CELLS

Reh B (1), Feng Y (1), Wang X (1,2), Bhandari RK (1)

(1) Department of Biology, University of North Carolina Greensboro, Greensboro, North Carolina

(2) College of Life Sciences, South China Normal University, Guangzhou, China

Perchlorate is a manufactured chemical compound commonly used in military artillery and equipment. It has been detected in drinking water, air, soil, and breast milk. Human exposure can occur in the theatre of war and areas adjacent to military training grounds. Higher concentrations of perchlorate have been found to affect reproductive system and whether lower environmentally realistic concentrations affect stem cells that produce sperm and eggs, also called primordial germ cells (PGCs), is not clearly understood. In the present study, I examined the effects of 0, 10, 100, and 1000 ug/L potassium perchlorate exposure on PGCs of medaka embryos and expression pattern of the genes that maintain PGC integrity and epigenetic processes. Expression of gene encoding germ cells nuclear antigen (gcna) and DNA methyltransferases (dnmt) was determined by qPCR. Perchlorate exposure delayed hatching time, reduced heartbeat, inhibited proper migration of PGCs, increased developmental deformities, and reduced body growth of embryos. Expression of dnmt3aa and dnmt3bb genes increased in PGCs of the 10 ug/L group; whereas expression of gcna showed a dose-dependent increase. The results suggest that perchlorate affects germ cell nuclear integrity and epigenetic modulators in PGCs and provide insights into possible perchlorate effects on germline stem cells in humans.

Acknowledgements: Funded by Department of Biology, Lloyd International Honor College, and UNCG-STAMPS program, UNC Greensboro

Session 7: Neuropeptides involved in invertebrate nutritional regulation and reproduction (Part 2)

CROSS-TALKS BETWEEN NUTRIENTS, NEUROENDOCRINE SYSTEM AND BIOLOGICAL CLOCKS IN THE REGULATION OF DIGESTION, FORAGING, CELL PROLIFERATION AND REPRODUCTION IN THE COCKROACH, PERIPLANETA AMERICANA

El-Gendy A (1), Mikani A (2), Kamrzzaman ASM (3), Yoshida Y (3), Mohamed AA (1), Makio Takeda (3)

(1) Department of Entomology, Faculty of Science, Cairo University, Giza 12613, Egypt

(2) Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran14115-336, Iran

(3) Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan

Cruiser type foragers like cockroaches depend on the encounter with foods and water for many aspects of life. Otherwise they must endure starvation to that they are well-adapted, like storage excretion in uric acid in the fat body and symbiotic blattabacterium to sustain their life for a month without food and water. This endurance mode quickly shifts to digestion, growth and reproduction when fed by means of sensing of nutrients by the midgut at microvilli. The midgut paraneurons or enteroendocrine cells releases CCAP into the hemocoel that reaches the brain and stimulates a massive release of CCAP from the CC to stimulates digestive enzymes from the enterocytes as a big feed-forward loop. The brain regulates behavior because the injection of CCAP stimulates sleep and peristaltic contraction of the digestive tract. CCAP also stimulates reproduction, since its injection stimulates vitellogenin synthesis. Upon starvation the stimulatory pathway is geared to a reversal direction and this time sNPF plays a key role. The injection of sNPF into the fed roach stimulated foraging and it

shut down the digestion and reproduction just like a Yin/Yang system. We next made immunohistochemical localizations of both CCAP-ir and sNPF-ir. Quite surprisingly we found these reactivities in the same cells, meaning the system operates as a negative autocrine feed-back loop, namely CCAP works a feed-forward switch to release CCAP on one hand but also as a negative feed-back switch to shut down sNPF release via CCAP-receptor located on the same endocrine cells and the reverse course occurs when starvation stimulates sNPF that shut down CCAP axis via sNPF receptor. Cross-talk gets more complicated because feeding/starvation affects aaNAT that alters a dynamic balance of serotonin (5HT) and downstream indolamines. Injection of 5HT stimulates cell proliferation. BrdU uptake was suppressed by starvation, while the latter stimulated transcription of aaNAT. On the other hand, starvation suppressed reproduction. Then, what is the role of JH or 20E that some consider as a primary regulator in reproduction? The injection of indoleamines or CCAP/sNPF stimulated/ suppressed JH and ecdysteroidogenic enzymes, meaning that JH/20E pathways are downstream of peptides and biogenic amines pathways. Also, these cross-talks are interlocked with the circadian system. aaNAT is a clock-controlled gene and both Vg1 and Vg2 have E-boxes. In conclusion, complex cross-talks occurs at many organ levels, e.g., the brain, stomatogastric nervous system, midgut, fat body, and gonads, and involved in biogenic amines, peptides as well as nutrients.

FUNCTIONAL AUTHENTICATION AND KNOCKDOWN OF A GASTROPOD ADIPOKINETIC HORMONE RECEPTOR IN THE SEA HARE

Tsai P-S, Laphyai P, Zhang H, Sanders KN

Departments of Integrative Physiology, University of Colorado, Boulder, CO, USA

Adipokinetic hormone (AKH) is a member of the gonadotropin-releasing hormone (GnRH) superfamily. AKH is widely distributed in ecdysozoans to regulate diverse functions including metabolism, stress, and reproduction, but its presence and function in lophotrochozoans are less understood. Our laboratory has previously identified a gastropod AKH in the sea hare, Aplysia californica, and found that this AKH (ac-AKH) suppressed feeding and induced a rapid weight loss due to an acute loss of hemolymph volume. Through data mining and validation by cloning, we have identified a full-length putative ac-AKH receptor (ac-AKHR) from the central nervous system (CNS) of A. californica. The goals of the present study were to functionally authenticate this receptor as an ac-AKHR and explore its functional roles through knockdown studies. The putative ac-AKHR was cloned into the pcDNA3.1+P2AeGFP expression vector, transfected into Chinese Hamster Ovary (CHO) cells, and tested in a radioreceptor assay (RRA) with an iodinated ac-AKH as a radioligand. In addition, transfected CHO cells were stimulated by ac-AKH and assessed for changes in inositol phosphate (IP) accumulation. Ac-AKHR-transfected cells exhibited specific binding displaced by unlabeled ac-AKH, but not ac-GnRH. In addition, ac-AKHR-transfected cells responded to the addition of ac-AKH with increasing accumulation of IP1. Lastly, we performed double-stranded RNA (dsRNA)-mediated knockdown (KD) of ac-AKHR in A. californica. Our results showed that this approach successfully knocked down 70% of ac-AKHR transcript. Although animals injected with target dsRNA did not exhibit significant changes in body weight, they showed diminished responsiveness to ac-AKH and increased hemolymph osmolality. Overall, this study has authenticated, for the first time, a molluscan AKHR. In addition, the KD studies corroborated the previous injection studies to suggest that ac-AKH may play a significant role in the volume/hemolymph regulation.

Acknowledgements: Supported by NSF Grant 1352944 to PST.

IDENTIFICATION OF A TACHYKININ RECEPTOR AND ITS IMPLICATION IN METABOLISM IN RHODNIUS PROLIXUS, A CHAGAS DISEASE VECTOR

Haddad A, Leyria J, Lange AB

Department of Biology, University of Toronto Mississauga, Canada

Neuropeptides and their receptors are fundamentally important for regulating many physiological and behavioural processes in insects, including in the Chagas disease vector Rhodnius prolixus. In this work, we have identified, cloned and sequenced the tachykinin receptor from R. prolixus (Rhopr-TKR) fifth instars. The receptor is a G protein-coupled receptor belonging to family A. The total length of the open reading frame of cDNA of Rhopr-TKR is 1014 bp, which translates into a receptor of 338 amino acids with seven transmembrane domains. Sequence analyses show high similarity and identity between Rhopr-TKR and other cloned invertebrate and vertebrate tachykinin receptors. Transcript expression level of Rhopr-TKR is highest in the central nervous system (CNS), followed by the fat body, an interchanging center remotely integrating with the CNS to regulate nutritional signals, suggesting a role of Rhopr-TKR in metabolism. Fluorescent in-situ hybridization for the Rhopr-TKR transcript shows a signal in a group of 4 bilaterally paired neurons in the protocerebrum of the brain. Using RNA interference, we generated insects with transcript knockdown of Rhopr-TKR to examine the Rhopr-TK signaling pathway's role in lipids and carbohydrates metabolism during the first 24 h after a blood meal. Knockdown of

Rhopr-TKR led to a decrease in the size of the blood meal and a significant increase in hemolymph carbohydrate and lipid levels. Further investigation revealed that those insects in which the Rhopr-TKR transcript had been knocked down had decreased transcripts levels for the R. prolixus insulin-like peptide (Rhopr-ILP), R. prolixus insulin-like growth factor (Rhopr-IGF) and R. prolixus insulin receptor (Rhopr-InR) in both the CNS and fat body. Taken together, these findings suggest that Rhopr-TKR interacts with the insulin-signaling pathway and is involved in regulating lipid and carbohydrate metabolism and mobilization.

Acknowledgements: This work was supported by an NSERC Discovery Grant to ABL.

HOST QUALITY INDUCES PHENOTYPIC PLASTICITY IN THE BROWN RICE PLANTHOPPER

Xinda Lin (1), Yili Xu (2), Jianru Jiang (2), Mark Lavine (2), Laura Corley Lavine (2)

(1) College of Biotechnology & Bioengineering, Zhejiang University of Technology, Hangzhou, China 310014

(2) College of Life Sciences, China Jiliang University, Hangzhou, China, 310018

(3) Department of Entomology, Washington State University, Pullman, WA, 99164-6382, USA

Any change in food quality is a critical environmental condition that impacts an animal's growth and development. Many insects facing this challenge have evolved a phenotypically plastic, adaptive response. For example, many species of insect exhibit facultative wing growth which reflects a physiological and evolutionary trade-off between dispersal and reproduction, triggered by environmental conditions. An important unsolved problem in evolutionary ecology is what the environmental cues are and how these cues are transduced to produce these alternative forms, and their associated ecological shift from dispersal to reproduction. In this study, we investigated the role that host quality has on the induction of wing development in the brown rice planthopper, a wing polyphenic insect exhibiting strong trade-offs in investment between dispersal and reproduction. As rice plants grow, the short-winged brown planthopper dominates the population but a shift occurs as the plants mature and senesce in the field such that long-winged brown planthoppers emerge and migrate to more suitable hosts. It remains unknown how changes in the rice plant induce development of the long wing morph, despite recent discoveries on the role of the insulin and JNK signaling in the control of wing morph development. In this study, we found that by mimicking the glucose concentration of senescing rice plants we significantly increased the proportion of long-winged female planthoppers. We also found that the effects of glucose on wing morph are additive with previously described effects of density. Our results show that host quality both directly regulates phenotypic plasticity and interacts with other factors such as density to produce the appropriate phenotype for these specific environmental conditions.

Session 8: Growth, Metabolism, Hormones and Behavior (Part 2)

BRAIN-DERIVED NEUROTROPHIC FACTOR INDUCES PRODUCTIVE OPTIC NERVE REGENERATION IN MICE DEFICIENT FOR KRÜPPEL-LIKE FACTORS 9 AND 13

Ávila-Mendoza J (1,2), Jessy D. Martinez (1), Sara J. Aton (1) and Robert J. Denver (1)

(1) Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, USA

(2) Current address: Department of Cellular and Molecular Neurobiology, Institute of Neurobiology, Universidad Nacional Autónoma de México, Querétaro, México

Several Krüppel-like factors (KLFs) have been shown to promote and maintain the differentiated state of neurons, and are implicated in the loss of regenerative capacity of adult mammalian neurons. Our previous work found that the paralogous KLFs 9 and 13 inhibit axon growth in hippocampal neurons, acting in part by repressing growth factor signaling pathways that act via cyclic AMP. Here, we investigated if loss of one or both KLFs affects brain-derived neurotrophic factor (BDNF)-induced axon regeneration in vivo using the mouse optic nerve crush (ONC) model. We administered intravitreal injections of the recombinant adeno-associated virus AAV2-Bdnf or AAV2-Egfp (control) in wild type (WT), Klf9-/-, Klf13-/- or Klf9-/-/Klf13-/- mice. Twenty one days later we conducted ONC, then 2 weeks later injected cholera toxin B subunit intravitreally to label axons, euthanized the animals 2 days later and conducted histochemistry. Ectopic BDNF expression had no effect in WT, but strongly enhanced axon regeneration in mice of each of the Klf deficient genotypes; this effect was largest in Klf13-/- mice. We next determined if regenerated axons were integrated into a functional neural circuit between the eye and the primary visual cortex (V1) by analyzing changes in ARC immunoreactivity in V1 after a novel visual stimulus. We found that optic nerve function, measured as an increase in ARC immunoreactivity in V1, was partly recovered in Klf13-/-

mice that had received AAV2-Bdnf, but not AAV2-Egfp before ONC; no functional recovery was seen in WT mice. Our findings show that BDNF can promote productive optic nerve regeneration in the absence of Klf9 and Klf13, and suggest that these Klfs can be targeted to promote growth factor induction of axon regeneration in adult mammalian neurons.

Acknowledgements: Supported by NIH grant R21 5R21 NS088062-02 and the College of LS&A at the University of Michigan to RJD. JAM was supported by a CONACYT postdoctoral fellowship, CVU-267642.

T3 EXERTS EARLY EFFECTS UPON MYELINATION IN THE ZEBRAFISH

Iván Lazcano, Yasmin Hernández Linares, Patricia Villalobos, Aurea Orozco.

Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Querétaro, México

During development, thyroid hormones (THs) are key during the myelination process by promoting the maturation of oligodendrocyte (OL) precursor cells (OPCs) as well as by regulating the expression of genes that encode for myelin structural proteins, which together lead to the myelination of neuronal axons. Given that the mechanisms involved in TH-dependent myelination are far from being elucidated, and that myelin formation is a process conserved from fish to mammals, we took advantage of the zebrafish model to counteract some questions about TH role in myelination. Zebrafish OPCs are present as early as 30 hours post-fertilization (hpf) and myelinating OL are functional after 72 hpf. The onset of TH responsiveness and its temporality have not yet been determined. With this in mind, we studied the effect of a single administration of T3 (0.025 nM) applied to F0 mbp:egfp transgenic zebrafish at different time points within the following 3 days post-fertilization (dpf) and followed the effect of T3 upon CNS myelination. Control group wild type uninjected or mbp:egfp inyected zebrafish were grown in E3 medium alone, and three different experimental groups were analyzed: T3 administered at 0 hpf (treatment 1), at 24 hpf (treatment 2) and at 48 hpf (treatment 3). In all cases, animals were sacrificed at 72 hpf and used for gene expression quantification, confocal microscopy and histology analysis. When compared to controls, only plp2, a gene associated to myelination was positively regulated in treatments 2 and 3 correlating with an increase of mbp:egfp signal in the brain of transgenic zebrafish. Interestingly and in contrast to these observations, only zebrafish exposed to treatment 1 showed a marked decrease of fluorescent signal, although no changes in the expression of the genes associated to myelination were observed. Since myelin synthesis starts after 60-72 hpf, it is possible that T3 could modify the fate of OPCs in the CNS. This was studied through immuno-staining techniques to identify neurons, radial glial cells and OPCs. Together, our results show that the positive effect of T3 on myelination depends on the developmental stage of the larvae, being detrimental when administered at the onset of zebrafish development, when the fate of OPC development and maturation could be disarrayed.

Acknowledgements: This work was supported by a grant from PAPIIT IN204920.

EFFECTS OF THYROID HORMONE ON FEEDING BEHAVIOR AND THE EXPRESSION OF APPETITE REGULATING NEUROPEPTIDES

Deal CK, Volkoff H

Departments of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

There is poor evidence for an association between thyroidal state, feeding and appetite regulation in fish. We assessed how an altered thyroid state influences feeding behavior, food intake and expression of hypothalamic appetite-regulating peptides (Klotho-α and Klotho-b; orexin, OX; cholecystokinin, CCK; agouti-related peptide, AgRP; cannabinoid receptor 1, CB1), and levels of total thyroxine (tT4) and total triiodothyronine (tT3) in goldfish. To achieve contrasting thyroidal conditions, we implanted goldfish with propylthiouracil (PTU) or T4 osmotic pumps and administered these continuously over 12 days. T4-implanted fish showed increased feeding behavior but not food intake, while PTU did not alter either. There was a poor association between an altered thyroid state and appetite regulators. We show a novel role for the Klotho protein in the hypothalamus, as its expression is downregulated under a high thyroid load, indicative of a decreased metabolic state. CCK expression was downregulated when peripheral THs were increased, suggesting a blunted hypothalamic response to regulate energy balance. AgRP, OX or CB1 were not affected by thyroidal state relative to controls. In consensus with other studies, PTU does not appear to be a sensitive thyroid inhibitor to create hypothyroid conditions in fish. Overall, we show that unlike in mammals, hyperthyroid conditions in goldfish do not lead to an increased desire or need to consume food, furthering evidence for a weak link between the thyroid and appetite.

Acknowledgements: Supported by an NSERC Discovery Grant (HV)

METABOLOMICS INVESTIGATION OF SEASONALLY RELATED CHANGES IN MALE GOLDFISH (CARASSIUS AURATUS) LIVER

METABOLISM

Ladisa C, Ma Y and Habibi HR

Department of Biological Sciences, University of Calgary, Calgary, Alberta Canada

Many teleost fish species such as goldfish are seasonal breeders displaying distinct reproductive and growth phases. Seasonal variations are controlled by hormones of brain-pituitary-peripheral axes and are accompanied by significant metabolic changes. Availability of metabolic energy is essential to support reproductive and growth processes. The main concept is that when an animal is reproducing it cannot energetically sustain maximal growth, and vice-versa. This shift is particularly important in oviparous species, such as fish, because of the substantial energy investment needed during the gametogenic process both in males and females. The main objective of this study was to investigate changes in metabolic profile and energy allocation patterns at different stages of reproduction, using male goldfish as a suitable model organism and LC-MS as analytical platform for metabolomics analysis. Emphasis is placed on changes in liver metabolic pathways to energetically sustain the physiological processes related to growth and reproduction. Results show a significant shift in the metabolic profile of male goldfish sampled during three stages of the seasonal cycle. In the regressed gonadal season, corresponding to the peak of the growth phase, carbohydrate and nucleotide metabolism were enhanced in support of anabolic processes whereas, during mid gametogenesis, energy was channeled towards lipid metabolism to sustain gonadal maturation. Late gametogenesis stage was characterized by an enhanced ketone bodies synthesis revealing the importance of these metabolites in the final stages of testis maturation.

The findings provide novel information and a framework for better understanding of the hormonally induced changes in metabolism to energetically sustain growth and reproduction in fish and other oviparous species undergoing seasonal cycle.

Acknowledgements: Supported by NSERC grants to HRH.

Wednesday, May 26, 2021

Session 9: Non-invasive methods to measure corticoids and sex steroids in domestic animals and wild fauna

NON-INVASIVE HORMONE MONITORING TO STUDY THE ENDOCRINOLOGY OF DOMESTIC, ZOO AND WILDLIFE SPECIES

Manel Lopez-Bejar

Department of Animal Health and Anatomy, Veterinary Faculty, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain; and College of Veterinary Medicine, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766, USA

The assessment of the endocrine status can provide valuable information of the individual welfare, sexual and health states and be important to estimate its resilience to adapt to the environment or management conditions. The use of non-invasive matrixes, such as saliva, feces, hair, feathers, scales, etc., as alternatives to blood samples, are being increasingly used for the hormonal monitoring in mammals, birds, reptiles and fishes. Glucocorticoids (GC) are steroid hormones secreted as an end product of the hypothalamic-pituitary-adrenal (HPA) axis in mammals, birds and reptiles or the hypothalamic-pituitary-interrenal axis in fish. Although GC are typically considered “stress hormones”, their actions will largely depend on a dynamic and complex profile of ultradian, circadian and stress reactive circulating levels of the hormone. At low or resting levels, GC have the continuous role of maintaining several life tasks such as growth, development, reproduction and disease resistance. Stress results when GC values increase above baseline levels in response to unpredictable or challenging events, also referred to as stressors, with the primary function to overcome the threat. Monitoring the HPA axis through non-invasive methods is useful to understand how handling, health and social conditions may affect the physiological status of domestic or zoo animals. The measurement of GC or its metabolites has an increased use in evaluating habitat quality and environmental impacts in free-living organisms. The non-invasive matrixes show also an important potential in the evaluation of the ovarian function and endocrine disorders based on serial analyses of sex hormones. Non-invasive hormone monitoring has become an ideal approach to study the endocrinology of domestic, zoo and wildlife species to assess their individual or collective welfare, sexual and health status, and also a potential instrument to assess the health state of their habitat or environmental conditions.

NOVEL INSIGHT INTO FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS) REPRODUCTIVE PHYSIOLOGY:

IMPLICATIONS FOR CONSERVATION BIOLOGY

Brammer-Robbins E (1,2,4), Nouri MZ (1,2), Denslow N (1,2) Larkin I (4), Martyniuk CJ (1,2,3)

(1) Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

(2) Center for Environmental and Human Toxicology, University of Florida, Gainesville, Florida, USA

(3) Genetics Institute, University of Florida, Gainesville, Florida, USA

(4) Aquatic Animal Health Program, Veterinary Medical CenterCollege of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

Determining reproductive status and overall health of Florida manatees (Trichechus manatus latirostris) is critical for species conservation. While there are some data on major sex hormones (e.g., 17β-estradiol and testosterone) in this species; no information exists for other hormones such as aldosterone and 17-OH-progesterone. We developed an LC MS/MS based approach to measure sex steroid hormones, cortisol, and aldosterone, in the blood of wild manatees. Plasma samples were collected from 8 male and 13 female healthy adult manatees from Crystal River, FL (2010-2019). For this study, the sample cohort was restricted to healthy adults. Morphometric data were collected on both male and female manatees. Plasma hormones measured included cortisol, aldosterone, progesterone, 17- hydroxyprogesterone, estrone, 17β-estradiol, testosterone, and dihydrotestosterone. Morphometric assessment showed that pregnant females were significantly heavier in weight and had higher body condition scores overall. As expected, pregnant females had significantly higher mean levels of progesterone (six-fold higher) compared to unpregnant females and males and higher levels of cortisol. Plasma aldosterone was not different among females or males. We were able to detect both T and DHT in some male manatees and 17-OH progesterone, was detectable in some animals of each group. Estradiol and estrone were not detectable. Pregnant females may have increased stress levels due to pregnancy or possibly due to factors related to habitat. Based on the body condition data, it is unlikely that the increased stress is associated with a lack of nutrition. Progesterone was a strong indicator of pregnancy, although non-pregnant manatees showed high variability in progesterone concentrations. Physiological health information is crucial to informing management efforts; therefore, this data is expected to contribute to the preservation of this unique and valuable species.

Acknowledgements: Supported by National Science Foundation Graduate Research Fellowship Program

THE MEASUREMENT OF STEROID HORMONES IN ATLANTIC SALMON MUCUS

Legacki E (1), Peterson B (2), Boggs A (1)

(1) National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, SC, USA

(2) National Cold Water Marine Aquaculture Center, Franklin, ME, USA 04469

The use of steroid hormones as biomarkers provides a snapshot of physiologies indicative of stress or reproductive status. However, in fish, sampling tends to be invasive, utilizing blood or tissue samples that are difficult to collect and stressful for all involved. Fish mucus, a complex material that contains a broad range of small molecules, is a non-lethal option for steroid hormone measurements. The primary goal of this study is to define mucus as an appropriate matrix for the measurement of pregnanes, glucocorticoids and androgens. Mucus was collected from sexually mature male (n=13) and female (n=40) Atlantic salmon through absorption onto filter paper and snap frozen. All samples were subjected to a methanol crash to precipitate out proteins and analyzed with a liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to measure 20 steroid hormones specific to reproductively active fish. Of those steroids measured, androstenedione and 19-norandrostenedione were the major androgens found in the mucus and were significantly greater in male vs female fish ($p \leq 0.05$). Glucocorticoids were also elevated in males ($p \leq 0.05$) and the major glucocorticoid detected was 11-deoxycorticosterone, followed by cortisol and cortisone. Steroid hormones specific to the maturation of milt and oocytes, 11 ketotestosterone and 17α,20β hydroxyprogesterone, were found in measurable concentrations in both male and female fish. Fish mucus appears to be an appropriate non-invasive matrix which is easily, non-lethally and minimally invasive to collect. Additional studies will be conducted to define the steroid hormone profile unique to reproductively active male and female Atlantic salmon ready to spawn. These data will further the knowledge base of fish reproductive endocrinology and provide potential biomarkers for future studies on fish reproductive and stress physiology.

Acknowledgements: Supported by National Research Council (NRC) Fellowship

DEAD NEWBORN CALVES AFFECTS CORTISOL, BEHAVIOR AND MILK PRODUCTION IN PRIMIPAROUS DAIRY COWS

González-de-la-Vara MR (1), De Anda FJ (2), Romano MC (3)

(1) Departamento de Etología, Fauna Silvestre y Animales de Laboratorio. Facultad de medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México (UNAM), Mexico City, México

(2) Agropecuaria el Gigante, Aguascalientes, México

(3) Departamento de Fisiología, Biofísica y Neurociencias, CINVESTAV, Instituto Politécnico Nacional, Mexico City, México

Delivery pathologies may impact several aspects of milk production in multiparous and primiparous cows. Therefore, the objective of this work was to compare serum and hair cortisol concentrations, behavior, and milk production in primiparous dairy cows that delivered dead or alive newborn calves.

Holstein cows were housed in an earth flooring corral (42.66m2/cow) and were assigned in 2 groups: (AC) Cows with alive newborn calves (n= 29), and (DC) cows with dead newborn calves (n=15), and studied throughout 90 days. Milk yield was measured in a milking parlor and social, sexual and maintenance behavior was observed daily for 4 hours. On days 1, 30, 60 and 90 of lactation, hair and serum cortisol concentrations considered as stress indicators were measured by RIA. Somatic cell count (SCC) and body condition (BC) were also registered.

Serum and hair cortisol concentration in DC cows was significantly higher than in AC cows on day 60 of lactation. Cows of AC group spent more time lying and ruminating than cows of DC group, but received significantly more aggressions and displayed more sexual behavior. No differences between groups were found in the proportion of time spent standing, walking or drinking. No statistically differences were observed in body condition, somatic cell counts, and days to first heat. As expected, milk production was higher in AC compared to DC cows.

The findings of this study indicate that the delivery of dead newborn calves impacts negatively and for long term the cortisol concentrations, behavior, and milk production of primiparous dairy cows. The important role of stress caused by delivery pathologies of primiparous cows should be considered and rectified.

Session 10: Neuroendocrine regulation of ionic, osmotic, and acid-base balance in vertebrates

IDENTIFICATION AND CHARACTERIZATION OF NEUROHYPOPHYSIAL HORMONE RECEPTORS IN THE HAGFISH, EPTATRETUS BURGERI

Yamaguchi Y (1), Takagi W (2), Kaiya H (3), Yoshida M (4)

(1) Institute of Agricultural and Life Sciences, Academic Assembly, Shimane University, Matsue, Shimane, Japan

(2) Laboratory of Physiology, Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa, Chiba, Japan

(3) Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan

(4) Marine Biological Science Section, Education and Research Center for Biological Resources, Faculty of Life and Environmental Science, Shimane University, Oki, Shimane, Japan

Neurohypophysial hormones (NHs), e.g., vasopressin- and oxytocin-family peptides, critically regulate body fluid homeostasis as well as other physiological processes. The versatile actions of NHs are mediated by distinct G protein-coupled receptors. The NH receptor family was classically thought to consist of four subtypes (V1aR, V1bR, V2R and OTR), while recent studies revealed that there are seven subtypes (V1aR, V1bR, V2a-V2dR and OTR; V2aR corresponds to the conventional V2R). The members of NH receptor family were diversified by multiple gene duplication events, which may be, at least in part, attributable to two rounds of whole genome duplication (WGD). However, despite intensive research effort in cartilaginous fishes and lampreys, the early evolution of NH receptor family has still been debated. In the present study, we focused on the hagfish (Eptatretus burgeri), another group of cyclostomes. Comprehensive searches against de novo transcriptome assemblies of nine tissues and genome assembly (Eburgeri_3.2; GCA_900186335.2) yielded two putative hagfish NH receptors, conveniently designated as DN7735 and DN20708. DN7735 was closely related to known V1Rs/OTRs, both in sequence and in function; when expressed in CHO cells, DN7735 induced Ca2+ signaling in response to vasotocin, the endogenous NH of hagfish. The mRNA of DN7735 was abundant in the brain, where intense signals were observed in the habenular, striatum, hypothalamus and adenohypophysis. On the other hand, DN20708, grouped in the clade of known V2Rs, was predominantly expressed in the systemic heart. To further investigate the orthologous relationships among the vertebrate NH receptors including newly identified hagfish molecules, comprehensive gene synteny analysis is underway. Our findings provide new insights into the molecular and functional evolution of vertebrate NH system.

Acknowledgements: Supported by JSPS KAKENHI Grant Number JP17H06876, JP19K16178, the Kao Foundation for Arts and Sciences, and the Atmosphere and Ocean Research Institute, the University of Tokyo.

THERMALLY-INDUCED CHANGES IN CELL VOLUME AND HORMONE RELEASE IN PROLACTIN CELLS OF MOZAMBIQUE TILAPIA

Woo DW (1), Malintha GHT (1), Celino-Brady FT (1), Breves JP (2), Yamaguchi Y (3), Seale AP (1)

(1) Department of Human Nutrition, Food and Animals Sciences, University of Hawai'i at Mānoa, Honolulu, HI, USA

(2) Department of Biology, Skidmore College, Saratoga Springs, NY, USA

(3) Institute of Agricultural and Life Sciences, Academic Assembly, Shimane University, Matsue, Shimane, Japan

Euryhaline fishes are capable of maintaining homeostasis in a wide range of salinities by regulating solute and water transport in osmoregulatory tissues. Discrete solute- and water-transporting processes are regulated by the neuroendocrine system. Prolactin (Prl) plays an important role when fish acclimate to freshwater environments by stimulating ion-retention/absorption and water secretion. Located within the rostral pars distalis (RPD) of the pituitary, Prl cells of the Mozambique tilapia (Oreochromis mossambicus) are sensitive to physiologically relevant changes in extracellular osmolality and continue to provide a unique model from which to identify the underpinnings of osmoreception. The short-term response by Prl cells to hyposmotic conditions is triggered by an increase in cell volume that enables extracellular Ca2+ to enter the cell through the transient receptor potential vanilloid 4, Trpv4, a cation channel that is also thermosensitive (in addition to being stretch-gated) in some vertebrates. While the cell-volume dependent secretory response of tilapia Prl cells to a hyposmotic stimulus is well established, the effects of temperature on Prl cells has not been characterized. We specifically incubated RPDs at 20, 25 and 30 °C and dispersed Prl cells at 26 and 32 °C. We found that Prl release from both whole RPDs and dispersed Prl cells showed a positive relationship with temperature. When dispersed Prl cells were subjected to an increase from 26 to 32 ºC, 30% of the total cells gradually increased in volume by ~10%; the remaining cells did not change in volume with this increase in temperature. We confirmed that all cells were competent to respond to a hyposmotic stimulus (280 mOsm/kg). Our findings suggest that thermally-induced Prl release may be mediated, at least in part, through a cell-volume dependent mechanism, similar to how osmoreception is achieved. And moreover, thermal-sensitivity may be a feature of only a sub-population of tilapia Prl cells.

Acknowledgements: Supported by grants from the NIH (1R21DK111775-01), NSF (IOS-1755016) NOAA (#NA18OAR4170347), and HATCH (#HAW02051-H) to A. P. Seale, and NSF (IOS-1755131) to J. P. Breves.

THE ROLE OF THE CORTICOTROPIN-RELEASING FACTOR SYSTEM DURING SMOLTIFICATION IN ATLANTIC SALMON

Culbert BM (1), Regish AM (2), Hall DJ (2), McCormick SD (2), Bernier NJ (1)

(1) Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

(2) U.S. Geological Survey, Leetown Science Center, Conte Anadromous Fish Research Laboratory, Turners Falls, MA, USA

Anadromous fishes undergo dramatic osmoregulatory changes in preparation of transitioning from freshwater to seawater. Cortisol is one of the primary hormones involved in mediating these changes and cortisol levels increase during the spring smoltification period prior to seawater migration in Atlantic salmon (Salmo salar). While the neuroendocrine mechanisms regulating cortisol synthesis during smoltification remain unclear, it is likely that the corticotropin-releasing factor (CRF) system is involved because it is one of the primary regulators of cortisol synthesis. Therefore, we measured transcript abundance of the major components of the CRF system in the hypothalamus, preoptic area, caudal neurosecretory system (CNSS), and pituitary of Atlantic salmon parr and smolts during spring and summer. To further determine the role of these genes in osmoregulation, we also assessed their response following transfer to seawater during peak smoltification in May. We found that smolts maintained ~2-fold higher transcript abundance of corticotropin-releasing factor b1 (CRF-b1) in the preoptic area throughout the spring compared to parr. Smolts also exhibited an ~7-fold upregulation in hypothalamic transcript abundance of urotensin 1a (UTS-1a) compared to parr in May through July. When transferred to seawater, UTS-1a transcript levels were further upregulated in smolts 24 h after transfer to seawater, while transcript levels did not increase until 96 h after transfer in parr. Additionally, smolts maintained ~4–6-fold higher transcript levels of UTS-1a, -1b, and CRF-b1 in the CNSS across the spring, potentially reflecting an important peripheral role for these hormones in promoting seawater tolerance. Overall, our results support UTS-1a as a key neural regulator of cortisol synthesis and/or ion regulation and suggest that the osmoregulatory scope of the CRF system extends beyond simply regulating cortisol synthesis.

EFFECTS OF FRESHWATER ACCLIMATION ON THYROID HORMONES AND BRANCHIAL DEIODINASES IN MOZAMBIQUE TILAPIA

Seale AP (1), Seale LA (2), Gilman, CL (3), Zavacki AM (4), Larsen PR (4), Inokuchi M (5), Breves JP (6)

(1) Department of Human Nutrition, Food and Animals Sciences, University of Hawai'i at Mānoa, Honolulu, HI, USA

(2) Pacific Biosciences Research Center, School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, Honolulu, HI, USA

(3) Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawai'i at Mānoa, Honolulu, HI, USA

(4) Division of Endocrinology, Diabetes and Hypertension, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA

(5) Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo, Japan

(6) Department of Biology, Skidmore College, Saratoga Springs, NY, USA

Euryhaline fishes are capable of maintaining osmotic homeostasis in a wide range of environmental salinities. Several pleiotropic hormones, including prolactin, growth hormone, and thyroid hormones (THs) mediate various adaptive responses to changes in environmental salinity. It is unclear, however, the extent to which THs and the pituitary-thyroid axis promote the adaptive responses of key osmoregulatory organs to freshwater (FW) environments. THs are primarily released into circulation as the prohormone thyroxine (T4), requiring conversion into active 3-5-3'-triiodothyronine (T3) to act upon target tissues. Conversion of T4 into T3 is carried out by iodothyronine deiodinases 1 and 2 (Dio1 and Dio2), while inactivation of T4 and T3 is carried out by iodothyronine deiodinase 3 (Dio3). In the current study, we characterized circulating TH levels in parallel with branchial deiodinase activities during the acclimation of Mozambique tilapia (Oreochromis mossambicus) to FW. Tilapia transferred from seawater (SW) to FW exhibited reduced plasma T4 and T3 levels by 6 h. These reductions coincided with an increase in branchial Dio2-like activity and decreased branchial dio1 gene expression. Gene expression of branchial dio1, dio2, and dio3 was not directly affected by extracellular osmotic conditions in vitro. Lastly, we observed that dio1 and dio2 expression in vivo was stimulated by thyroid-stimulating hormone in hypophysectomized tilapia. Our collective findings suggest that THs are involved in mediating the FW acclimation of Mozambique tilapia through their interactions with branchial deiodinases in the gill.

Acknowledgements: This project was supported by grants from the NIH (R21DK111775-01), NSF (IOS-1755016), NOAA (#NA18OAR4170347), and HATCH-NIFA (#HAW02051-H) to A.P.S., NIH (R1DK128390) to L.A.S., and NSF (IOS-1755131) to J.P.B.

Session 11: Developmental roles of corticosteroids and their receptors

COORDINATION OF STRESS-RELATED BEHAVIOUR IN LARVAL ZEBRAFISH: THE ROLE OF CRHR1, MR AND GR

Faught E, Vijayan MM

Departments of Biological Sciences, University of Calgary, Calgary, CAN

The stress response, mediated by the hypothalamus-pituitary-adrenal (HPA) axis, results in the release of glucocorticoids (GCs), causing metabolic, endocrine, and behavioural adaptations that allow an organism to cope with stress. GCs mediate their actions through two corticosteroid receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Both receptors have well-established roles in mediating stress-induced behaviour in mammals and fish. However, these behavioural responses are often evaluated with exogenous treatment of GCs and little is unknown about the integration of MR/GR mediated responses with those of other HPA axis intermediates. Indeed, corticotropin-releasing hormone (CRH), the primary step in HPA activation, is known to play a role in stressor-mediated behaviour. Therefore, we tested the hypothesis that CRH via the CRH-receptor 1 (CRHR1) will initiate GC release allowing for GR/MR mediated modulation of behavioural response in vivo. In this study, we used zebrafish larvae, which exhibit well-characterized changes in locomotion in response to acute light exposures. Given the distinct temporal release and signalling of CRH versus cortisol during stress, we assessed the changes to larval activity over the short term (15 min) and long term (1 and 4 h) post-stress. We first generated and validated zebrafish containing a functional CRHR1 knockout (CRHR1-KO). Larvae lacking crhr1 did not increase cortisol, the primary GC in fish, post-stress and they also lacked stress-induced hyperactivity. To determine whether this was due to a lack of CRH or cortisol, wildtype larvae were treated with metyrapone (MET),

which also effectively blocked the attendant rise in cortisol post-stress; however, only abolished long-term hyperactivity. To further determine the temporal role for cortisol signalling, we assessed activity in larvae lacking either the GR (GRKO) or the MR (MRKO). Overall, the results suggest that the acute stress-induced hyperactivity is mediated by the CRH/CRHR1 system, while the subsequent cortisol production and the associated GR/MR signalling is essential for prolonging this stressor-induced hyperactivity. This study underscores the importance of systems-level approach in assessing the mechanisms involved in stress responsivity.

Acknowledgements: Supported by NSERC Discovery Grant (MMV)

THE ROLES OF CORTICOSTEROIDS DURING THE SEA LAMPREY METAMORPHOSIS: OSMOREGULATORY AND GLUCONEOGENIC ACTIONS

Shaughnessy CA (1,2), McCormick SD (2,3)

(1) Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA, USA

(2) U.S. Geological Survey, Eastern Ecological Science Center, S.O. Conte Anadromous Fish Research Laboratory, Turners Falls, MA, USA

(3) Department of Biology, University of Massachusetts, Amherst, MA, USA

Lamprey represent the most basal living example of a vertebrate animal which regulates its internal fluid and ion homeostasis. This phylogenetic position among vertebrates makes lamprey an important model organism for understanding the basal vertebrate state, and thus the evolution of vertebrate physiology. Sea lamprey (Petromyzon marinus L.) are a semelparous, anadromous species with a fascinating life history. As filter-feeding larvae, sea lamprey live in freshwater (FW) for 4–6 years before undergoing a true metamorphosis during Summer and Autumn months, which includes the appearance of eyes, a toothy oral disc and the development of seawater (SW) tolerance. Emerging in late autumn as metamorphosed juveniles, sea lamprey then migrate to SW where they spend 1–3 years parasitizing other aquatic vertebrates and exhibiting rapid growth before returning upstream as mature adults to spawn and then die. Broadly, this presentation examines the roles of corticosteroids and their receptor(s) in controlling osmoregulation and gluconeogenesis during the sea lamprey metamorphosis. Specifically, this presentation compares the roles of two endogenous corticosteroids found in sea lamprey serum (11-deoxycortisol and deoxycorticosterone) and the lamprey corticosteroid receptor in controlling osmoregulatory mechanisms in the gill and intestine, as well as hepatic gluconeogenesis after temperature and handling stressors. Special attention is given to describing physiological changes during metamorphic progression as well as comparing larvae and juvenile developmental life stages. We will also point out future areas of research that will increase our understand the physiology and endocrinology of vertebrate metamorphosis.

Acknowledgements: Supported by a National Science Foundation grant to SDM.

BRIEF DEVELOPMENTAL EXPOSURE TO FLUOXETINE CAUSES LIFE-LONG DISRUPTION OF THE STRESS RESPONSE IN ZEBRAFISH

Amin Nozari (1), Vance L. Trudeau (1), Carole Yauk (1,2), Remi Gagné (3)

(1) Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

(2) Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada

(3) Formerly at Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada

Antidepressants like fluoxetine (FLX), are widely used to treat depressive disorders during pregnancy. Early-life exposure to FLX is known to disrupt the normal function of the stress axis in humans, rodents, and teleosts. We used a zebrafish line with a cortisol-inducible fluorescence transgene to study the effects of developmental daily exposure to FLX (54 µg/L) on the transcriptomic profile of brain tissues in exposed larvae and developmentally exposed-adults. High throughput RNA sequencing was conducted on brain tissues in unstressed and stressed conditions. Long-lasting effects of FLX were observed in telencephalon and hypothalamus of adult zebrafish with 1927 and 5055 genes significantly (≥1.2 fold-change, false-discovery rate $p < 0.05$) dysregulated in unstressed condition, respectively. Similar findings were observed in hypothalamus with 1245 and 723 genes being significantly dysregulated in stressed adults, respectively. The Homo sapiens orthologue analysis on the differentially expressed genes using Ingenuity Pathway Analysis (IPA) showed alterations in neuroendocrine signalling, cholesterol metabolism, and synaptogenesis. Enriched networks affected include lipid metabolism, molecular transport, and nervous system development. Analysis of upstream transcription regulators showed potential dysregulation of clocka and nr3c1 which regulate circadian rhythm, stress response, cholesterol metabolism, and histone modifications. Several genes involved in epigenetic regulation were also affected by FLX, including dnmt3a, adarb1,

adarb2, hdac4, hdac5, hdac8, and atf2. We show that life-long disruptive effects of FLX on pathways associated with neuroendocrine signalling, stress response and the circadian rhythm; all of which are implicated in the development of depressive disorders in humans. Our results raise concern for the endocrine-disrupting potential of antidepressants during embryonic development.

GLUCOCORTICOID RECEPTOR MEDIATES CORTICOSTERONE AND THYROID HORMONE SYNERGY DURING FROG METAMORPHOSIS

Sterner Z, Buchholz D

Department of Biological Sciences, University of Cincinnati, Cincinnati, OH

Amphibian populations have been experiencing major declines, in part due to chemical pollution. In particular, certain pesticides cause endocrine disruption through antagonistic effects on hormone receptors thereby negatively impacting growth and development. Two potential targets of endocrine disruption are thyroid hormone and corticosterone, which interact synergistically to accelerate developmental progression. To examine potential mechanisms of chemical disruption, we used glucocorticoid receptor knockout (GRKO) tadpoles to examine the role of glucocorticoid receptor (GR) in synergy between corticosterone and thyroid hormone. Synergistic upregulation was observed in gene expression of a thyroid hormone response gene in the tail (thrb) in wild-type individuals but not in GRKO. Synergy was also observed in morphology through acceleration of tail and gill resorption in wild-types, but not in GRKO. Such synergy did not occur in all tissues, such that GRKO tadpoles had impaired synergy in the tail and gills, but not in the liver and hindlimb. Surprisingly, GRKO tadpoles treated with supraphysiological doses of thyroid hormone survived through metamorphosis showing that synergy mediated by GR is essential for the completion of metamorphosis. Therefore, chemicals interfering with GR may affect synergy between corticosterone and thyroid hormone and potentially cause detrimental developmental issues to natural populations.

Session 12: Avian Endocrine and Metabolic Responses to Urbanization

URBANIZATION AND AVIAN ENDOCRINE RESPONSES

Vitousek MN

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY and Cornell Lab of Ornithology, Ithaca, NY

As a result of the rapid increase in urbanization across most of the globe many avian populations are facing novel or dramatically altered environments. A swiftly growing literature has begun to document how phenotypes differ in urban environments and in response to anthropogenic stimuli – patterns that likely reflect both plastic and evolutionary responses. Endocrine traits have received particular attention; as central mediators of phenotype, endocrine systems can act as sensitive indicators of context, and also mediate coordinated responses to changing environments. Comparative and experimental studies have supported the potential for urbanization to influence endocrine responses. However, findings to date have revealed variable and often inconsistent patterns across populations or species – likely reflecting differences in the degree to which stimuli are perceived as challenging, the chronic stress response, and how anthropogenic stimuli increase or decrease resource availability. Likewise, it is important to consider the potential for the same stimulus to have different effects at different timescales (e.g., within-individual plasticity or flexibility vs. an evolved response). Here I describe some recent work linking noise and other anthropogenic stimuli to HPA axis regulation in birds across scales. These and other studies support the potential for urbanization to influence endocrine responses, but also highlight the need for a predictive, scale-dependent, and organism-centered framework of anthropogenic effects. Such a framework could prove valuable for identifying the sublethal effects of anthropogenic challenges, identifying at-risk populations and communities, and elucidating how urbanization may be exerting selective pressures on the endocrine mediators of phenotype in ways that could affect the capacity to cope with a diversity of challenges.

Acknowledgements: This work is supported by grants from NSF-IOS (1457251), DARPA (YFA-D17AP00033), and USDA NIFA Hatch (1017321).

A FOUR-WEEK URBAN DIET IMPAIRS VASODILATION BOT NOT NUTRITIONAL OR METABOLIC PHYSIOLOGY IN WILD-CAUGHT MOURNING DOVES (ZENAIDA MACROURA)

Basile AJ (1), Renner MW (1), Kayata L (1), Mohr AE (2), Deviche P (1), Sweazea KL (1,2)

1) School of Life Sciences, Arizona State University, Tempe, AZ, USA

(2) College of Health Solutions, Arizona State University, Phoenix, AZ, USA

Birds living in urban areas routinely consume anthropogenic foods, but the physiological consequences of this consumption are poorly understood. To address this question, we investigated the effects of an urban diet (UD) in wild, urban-caught mourning doves in a controlled environment. Since anthropogenic foods often contain a high proportion of refined carbohydrate and fat, we predicted that UD consumption alters body mass as well as plasma and tissue nutritional physiology markers, metabolites, and metabolic pathways. To test this prediction, we compared the nutritional physiology and peripheral vasodilation of doves fed an UD (1:1 ratio of bird seeds and French fries; n=6) with those of doves receiving a control diet (CON: bird seed diet; n=7) for four weeks. At the end of the dietary manipulation period, birds were euthanized, and differences in body mass, various nutritional physiology markers, metabolites, and metabolic pathways were analyzed in plasma, pectoralis muscle, liver, kidney, gastrocnemius muscles, and adipose samples. In addition, cranial tibial arteries were dissected to measure ex vivo vasodilation in response to acetylcholine treatment after phenylephrine-induced vasoconstriction. Neither body mass, plasma osmolality, glucose, sodium, insulin, triglyceride, uric acid, liver glycogen and triglycerides, nor muscle glycogen differed between diet groups. Only one metabolite (myoinositol; $p=0.017$) and three metabolic pathways (inositol phosphate metabolism, phosphatidylinositol signaling system, ascorbate and aldarate metabolism; $p=0.007$ for all) were altered in pectoralis muscle between diet groups. Vasodilation of tibial arteries was impaired in UD compared to CON-fed birds ($p=0.004$), suggesting the potential for UD consumption to alter cardiovascular function. Overall, short term consumption of a diet comprised of 50% anthropogenic foods did not result in major nutritional and metabolic perturbations suggesting that urban mourning doves may be well adapted to consuming anthropogenic foods.

Acknowledgements: Funded by the ASU School of Life Sciences, Central Arizona Phoenix Long Term Ecological Research Graduate Student Research Grant funded via the National Science Foundation (1832016), and ASU Barrett, The Honors College.

ARE CORTICOSTERONE LEVELS A PROXY OF ENVIRONMENTAL CONSTRAINTS IN A DECLINING URBAN EXPLOITER, THE HOUSE SPARROW

Angelier F (1), Grace JK (2), Dupont S (1), Beaugeard E (1), Meillère A (1), Brischoux F (1)

(1) Centre d'Etudes Biologiques de Chizé, CNRS-LRU, 79360 Villiers en Bois, France

(2) Dept. of Ecology and Conservation Biology, Texas A&M University, College Station, TX 77843, USA

Urbanization is known to be associated with drastic environmental changes for wild vertebrates. Although most vertebrates are energetically constrained and stressed by this anthropogenic habitat, a few species are able to benefits from human activities and to thrive in cities. It is often predicted that this ability to cope with the stressful urban environment could be linked to physiological and hormonal changes. In that respect, the Hypothalamus-Pituitary-Adrenals (HPA) axis deserves a specific attention because it is one of the main mediators of allostasis and corticosterone levels have often been linked to energetic constraints. We investigated the functional link that exist between urbanization, corticosterone and energetic constraints in a declining urban exploiter, the house sparrow (Passer domesticus). House sparrows have co-evolved with humans for centuries, but they have recently undergone an unexplained drastic decline in most urbanized areas. We captured and sampled hundreds of house sparrows along an urbanization gradient to monitor not only several indices of energetic condition (body condition, body size, plumage quality, and diet through stable isotopes) but also the functioning of their HPA axis (baseline and stress-induced plasma corticosterone levels, feather corticosterone levels). Although we did not find any evidence of any impact of urbanization on adult sparrows, we found that urban juvenile sparrows were in poorer condition and had higher feather corticosterone levels relative to rural juvenile sparrows. This suggests that sparrows may be energetically constrained during their development in urban areas. In further experimental captive studies, we aimed to better understand the metabolic and endocrine consequences of having higher corticosterone levels for developing sparrows. By manipulating corticosterone levels in sparrow chicks, we demonstrated that exposure to elevated corticosterone levels during early life can have important long-lasting consequences on multiple organismal systems (reduced metabolism, alteration of the HPA axis). Altogether, our results suggest that the functioning of the HPA axis can reflect the energetic constraints of the urban environment and can also mediate specific phenotypic responses to urban constraints.

Acknowledgements: Supported by the ANR URBASTRESS (FA), the Région Nouvelle-Aquitaine (FA), and the FYSSEN

Foundation (JKG, FA).

PAST AND FUTURE: THE IMPACT OF URBANIZATION ON AVIAN PHYSIOLOGY

Deviche P, Sweazea K

School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

Urban environments are evolutionarily novel and differ from natural environments in many respects including food and/or water availability, predation, noise, light, air quality, pathogens, biodiversity, and temperature. The success of organisms in urban environments requires physiological plasticity and adjustments that have been described extensively, in particular in birds residing in geographically and climatically diverse regions. These studies have revealed some relatively consistent differences between urban and non-urban conspecifics. For example, seasonally breeding urban birds often develop their reproductive system earlier than non-urban birds, perhaps in response to more abundant trophic resources. In most instances, however, analyses of existing data indicate no general pattern distinguishing urban and non-urban birds. For example, it is often hypothesized that urban environments are stressful, yet the activity of the hypothalamus-pituitary-adrenal axis does not differ consistently between urban and non-urban birds. A similar conclusion is reached by comparing blood indices of metabolism. The origin of these disparities remains poorly understood, partly because many studies are correlative rather than aiming at establishing causality, which effectively limits our ability to formulate specific hypotheses regarding the impacts of urbanization on wildlife. We suggest that future research will benefit from prioritizing mechanistic approaches to identify environmental factors that shape the phenotypic responses of organisms to urbanization and the neuroendocrine and metabolic bases of these responses. Further, it will be critical to elucidate whether factors affect these responses (a) cumulatively or synergistically; and (b) differentially as a function of age, sex, reproductive status, season, and mobility within the urban environment. Research to date has used various taxa that differ greatly not only phylogenetically, but also with regard to ecological requirements, social systems, propensity to consume anthropogenic food, and behavioral responses to human presence. Researchers may instead benefit from standardizing approaches to examine a small number of representative models with wide geographic distribution and that occupy diverse urban ecosystems.

Thursday, May 27, 2021

Session 17: The relevance of (neuro)steroids and steroidogenic enzymes in comparative endocrinology

THE ROLE OF AROMATASE IN THE GATEWAYS OF SOCIAL PERCEPTION IN TELEOSTS

Shaw K, Trudeau VL

Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

Sex steroids play critical roles in modulating sensory perception for mate identification and assessment. Aromatase (Cyp19a1) is a steroidogenic enzyme that functions to convert aromatizable androgens into bioactive estrogens, and hence is in a pivotal position to mediate pathways for social perception. In teleosts, there are two paralogs encoding aromatase, cyp19a1a and cyp19a1b, with highest expression in the gonad and brain, respectively. Though Cyp19a1a in granulosa cells is critical for ovarian differentiation, the importance of Cyp19a1b in radial glial cells (RGCs) in the nervous system for sexual behaviour is yet unknown. Neural aromatase is concentrated in sensory afferent nerve fibres and forebrain regions critical for sensory perception of social stimuli, even among teleost species reliant on different sensory modalities for social communication. Since aromatase expression is restricted to RGCs in the teleost brain, these findings beg the question of the function of locally produced estrogens in these central neural circuits for social behaviour. The zebrafish is an amenable model organism to address this question due to the existence of a transgenic zebrafish (Tg(cyp19a1b:GFP) expressing GFP under the control of the neural aromatase promoter and TALEN-mediated cyp19a1 mutant lines that enable assessments of how independent loss of cyp19a1a and cyp19a1b may affect social behaviour. RGCs expressing cyp19a1b:GFP are found in close proximity to cell bodies producing isotocin (IST) in the preoptic area, a brain region important for social behaviour. IST is a nonapeptide that functions in mate preference through modifying the salience of social information. The presence of estrogen response element half-sites in the zebrafish ist gene suggest that locally produced estrogens in RGCs could diffuse to modulate IST levels to affect neural circuits responsible for perceiving the

salience of social information for behaviour.

Acknowledgements: Supported by Ontario Graduate Scholarship (KS), NSERC CGS-D (KS), and NSERC Discovery Program (VLT). Generous contribution of cyp19a1 mutant lines from X. Liu (Sun Yat-Sen University) are appreciated.

STEROIDS AND NEUROSTEROIDS-LIKE HORMONES SYNTHESIS IN TAENIA CRASSICEPS WFU CYSTICERCI

Patricio-Gómez JM (1), Valdez R (1), Figueroa M (2) and Romano MC (1)

(1) Department of Physiology, Biophysics and Neuroscience, CINVESTAV del I. P.N., Mexico City, Mexico

(2) Department of Pharmacy, Faculty of Chemistry, UNAM, Ciudad de Mexico, Mexico

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, which holds cerebral ganglia and germinal cells. We previously reported that T. crassiceps ORF, WFU, and T. solium cysticerci transform tritiated steroid precursors to androgens, estrogens, and corticosteroids. In this work, we analyzed the spontaneous synthesis of steroids in two larval stages of T. crassiceps WFU cysticerci by UPLC-HRESIMS-MS/MS. We also evaluated the synthesis after incubation with the steroid precursor progesterone (P4). For this purpose, cysticerci were obtained from the abdominal cavity of female mice and preincubated for 24 h in DMEM plus antibiotics/antimycotics. After preincubation, the parasites were manually separated in invaginated (IC) and evaginated cysticerci (EC). Then, cysticerci were incubated for 43 h in fresh media with and without progesterone (20 µM). Blanks containing the culture media alone or plus the P4 precursor were simultaneously incubated. Culture media were recovered, and steroids extracted. The reconstituted samples were analyzed by UPLC-HRESIMS-MS/MS. We identified steroid production per se in both the IC and EC culture media. IC and EC secrete 17 and 23 hormones, respectively, ten of these compounds have not been previously described in the parasite. This is the first report of the synthesis of potent androgens, such as DHT, and neurosteroids-like molecules, such as allopregnanolone, allotetrahydrodeoxycorticosterone, and pregnanolone, by the parasites. Data showed that the synthesis of such potent sexual steroids occurs in both larval developmental stages of T. crassiceps WFU cysticerci.

Acknowledgements: Supported in part by CINVESTAV del I.P.N.

USING SCALE CORTISOL CONTENT AS A BIOINDICATOR OF SOCIAL STATUS AND LONG-TERM HEALTH IN WILD FISH

Bernier NJ, Culbert BM, Yin-Liao I, Balshine S, Laberge F

(1) Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

(2) Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario, Canada

Recent studies have shown that cortisol can be detected in fish scales. Results from our laboratory experiments aimed at characterizing the temporal profiles of cortisol accumulation and clearance from the scales of goldfish suggest that scale cortisol content can serve as an indicator of chronic stress in fish. Whether scale cortisol content may also serve as a time-integrated measure of cortisol production in wild fish remains to be determined. Since cortisol levels typically differ among ranks in social hierarchies, we observed wild social groups of the cooperatively breeding cichlid Neolamprologus pulcher in Lake Tanganyika (Zambia) and evaluated how scale cortisol content varies across group members. While scale cortisol was detectable in ~50% of dominant males and dominant females, we did not detect any cortisol in the scales of subordinates. Consistent with these differences in scale cortisol content, we also detected transcriptional differences across social ranks for several genes involved in regulating the actions of cortisol in the brain and liver. Additionally, to assess whether chronic exposure to contaminants is stressful in wild fish, we quantified scale cortisol content from yellow perch originating from three sites that differ in degrees of legacy industrial pollution and from one reference site in the Detroit River and Lake St. Clair area (Canada and USA). Integrated with individual measures of organ sizes, toxicant exposure, and trophic position, scale cortisol content may provide insight into the factors that contribute to the long-term health of wild fish exposed to industrial pollution. Although improvements in assay sensitivity will be needed before scale cortisol content can be considered a minimally invasive method for the evaluation of glucocorticoid levels in fish, our results provide novel insight into the potential uses and current limitations of scale cortisol content as a bioindicator in wild fish.

Acknowledgements: Supported by NSERC Discovery Grant funding to NJB, FL and SB, Canada First Research Excellence

Funds to NJB and FL, a NSERC CGS scholarship to BMC, and both OGS and QEII-GSST scholarships to IYL.

AGE, SEX, AND SEASONAL COMPARISONS OF URINARY ESTRADIOL IN THE BIG BROWN BAT

Greville LJS (1), Bueno LM (2), Pollock T (1), Faure PA (1)

(1) Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario, Canada

(2) Virology Research Center, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Hormone analysis in bats and other small mammals often occurs via blood draw and plasma analyses. Urinary steroid levels are frequently used in large mammals to track the reproductive state of individuals. Urine analysis is non-invasive and allows for repeated testing in small mammals. We quantify unconjugated 17B-estradiol (E2) in the urine of male and female big brown bats (Eptesicus fuscus) across their annual reproductive cycle in a captive breeding colony. Male bats had higher levels of urinary E2 than female bats, and adults higher than yearlings, following creatinine adjustment for hydration. In non-pregnant females, a number of seasonal differences in both creatinine-adjusted and unadjusted urinary E2 were observed. Male E2 was higher than females in the winter in both conditions, as well as in autumn with creatinine-adjustment. Urinary progesterone (P4) levels in non-pregnant female bats remained constant across seasons with the exception of unadjusted P4 levels being higher in the summer compared to other seasons. In pregnant female bats we observed a peak in both urinary E2 and P4 beginning ~20 days prior to parturition, with both steroid hormones returning to baseline levels in the following weeks. Our findings are the first to track urinary steroid levels throughout the reproductive cycle of a bat species and are the first step towards using urinary analysis to detect pregnancy. Results also suggest a physiological delay of reproductive maturation in big brown bats. Importantly, the quantification of urinary steroid levels is critical in interpreting their roles in "primer pheromonal effects" in bats.

Acknowledgements: Supported by NSERC Discovery Grant (PAF), NSERC PGS-D (LJSG), CAPES (LMB), and NSERC CGS-D (TP)

Session 18: Hormone mediated control of ion and fluid homeostasis in invertebrates

EXAMINING THE ROLE OF THE V-TYPE H+-ATPASE IN CAPA-MEDIATED INHIBITION IN AEDES AEGYPTI MALPIGHIAN TUBULES

Sajadi F, Paluzzi JP

Department of Biology, York University, Toronto, Ontario, Canada

Haematophagus insects, such as the female Aedes aegypti mosquito, face the challenge of excess ion and water intake after a blood meal. To cope with this, adult female A. aegypti have a specialized excretory system that includes the Malpighian 'renal' tubules (MTs), which are under rigorous control by several endocrine factors to regulate transepithelial movement of ions. Active ion transport in A. aegypti MTs is driven by the V-type H+-ATPase (VA), serving as the primary energizer for transepithelial secretion. CAPA peptides inhibit fluid secretion of MTs stimulated by select diuretic factors, 5HT and DH31 through the NOS/cGMP/PKG pathway. However, the anti-diuretic signalling mechanism remains unclear. Due to the predominant role of the VA in fluid secretion, the objectives of this study were to investigate the role of VA in CAPA inhibition. Bafilomycin, a VA inhibitor, was found to significantly inhibit DH31-and 5HT-stimulated secretion, while having no effect on CRF-stimulated MTs. CAPA and bafilomycin treatment led to alkalization of the secreted fluid suggesting inhibition of the VA, which may lead to constrained entry of cations across the apical membrane. Additionally, adult female MTs treated with DH31 resulted in an increase of VA activity, while tubules incubated with both DH31 and CAPA had a lower VA activity resulting in activity levels comparable to saline. To determine whether CAPA causes V1 dissociation from the membrane, cytosolic and membrane protein fractions were isolated from DH31- and CAPA-incubated MTs. V1 protein expression was found higher in the membrane fractions of DH31-incubated MTs while higher levels were seen in the cytosolic fractions of CAPA-treated tubules. The results thus far could suggest a novel mechanism for CAPA inhibition, blocking the VA to hinder fluid secretion. Investigating the pathway of CAPA inhibition and its role in countering diuresis will help provide a deeper understanding of this critical physiological process.

Acknowledgements: Supported by an Early Research Award from the Ontario Ministry of Research & Innovation and an

NSERC Discovery Grant (JPP) as well as NSERC CGS, Vernon Stong, and Carswell Scholarships in Science (FS).

THE REGULATION OF AQUAPORIN ABUNDANCE BY DIURETIC HORMONES IN THE MALPIGHIAN TUBULES OF THE LARVAL DISEASE VECTOR MOSQUITO, AEDES AEGYPTI

Picinic BN, Donini A, Paluzzi JP

Department of Biology, York University, Toronto, Ontario, Canada

The disease vector mosquito, Aedes aegypti is responsible for the transmission of deadly arboviral diseases such as Dengue, Chikunguya, Zika virus, and Yellow fever. Larval A. aegypti are aquatic insects, typically inhabiting hypotonic freshwater however, they have been shown to inhabit brackish water and sewage water as well. The adult A. aegypti are terrestrial animals and the females of the species are hematophagous, therefore they require a blood meal to produce mature, viable eggs. Hence, both the larvae and adults are faced with unique osmoregulatory challenges. Specifically with larvae, they must eliminate excess water from their hemolymph, which is accomplished in part by regulating the water flux across the epithelia of organs such as the midgut, Malpighian tubules (MTs), hindgut, and anal papillae. The MTs and hindgut together are functionally analogous to the renal tubes of mammalian kidneys where urine is produced; however, the mechanisms by which it is produced are different. In the MTs of A. aegypti, urine production is accomplished by ion secretion, driven by an apical V-type H+-ATPase in the principal cells of this epithelium. Water is drawn into the lumen of the MTs by osmosis, following ion secretion. There is a plethora of literature that describes the regulation of fluid secretion by the MTs as a result of ion transport. However, not much is known about how the transport of water across the epithelia of MTs is regulated. There are six aquaporin (water transport proteins) genes (AaAQP1-6) in the A. aegypti mosquito, all of which are expressed in the MTs. In this study, we have begun to examine the effects of select diuretic hormones and their second messengers on the AaAQP abundance in the MTs. Diuretic hormones, such as serotonin (5-HT), are released into circulation in larval A. aegypti, in order to increase the secretory capacity of the MTs during times of osmotic stress. When isolated larval MTs were incubated with 10-7M 5-HT for ~1hr at RT in vitro, there were no significant changes in AaAQP1 protein levels. Interestingly, when isolated larval MTs were incubated with the second messenger of 5-HT, cAMP, for ~1hr at RT, there was a significant increase in AaAQP1 abundance in the MTs. However, immunohistochemistry used to localize AaAQP1 in the MTs following cAMP treatment has shown no change to their localization in this epithelium. The effects of these and other factors on the AaAQP abundance and their localization in the MTs is the subject of continuing work.

Acknowledgements: Support by York University, NSERC Discovery Grant

EXPLORING THE ROLE OF GLYCOPROTEIN HORMONES GPA2/GPB5 ON THE DIURETIC PROCESS IN THE MEDICALLY IMPORTANT INSECT, RHODNIUS PROLIXUS

Al-Dailami A, Leyria J, Orchard I, Lange AB

Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

Glycoprotein hormones are formed by the heterodimerization of alpha and beta subunits. They mediate a wide range of physiological functions such as metabolism, reproduction, and development. In vertebrates, glycoprotein hormones include follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), among others. The last glycoprotein hormone discovered was thyrostimulin, which is formed by the dimerization of GPA2 with GPB5 subunits. To date, the functional role of thyrostimulin (GPA2/GPB5) in vertebrates has not been fully elucidated. However, recent reports in invertebrates, specifically in holometabolous insects, suggest that GPA2/GPB5 plays a critical role in development and diuresis. In this study, we characterize the glycoprotein hormone (GPA2/GPB5) and its receptor (LGR1) in Rhodnius prolixus, which is a hemimetabolous insect and the vector of Chagas disease. We also investigate the physiological roles for this glycoprotein hormone-signalling pathway in fifth instar R. prolixus, specifically in relation to feeding and diuretic processes, both of which have high epidemiological relevance. Both subunit transcripts, GPA2 and GPB5, and LGR1 transcripts are present in a variety of tissues, with greatest expression of the subunits in the central nervous system (CNS), whereas LGR1 expression is highest in the Malpighian tubules (MT). Results from temporal qPCR analyses are consistent for the subunits and the receptor, showing a decrease in transcript expression 24 h after feeding in the CNS and MTs, respectively, followed by an increase in transcripts as the days after feeding advance. In order to assess the role of this glycoprotein hormone in diuresis and feeding, we silenced the expression of the LGR1 transcript using RNA interference,

and at 7 days post-injection, insects with reduced LGR1 expression showed greater weight loss and mortality rate in both fed and unfed nutritional states. In addition, insects with reduced LGR1 consumed a significantly smaller blood meal and the mortality rate was greater. The results suggest that GPA2/GPB5 may play a role controlling R. prolixus feeding and diuresis.

Acknowledgements: This work was supported by NSERC Discovery Grants to ABL and IO.

CHARACTERIZATION OF RYAMIDE NEUROPEPTIDE AND ITS RECEPTOR IN THE HUMAN DISEASE-VECTOR, AEDES AEGYPTI

Nguyen T, and Paluzzi JP

Department of Biology, York University, Toronto, Ontario, Canada

The regulation of physiological functions by neuropeptides allows insects, like other animals, to maintain homeostasis. Different neuropeptides have varying roles with specific effects on their target organs mediated by the activation of their cognate receptors. The RYamide family of neuropeptides was recently identified among insects, including the silkworm Bombyx mori, red flour beetle Tribolium castaneum, and several Drosophila species. This research will focus on the physiological relevance of RYamides in the yellow fever mosquito, Aedes aegypti. Previous studies in other insects have indicated a role in the modification of feeding behaviour. Moreover, the CG5811 gene that encodes the RYamide receptor in Drosophila melanogaster was found to be significantly abundant in the hindgut. Similarly, significant enrichment of the RYamide receptor transcript was detected in the hindgut of Bombyx mori. Consequently, RYamide receptor expression in the hindgut suggests its peptidergic ligand may be involved in the regulation of water and ion reabsorption. Preliminary results of the current research focused on adult Aedes aegypti indicate receptor transcript expression in the hindgut, consistent with observations in other species. Further research will be done to quantify RYamide receptor transcript abundance in various tissues and organs in adult A. aegypti as well as confirm the authenticity of the RYamide receptor using receptor functional activity assays. In the future, my research will examine the activity of RYamide neuropeptides on the hindgut in order to determine their physiological role on this organ. Altogether, my research aims to characterize the RYamide and its receptor in A. aegypti and confirm its proposed role in hindgut regulation.

Acknowledgements: Supported by York University, and the Canada Graduate Scholarships Master's program.

Session 19: Novel neuropeptides: what can the comparison of invertebrate and vertebrate systems teach us?

THYROSTIMULIN AND ITS INVERTEBRATE HOMOLOG, GPA2/GPB5: A SPECIAL FOCUS ON THE GPA2/GPB5 HORMONE RECEPTOR IN DROSOPHILA MELANOGASTER

Thorat L, Patel S and Paluzzi JP

Department of Biology, York University, Toronto, Ontario, Canada

Classic heterodimeric glycoprotein hormones belong to a family of cystine-knot containing proteins possessing a common glycoprotein α subunit (GPA1) and a hormone-specific glycoprotein β subunit (GPB1-4). Recently, GPA2/GPB5 was discovered as a novel glycoprotein hormone in mammals and other vertebrates with the presence of homologs across all bilaterian phyla. Leucine-rich repeats containing G protein-coupled receptors (LGRs) act as receptors for glycoprotein hormones and are characterized by a large N-terminal ectodomain that confers receptor-ligand specificity. Unlike the GPA1/GPB1-4, our understanding of the functional roles of GPA2/GPB5 is rather fragmentary. Here, we present an overview of the vertebrate GPA2/GPB5 (referred to as thyrostimulin) versus its invertebrate homolog. For the invertebrate homolog (i.e. GPA2/GPB5), LGR1 serves as the receptor and is crucial in reproduction and development. In this study, we aimed to further investigate the physiological roles of the D. melanogaster LGR1, which happens to be the closest fruit fly homolog of the vertebrate glycoprotein hormone receptors. Using appropriate transgenic fly lines, we achieved LGR1 knockdown and demonstrated that LGR1 deficient progeny are enormously susceptible to desiccation stress compared to their control counterparts. LGR1 was notably enriched in the Malpighian tubules (MTs) and reproductive organs. Given the prominent sexual dimorphism in desiccation tolerance responses, we will explore the dynamic regulation of LGR1 in a tissue- and sex-specific manner. D. melanogaster MTs are (in part) functionally analogous

to vertebrate renal tubules and serve excretory as well as osmoregulatory functions. Therefore, we are employing fluid secretion and efflux bioassays to determine the critical role of LGR1 on the function of MTs. Additionally, mating assays, assessment of fertility and fecundity and life history analysis will enable us to track the ramifications of LGR1 knockdown in reproductive fitness and development. In summary, our study provides the first direct evidence of the role of LGR1 on fly reproductive biology and desiccation stress tolerance. These findings can be extended to other insects and invertebrates to advance our knowledge of this evolutionarily ancient yet understudied glycoprotein hormone.

Acknowledgements: Supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant and an Ontario Ministry of Research, Innovation, and Science Early Researcher Award to J-PP.

FUNCTIONAL CHARACTERIZATION OF CHOLECYSTOKININ/SULFAKININ TYPE NEUROPEPTIDE SIGNALLING IN AN ECHINODERM REVEALS EVOLUTIONARILY ANCIENT ROLE IN INHIBITORY REGULATION OF FEEDING PROCESSES

Tinoco AB (1), Barreiro-Iglesias A (1,3), Yañez-Guerra LA (1,4), Delroisse J (1,5), Zhang Y (1), Gunner EF (1), Zampronio C (2), Jones LM (2), Egertová M (1), Elphick MR (1)

(1) Queen Mary University of London, School of Biological & Chemical Sciences, Mile End Road, London, E1 4NS, UK, (2) School of Life Sciences and Proteomics, Research Technology Platform, University of Warwick, Coventry, UK, (3) Department of Functional Biology, CIBUS, Faculty of Biology, University of Santiago de Compostela, 15704, Santiago de Compostela, Spain, (4) Living Systems Institute, University of Exeter, Exeter EX4 4QD, UK, (5) University of Mons, Biology of Marine Organisms and Biomimetics Unit, 7000 Mons, Belgium

Cholecystokinin (CCK) and gastrin are structurally related peptide signalling molecules that regulate digestive physiology and/or mediate satiety in mammals and other vertebrates. Similarly, CCK-type neuropeptides known as sulfakinins (SK) inhibit food intake and affect gut activity in insects. The evolutionary origin of CCK/SK-type neuropeptide signalling can be traced to the urbilaterian common ancestor of protostomes and deuterostomes, but little is known about CCK/SK-type signalling in non-chordate deuterostomes. Here we have characterised CCK/SK-type signalling for the first time in an echinoderm - the starfish Asterias rubens, which feeds by everting its stomach out of its mouth and over the digestible soft tissues of prey (e.g. mussels). A cDNA encoding an A. rubens CCK-type precursor protein (ArCCKP) was sequenced and two ArCCKP-derived neuropeptides with sulfated tyrosines (Y*) were detected in extracts of A. rubens radial nerves - ArCCK1 (pQSKVDDY*GHGLFW-NH2) and ArCCK2 (GGDDQY*GFGLFF-NH2). Both ArCCK1 and ArCCK2 act as potent ligands for ArCCKR, an A. rubens ortholog of CCK/SK-type receptors. Analysis of the distribution of the ArCCKP transcript and ArCCK1 in A. rubens revealed expression in the central nervous system, digestive system, tube feet and body wall. Furthermore, sites of action of CCK/SK-type peptides in these organs/systems were revealed immunohistochemically using novel antibodies to ArCCKR. In vitro pharmacological experiments revealed that both ArCCK1 and ArCCK2 cause dose-dependent contraction of cardiac stomach, tube foot and body wall apical muscle preparations. Furthermore, injection of ArCCK1 or ACCK2 in vivo triggers cardiac stomach retraction and inhibition of the onset of feeding in A. rubens. These findings provide new insights into the mechanisms of neuropeptidergic regulation of the unusual extra-oral feeding behaviour of starfish. Furthermore, we have discovered that an evolutionarily ancient role of CCK/SK-type neuropeptides as inhibitory regulators of feeding-related processes in the Bilateria has been conserved in the unique context of the pentaradial body plan of an echinoderm.

Acknowledgements: Supported by: BBSRC, Leverhulme Trust, Queen Mary University of London, Fund for Scientific Research of Belgium (F.R.S.-FNRS), Mexican Council of Science and Technology (CONACyT) and China Scholarship Council.

A COMPLETE PACKAGE OF METHODS TO SIMULTANEOUSLY EXTRACT AND QUANTIFY SECRETONEURIN, OTHER NEUROPEPTIDES AND STEROID HORMONES FROM SMALL TISSUE SAMPLES USING LC-MS

Lu C (1), Peng D (1), Hu W (2), Trudeau VL (1)

(1) Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

(2) State Key Laboratory of Freshwater Ecology and Biotechnology,

Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

The detection and quantification of hormones are important to assess the reproductive and stress status of experimental

models and for the diagnosis of diseases in human and veterinary clinics. Secretoneurins (SN) has been proposed as a new sex hormone but effective quantification methods are challenging. Traditional methods require the use of antibodies with either radioactive or non-radioactive tracers. There are difficulties with these methods in terms of sensitivity, specificity, and inter-laboratory repeatability. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) can circumvent many of these challenges. Another source of variation is the extraction of lipophilic steroidal compounds, which is incompatible with the extraction of hydrophilic peptide hormones. We have developed efficient extraction and sensitive detection methods of SN with numerous other peptide and steroid hormones in the same tissue sample in mice and zebrafish. The extraction efficiency for both peptide and steroid analytes is over 85% and the average extraction cost is about 1 USD per sample. The standard deviation for extraction and LC-MS/MS analysis for each compound varies between 5-10%. The steroid hormones can be quantified in the low to medium fmol/uL range. We quantified peptide hormones are in the high fmol/uL to low pmol/uL range. For the first time, our group measured SNa in the2-8 pmol/uL range from the brain or pituitary harvested from a single female zebrafish. We detected SNb in the 3-4 pmol/uL range from the same samples. This makes it feasible to study the correlation between SNs and other peptide and steroid hormones by quantifying them simultaneously. The basic workflow will be outlined. This cost-effective package will be applied to the assessment of hypothalamo-pituitary-gonadal function in normal and mutant mice and zebrafish and may be adaptable to many other hormones across species.

Acknowledgements: Supported by NSERC (VLT), University of Ottawa Research Chair in Neuroendocrinology (VLT) and ther Chinese Academy of Sciences (WH).

CHARACTERIZATION OF THE SECRETOGRANIN-II/SECRETONEURIN SYSTEM IN THE BRAINPITUITARY-GONADAL AXIS OF ZEBRAFISH

Peng D (1), Lu C (1), Levavi-Sivan B (2), Hu W (3), Trudeau VL (1)

(1) Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

(2) Department of Animal Sciences, The Hebrew University of Jerusalem, Israel

(3) Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

Secretoneurin (SN) is a 31-34 amino acid neuropeptide, derived from the precursor protein secretogranin-II (Scg2). Teleosts have two types of Scg2 precursors named Scg2a and Scg2b that are processed into SNa and SNb, respectively. The previous studies in our lab demonstrated that exogenous SNa stimulated the release of luteinizing hormone (Lh) in goldfish, while mutation of scg2a and scg2b genes led to decrease lhb and cga subunit mRNA levels in adult zebrafish pituitary. The objective of this study was to determine the distribution of SNa in relation to other known reproductive hormones in zebrafish brain and pituitary by double immunofluorescent staining and to quantify the variation of the SN peptides as well as other reproductive hormones during the zebrafish ovulation by mass spectrometry. SNa-immunoreactivity (ir) was observed in neurons in the olfactory bulbs, ventral telencephalon, preoptic area (POA) and hypothalamus. SNa colocalizes with isotocin in magnocellular cells in the POA and fibers pass through the pituitary stalk and terminate largely in the neurointermediate lobe (NIL). The SNa-ir fibers were less abundant but clearly present in the pars distalis. Using the lhb-RFP x fshb-eGFP transgenic zebrafish line, we observed SNa-ir near gonadotrophs but not in them. Some endocrine cells within the NIL and rostral pars distalis also express SNa-ir, but their identity is currently under investigation. The mass spectrometry results revealed the natural variation of SNa peptide levels as well as the fluctuation of GnRH3, isotocin, estrone and estradiol during ovulation. Quantification of SNa1-14, SNa1-18, SNa19-34 also indicates time-dependent processing of the SNa peotide during the course of ovulation. Significantly lower levels of SNa and SNb peptides were quantified in males compared to females. These results indicate that brain and pituitary SNa levels vary in relation to the ovulatory cycle, further implicating it in the control of fish reproduction.

Ackowledgements: Supported by China Scholarship Council (DP), NSERC Discovery Grants (VLT), the Institute of Hydrobiology, Chinese Academy of Sciences-University of Ottawa Collaboration (WH and VLT).

Session 20: Recent topics in comparative endocrinology

SEROTONIN: A UBIQUITOUS YET OBSCURE HORMONE IN TELEOST FISH

McDonald, M. Danielle

Department of Marine Biology and Ecology, RSMAS, University of Miami, Miami, FL, USA

The neurochemical serotonin (5-HT, 5-hydroxytryptamine) is most commonly known as a neurotransmitter. In fish, 5-HT plays a role in the stress response, oxygen sensing, cardiovascular physiology, reproduction, aggression, and other processes. Over the years, little research has been done investigating the role of circulating 5-HT in the control of various physiological and behavioral processes in fish, despite there being measurable concentrations of 5-HT in the blood. The hypothesis of our work is that 5-HT plays a significant role as a hormone and that circulating levels of 5-HT are tightly controlled. Using the Gulf toadfish, Opsanus beta, as a model system, we have found that circulating 5-HT concentrations fluctuate in times of stress, during the control of certain physiological processes, and upon exposure to pollutants that directly and, perhaps, indirectly target serotonergic processes, such as selective serotonin reuptake inhibitors (SSRIs) and polycyclic aromatic hydrocarbons (PAHs), respectively. We have also determined that 5-HT can be cleared from the blood via transport-mediated uptake into tissues, degradation, and excretion into the urine and bile. Current work is investigating whether hormonal 5-HT plays a role in controlling the cardiovascular response to hypoxia and determining mechanisms of 5-HT homeostasis within the heart, gill, and blood vessels.

Acknowledgements: This research is supported by the National Science Foundation (Grant # IOS-1754550).

EVOLUTIONARY CONSERVED ACTIONS OF LEPTIN ON IMMUNE FUNCTION ACROSS DEVELOPMENTAL STAGES IN XENOPUS LAEVIS

Robyn E. Reeve and Erica J. Crespi

School of Biological Sciences, Center for Reproductive Biology, Washington State University, Pullman, WA 99164

The cytokine hormone leptin is well established as an immunomodulator in mammals, but much less is known about its role in the regulation of immune function in other vertebrates. To address this gap, we conducted a series of experiments exploring the role of leptin on immune function across developmental stages in the South African clawed frog (Xenopus laevis). Prior work has shown that leptin treatment rescued larvae exposed to pathogenic bacteria from death and restored growth and development. After metamorphosis, leptin administration stimulates inflammation at the site of a wound and stimulates proliferation of and stimulates proliferation of splenocytes in culture. To gain a better understanding of how leptin signaling may influence immunity before and after metamorphosis, we localized leptin receptor expression using in situ hybridization and fluorescent immunocytochemistry of pSTAT-3 (signal of leptin receptor activation) across immune organs in X. laevis larvae and juveniles. We showed that the leptin receptor is expressed in the skin, thymus, and spleen at both larval and juvenile stages. Expression patterns changed with life stage from being more ubiquitously expressed in the larvae to being associated with more specific structures in juvenile frogs. In the juvenile skin, leptin receptor is highly expressed in glandular cells and in the epidermis. In the spleen leptin is expressed in the outer capsule and dispersed cells within the pulp. Taken together, these results suggest that the immunomodulatory roles of leptin arose early in vertebrate evolution and are conserved. Our findings also show that leptin may have novel roles in the development of the immune system and the epidermis that have not been previously studied.

Acknowledgements: Supported by AMS Student Research Fellowship (RER) and WSU Seed Grant (EJC).

CHARACTERIZING THE G PROTEIN-COUPLED RECEPTOR FAMILY SREB (GPR27, GPR85, GPR173), AND A NEW MEMBER (SREB4), ACROSS FISH EVOLUTION

Breton T (1), Sampson W (1), Clifford B (2), Phaneuf A (1), Smidt I (3), True T (1), Wilcox A (1), Lipscomb T (4, 5), Murray, C (4), DiMaggio M (4)

(1) Division of Natural Sciences, University of Maine at Farmington, Farmington, ME, USA

(2) Science Department, Southern Maine Community College, South Portland, ME, USA

(3) Department of Biology, Bates College, Lewiston, ME, USA

(4) Tropical Aquaculture Laboratory, Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, FL, USA

(5) Livingston Stone National Fish Hatchery, US Fish and Wildlife Service, Shasta Lake, CA, USA

The SREB (Super-conserved Receptors Expressed in Brain) family of G protein-coupled receptors is highly conserved across vertebrates and consists of three members: SREB1 (orphan receptor GPR27), SREB2 (GPR85), and SREB3 (GPR173). Ligands for these receptors are largely unknown or only recently identified, and functions for all three are still beginning to be understood, including roles in glucose homeostasis, neurogenesis, and hypothalamic control of reproduction. In addition to the brain, all three are expressed in gonads, but relatively few studies have focused on this, especially in non-mammalian models or in an integrated approach across the entire receptor family. The purpose of this study was to more fully characterize sreb genes in fish, using comparative genomics and gonadal expression analyses in five diverse ray-finned (Actinopterygii) species across evolution. Several unique characteristics were identified in fish, including: 1) a novel, fourth euteleost-specific gene (sreb4) that likely emerged from a copy of sreb3 in a separate event after the teleost whole genome duplication, 2) sreb3 gene loss in Order Cyprinodontiformes, and 3) expression differences between an ancestral gar species and teleosts. Overall, gonadal patterns suggested an important role for all sreb genes in teleost testicular development, while gar were characterized by greater ovarian expression that may reflect similar roles to mammals. The novel sreb4 gene was also characterized by several unique features, including divergent but highly conserved amino acid positions, and elevated brain expression in puffer (Dichotomyctere nigroviridis) that more closely matched sreb2, not sreb3. These results demonstrate that SREBs may differ among vertebrates in genomic structure and function, and more research is needed to better understand these roles in fish.

Acknowledgements: Research reported in this project was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103423.

NEUROPROTECTIVE EFFECTS OF GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR TYPE 1 (IGF-1) AFTER HYPOXIC-ISCHEMIC INJURY IN CHICKEN CEREBELLAR CELL CULTURES

Baltazar-Lara R, Ávila-Mendoza J, Martínez-Moreno CG, Carranza M, Pech-Pool S, Vázquez-Martínez O, Díaz-Muñoz M, Arámburo C, and Luna M

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro 76230, México

Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) exert protective and regenerative actions in response to neural damage. These peptides are expressed locally in the central nervous system (CNS), and their expression is increased when there is damage induced by hypoxia-ischemia (HI). In this study, we explored the neuroprotective effects of GH and IGF-1 administration as well as the involvement of these endogenously expressed hormones in embryonic chicken cerebellar cell cultures exposed to an acute HI injury. Primary cultures of cerebellar neurons were subjected to hypoxic-ischemic conditions (<5% O2, 1 g / L glucose) for 12 h (HI) and subsequently to reoxygenation and normal glucose conditions (HI+Ox) for another 24 h. GH and IGF-1 were added either during or after HI, and their effect upon cell viability, apoptosis, or necrosis was evaluated. In comparison with normoxic controls (Nx), a significant decrease of cell viability (54.1 ± 2.1%) and substantial increases in caspase-3 activity and LDH release (1.78 y 5.3 times respectively) were observed in the reoxygenation group. On the other hand, both GH and IGF-1 treatments after injury (HI+Ox) significantly increased cell viability (77.2 ± 4.3% and 72.3 ± 3.9%, respectively) and decreased both caspase-3 activity (1.18. and 1.27 times respectively) and LDH release (1.8 and 2.6 respectively). Incubation under reoxygenation conditions provoked an important increase in the local expression of GH (3.2-fold) and IGF-1 (2.5-fold) mRNAs. However, GH gene silencing with specific siRNAs decreased both GH and IGF-1 mRNA expression (1.7- and 0.9-fold, respectively) in the HI+Ox group, indicating that GH regulates IGF-1 expression under these incubation conditions. In addition, GH knockdown significantly reduced cell viability (35.9 ± 2.1%) and substantially increased necrosis (1011 ± 276.6%). In contrast, treatments with GH and IGF-1 stimulated a partial recovery of cell viability (45.2 ± 3.7% and 53.7 ± 3.2%) and significantly diminished the release of LDH (320.1 ± 25.4% and 421.7 ± 62.2%), respectively. Our results show that GH, either exogenously administered and/or locally expressed, can act as a neuroprotective factor in response to hypoxic-ischemic injury and that this effect may be mediated, at least partially, through IGF-1 expression.

Acknowledgements: We thank Pilgrim's Pride, Querétaro, México, who donated fertilized broiler eggs used in this research. Supported by PAPIIT-DGAPA UNAM (IN208419, IN227020, IN209621,) and CONACYT (285004, 214971). SMPP received a PhD fellowship (696979) from CONACYT.

Lightning Rounds Abstracts

#13-1: COULD THE IGF-1 ACT AS AN ENDOCRINE MEDIATOR BETWEEN CIRCADIAN OSCILLATORS IN GOLDFISH?

Alonso-Gómez A, Madera D, Alonso-Gómez AL, Delgado MJ, Valenciano AI

Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Madrid, Spain

The circadian system determines the temporal organization of physiological processes. The key elements of this circadian system are the biological clocks or oscillators, its output signals, and the exogenous and endogenous synchronizers that adjust its circadian rhythm. Due to the non-hierarchical organization of the circadian system in fish, it is necessary the existence of signals that mediate the communication among biological clocks. Previous data of our group demonstrate that the insulin-like growth factor-1 (IGF-1) shows a daily rhythm of expression in the liver of goldfish (Carassius auratus), entrained by both external zeitgebers, photoperiod and scheduled feeding. The aim of this work is to study the possible role of the IGF-1 as an output signal from the hepatic oscillator that might potentially synchronize brain circadian clocks in goldfish. For that purpose, we study the effect of IGF-1 addition on clock genes expression at two different times of daily photocycle, at the light period (ZT4) and at the darkness (ZT18), in organotypic cultures of functional circadian oscillators in brain, hypothalamus, pituitary, optic tectum, cerebellum and vagal lobe. In the vagal lobe, the administration of IGF-1 (10 nM) during the photophase (ZT4) reduces the expression of per-1a and rev-erbα after 2 hours of culture time, meanwhile the addition of the IGF-1 during scotophase (ZT18) increases the expression of per1-b and rev-erbα after 2h, and per1b and bmal1a after 4h of culture time. In the cerebellum, the addition of IGF-1 (10 nM) during the photophase reduces the expression of per-1a and rev-erbα after 2 hours of culture time and reduces bmal1a when is present in the culture medium for 8 hours during scotophase. In hypothalamus, pituitary and optic tectum the addition of IGF-1 during the light period does not modify clock genes expression. In conclusion, this is the first study that demonstrates the effect of the IGF-1 on the expression of clock genes in the vagal lobe and cerebellum supporting the possible role of this peptide as a rhythmic output of the hepatic oscillator that entrains neural oscillators in goldfish.

Acknowledgements: Supported by the Spanish MICIU (PID2019-103969RB-C32). A. Alonso-Gómez and D. Madera are predoctoral fellows FPI (BES-2017-081398) and UCM (CT42/18-CT43/18), respectively.

#13-2: EVALUATION OF THE NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE NEURAL RETINA

Epardo D (1), Balderas-Márquez JE (1), Fleming T (1,2), Carranza M (1), Luna M (1), Harvey S (2), Ávila-Mendoza J (1), Arámburo C (1), Martínez-Moreno CG (1)

(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro. 76230, México

(2) Department of Physiology, University of Alberta, Edmonton T6G 2H7, Canada

Growth hormone (GH) is a pleiotropic hormone with well accepted roles in the central nervous system. Using a comparative and integrative approach, we found a strong neurotrophic effect of GH that includes neuroprotection, the activation of several signaling pathways, synaptogenesis, axogenesis and cell survival. The objective of this study was to determine if GH enhances retinal regeneration in a neural damage model of kainate mediated excitotoxic injury. In chickens, we found that after a single intravitreal KA (20 mg) injection at postnatal day 1 (P1), followed by 10 post-injury GH injections (300 ng) from P2 to P16, GH exerted a neuroprotective and neuroregenerative effect. This was confirmed through retinal morphometry and by analyzing the relative changes of transdifferentiation several neurotrophic factors (FGF2, PCNA, Bcl-2, BDNF, TNF-R2, SNAP25, GAP43 and Sox2) by qPCR or Western blot. In postnatal avian retinas, the activation of notch signaling pathway was observed since Notch1, Hes5 and Ascl-1a mRNA expression significantly changed. We also studied the effect of GH on

relative changes of glutamate receptors NMDA-R (NR-1 subunit) and KA-R (GRIK4 subunit). Interestingly, GH promoted retinal tissue recovery and increased the expression of FGF2 mRNA, although no effect for Ascl-1a was observed. In this model, notch signaling was activated by GH, promoting Notch1 and Hes5 expression. In addition, GH increased BDNF, Sox2, NR-1, GRIK4 and DLG1 mRNA levels, but not SNAP25. In concordance, GH increased Bcl-2 and GAP43 protein levels, and recovered intact TNF-R2 protein levels. In summary, chronic treatment with GH was able to induce the upregulation of neurotrophic, synaptogenic and transdifferentiation markers in the chicken retina after excitotoxicity.

Acknowledgements: Supported by PAPIIT-DGAPA UNAM (IN201817, IN208419, IN227020) and CONACYT (285004, 214971). DE and JEBM received a PhD fellowship (1083209 and 921788) from CONACYT.

#13-3: IS NOCTURNIN EXPRESSION MODIFIED BY DIFFERENT THERMAL CONDITIONS IN GOLDFISH?

Madera D, Alonso-Gómez A, Valenciano AI, Delgado MJ, Alonso-Gómez AL

Department of Genetics, Physiology and Microbiology. Faculty of Biology, Complutense University, Madrid, Spain

Nocturnin (NOC) is a phosphatase discovered in Xenopus laevis retina displaying a daily rhythmic expression. Recent studies suggest its implication in the transformation of NADP(H) to NAD(H), being a key molecule for the regulation of metabolism. In mammals, different works point to a relationship among nocturnin, the circadian system and the thermal variations of the environment. However, nothing is known about the possible physiological role of this enzyme in poikilotherms. Our previous studies described the expression of three noc paralogs, due to the whole genome duplications experimented by teleosts and Cyprininae Subfamily. Therefore, our aim was to investigate the effect of temperature on the relative abundance of all noc paralogs (noc-a1, noc-a2 and noc-b1) expressed in goldfish (Carassius auratus). For this purpose, fish were kept under different thermal conditions (12, 17, 22 and 27ºC), with 12L:12D photoperiod and satiating feeding conditions, scheduled at ZT1, ZT4, ZT7, ZT10. After 30 days, hypothalamus, pituitary, retina, muscle, liver and adipose tissue were sampled and noc paralogs expression was analyzed by RT-qPCR. Noc-a1 and noc-a2 transcripts were increased by high temperatures in pituitary, indicating a reverse compensation response, while noc-b1 differences were not significant. In the hypothalamus and retina, no differences were found in any paralog. On the other hand, in the liver and adipose tissue, noc-a1 levels were higher in fish at 27ºC, however in the muscle, its expression was induced by 12ºC acclimation. Liver expression of noc-a2 and noc-b1 increased at temperatures below 22ºC, while the differences found were lower in muscle and not significant in adipose tissue. Our results show a differential regulation of each noc paralog in goldfish, suggesting a functional specialization, mediated by direct and tissue-specific reverse compensation mechanisms. This study demonstrates, for the first time in fish, the effect of different thermal acclimations on noc expression and reveals to be an important reference studying the relationship of this enzyme with metabolism and circadian system.

Acknowledgements: Supported by the Spanish MICIU (PID2019-103969RB-C32). Madera D and Alonso-Gómez A are predoctoral fellows UCM (CT42/18-CT43/18) and FPI (BES-2017-081398), respectively.

#13-4: SEX-DEPENDENT MODULATION OF GENES INVOLVED IN GROWTH AND REPRODUCTION BY GROWTH HORMONE AND LUTEINIZING HORMONE IN TILAPIA

Celino-Brady FT (1), Breves JP (2), Seale AP (1)

(1) Department of Human Nutrition, Food and Animal Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i, USA

(2) Department of Biology, Skidmore College, Saratoga Springs, New York, USA

Trade-offs between growth and reproduction underlie sexual dimorphism in fishes. Nonetheless, the hormonal underpinnings of these tradeoffs have not been fully resolved. The growth hormone/insulin-like growth factor (GH/IGF) system plays a central role in regulating growth and development, while gonadotropins support gonadal development. To understand the interactions between factors that control growth and reproduction, we examined how the activities of luteinizing hormone (LH) are modulated by GH, and conversely, how targets of GH are affected by LH in Mozambique tilapia (Oreochromis mossambicus). Using our hypophysectomy model, we tested the effects of ovine GH (oGH) and oLH on the expression of gh receptor 2 (ghr2), igf1, and igf2 in muscle and ghr2, igf1, igf2, igf3, estrogen receptors α and β (erα, erβ), and androgen receptors α and β (arα, arβ) in the gonads. Gonadosomatic index (GSI) was reduced in females following hypophysectomy; the combined administration of oGH and oLH restored GSI to control levels. In muscle, ghr2 was more responsive to oGH in males, while igf2 was more responsive to oLH in females. In the gonads of control fish,

ghr2 and erβ were higher in females, whereas igf1, igf3, erα, and arß were higher in males. Among these transcripts, the effects of hypophysectomy were most pronounced on igf3. Reduced igf3 levels following hypophysectomy were restored by both oGH and oLH alone, or in combination, in males. In females, the combination of both hormones was required to recover igf3 levels. The combination of oGH and oLH restored the hypophysectomy-induced decrease in arβ in females, while there were no effects of oGH or oLH on arβ in males. Our results indicate that genes associated with growth and reproduction exhibit sexually-dimorphic responses to oGH and oLH in tilapia.

Acknowledgements: Supported by the NOAA/University of Hawaii-Sea Grant (#NA14OAR4170071, R/SB-18), NIFA Hatch (HAW02051-H), NIDDK NIH (R21DK111775) to A.P. Seale and NSF (IOS-1755131) to J.P. Breves.

#13-5: LEPTIN SIGNALING STIMULATES PERIPHERAL ANGIOGENESIS DURING XENOPUS LARVAL DEVELOPMENT

Curtis GH, Reeve RE, Whitfield K, Crespi EJ

School of Biological Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA 99164-4236

Leptin signaling has been shown to increase blood vessel formation in adipose tissue and tumors in mammals, as well as vascularization of chick chorioallantoic membranes. Our lab has shown that leptin injection induced up-regulation of phosphorylated-STAT3 in the blood vessels of the ventral tail fin, indicating leptin receptor activation of Jak-STAT signaling in these vessels. Using a transgenic line of Xenopus laevis that expresses a green fluorescent protein on vascular endothelial cells (flk-1:GFP), we tested whether leptin promotes angiogenesis in the ventral tail fin during larval development. First, we reared larvae in 0.1% MMR containing 0, 50, or 100 ng/mL leptin from Nieuwkoop and Faber (NF) stage 35 until by NF 43, when vessels start to grow into the ventral tail fin. We found that both doses of leptin increased the number of blood vessels extending into the tail fin by 33% by NF 47, and more than doubled the number of vascular loops, or vessels that reconnect with the posterior cardinal vein of the tail. In a second experiment, flk1:GFP larvae were reared in 0.1% MMR containing leptin antibody in two concentrations (1:100 or 1:500) to immunoneutralize leptin signaling, with vehicle controls of rabbit serum at the same dilutions and an untreated control. The 1:100 dose of leptin antibody significantly reduced the number of vessels and loops in the tail fin, but also reduced development rate and induced abnormalities, which could indirectly contribute to the reduced tail vasculature. These results provide the first evidence that leptin signaling stimulates peripheral angiogenesis during vertebrate embryonic/larval development.

Acknowledgements: Supported by Grants in Aid of Research from Sigma Xi and the Society for Integrative and Comparative Biology awarded to G. Curtis.

#13-6: **CHARACTERIZING THE SKIN MICROBIOTA IN RANA CATESBEIANA ACROSS NATURAL METAMORPHOSIS AND HORMONE EXPOSURE**

Imbery JJ (1), Jia B (2), Van Rossum T (3), Lo R (2), Brinkman FSL (2), Helbing CC (1)

(1) Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

(2) Department of Molecular Biology and Biochemistry, Simon Fraser University, British Columbia, Canada

(3) European Molecular Biology Laboratory, Structural and Computational Biology Unit, Heidelberg, Germany

Induction of frog metamorphosis by thyroid hormones (THs) has been well characterized, making tadpoles an excellent model for studying the effects of THs on vertebrate growth, development, and metabolism. The genetic and morphological shifts of this TH-dependent process have been extensively researched, however, there is little information on the effects of metamorphosis on the tadpole microbiome. To investigate these effects, we used 16S rRNA sequencing of swabs from skin between the eyes and nares to characterize the skin microbiome of Rana catesbeiana (American bullfrog) at different stages of natural metamorphosis. A significant shift in bacterial community composition and a decrease in alpha diversity was seen as tadpoles progress through metamorphosis, with a remarkable enrichment of bacteria from the Verrucomicrobia phylum. To test if this shift in microbial communities was hormone dependent, premetamorphic tadpoles underwent 48 h exposure to biologically relevant concentrations of THs: 3,5,3'-triiodothyronine (T3) and L-thyroxine (T4), and exposure to 17-beta estradiol (E2) as a hormone control. In addition to 16S rRNA sequencing, we developed a metatranscriptomic pipeline to investigate the differential expression of bacterial and viral transcripts using RNA-Seq performed on back skin (BS), olfactory epithelium (OE), and tail fin (TF) tissue from these tadpoles. No significant shift in bacterial communities after hormone exposure with 16S rRNA sequencing was observed indicating hormone independence. Metatranscriptomic data captured little differential expression of bacterial transcripts, confirming the 16S rRNA results, and demonstrates a similar distribution of bacterial transcripts in the BS, OE, and TF.

Moreover, T4 and E2 exposure did not result in the differential expression of any viral transcripts, however, T3 exposure results in the differential expression of a Ranavirus protein-encoding transcript in the TF indicating that T3 exposure may influence the impact of this pathogen. Overall, these data present an interesting insight into the maturation of the tadpole microbiome over the course of metamorphosis and demonstrates that these microbial communities appear to change in tandem with natural morphological changes instead of hormone presence.

Acknowledgements: Supported by a NSERC Strategic Grant (CCH, FB), a NSERC CGS-M Graduate Student Award (JJI), and a CIHR - Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award (TVR).

#13-7: NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE CHICKEN RETINA DURING INFLAMMATION

Balderas-Márquez JE, Epardo D, Carranza M, Luna M, Arámburo C, Martínez-Moreno CG

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro. 76230, México

Growth hormone (GH) has neurotrophic actions in the nervous system that include neurogenesis, neuroprotection and neuroregeneration. It is well established that GH promotes axonal growth, synaptogenesis, and cell survival in neuroretinal cells. However, the molecular mechanisms underlying the protective and regenerative effects of GH are still largely unknown. We propose that GH could modulate the inflammatory response to prevent cell death and tissue injury in retina, since an exacerbated inflammatory process leads to progressive retinal damage. In this work we developed an in vivo model to evaluate the anti-inflammatory effect of GH in response against LPS-induced inflammation. To that end, an acute dose of LPS (10 µg) was injected in the vitreous of 1-day old chicks to provoke local inflammation, and changes in the mRNA expression of cytokines were determined after 6 h. LPS induced a significant increase on the expression of IL1β, IL6, IL8, LITAF, iNOS and IFNγ mRNAs. We found that GH substantially reduced the expression of IL1β, IL6, IL8, iNOS and IFNγ mRNAs in LPS+GH co-treated retinas. On the other hand, LPS significantly decreased TRL4 mRNA expression. The effect of GH in microglia was analyzed in a mouse cell line (SIM-A9) after confirming that GHR was expressed and functional. In microglial cells incubated with both GH+LPS, the expression of TLR4 was similar to controls but there were no effects on GHR. In addition, GH decreased LPS-phospho-p65 induced immunoreactivity, suggesting a decrease in the translocation of NF-kB into the nucleus. Anti-inflammatory actions of GH against LPS were evaluated through cytokine production in microglia. We observed that LPS induced an increase on IL1β, IL6, TNFα, and iNOS mRNAs expression in SIM-A9 cells. Co-treatments with LPS+GH reduced TNFα and increased TGFβ and IL10 expression. In conclusion, GH exerts protective actions as a part of a complex intercellular network that includes pro- and anti-inflammatory cytokines.

Acknowledgements: Supported by PAPIIT-DGAPA UNAM (IN207018, IN208419, IN227020) and CONACYT (285004, 214971). JEBM received a M.Sc. fellowship (921788) from CONACYT.

#13-8: CHARACTERIZATION OF METABOLIC DEFECTS IN INSULIN-SIGNALING IMPAIRMENT DROSOPHILA MELANOGASTER

Alvarez-Rendón JP, Riesgo-Escovar JR

Department of Developmental Neurobiology and Neurophysiology, Instituto de Neurobiología, UNAM, Querétaro, México

Human diabetes encompasses a group of metabolic disorders characterized by hyperglycemia. This is generally caused by the lack of insulin production by the pancreas (type I diabetes) or its inefficient signaling (type II diabetes). Influenced by genetic and environmental factors, diabetes, an uncurable disease, entails detrimental consequences for the patient's quality of life both in the short and the long terms.

Drosophila melanogaster has a short life-cycle and an evolutionarily conserved insulin signaling pathway. We characterize defects present in fly diabetic states using adults, consequence of an organismal deficiency in insulin signaling, through development and aging, using viable heteroallelic mutants. We quantitated lipids and sugars levels of InR (the insulin receptor fly homologue), Dp110 (the catalytic subunit of the phosphoinositol-3-kinase fly homologue, downstream of InR), and dS6K (the ribosomal protein S6 kinase beta-1 fly homologue, under the control of TORC1, also regulated by insulin signaling) in heteroallelic mutants, as well as wildtype controls with the same genetic background, in both sexes, and at different times post-eclosion. We consistently observed a weight decrease in all heteroallelic mutants. We also observed significantly higher lipid levels in all mutant combinations. InR mutants show more severe defects, particularly in carbohydrate levels; dS6K mutants have higher lipid levels but no dysregulation in total carbohydrate levels. Dp110 mutants have defects particularly in the first post-eclosion days.

Diabetic longitudinal studies, looking at the flies as they age, as we do, describing the evolving defects consequences of low insulin signaling, are scarce. This study describes evolving metabolic mutant phenotypes in three heteroallelic mutants with different types of insulin signaling defects, thus enabling characterization of their consequences throughout adult life.

Acknowledgements: Funding: Papiit # IN207719 to JRR-E and CONACYT scholarship #612316 to JPAR.

#13-9: PHOENIXIN-20 (PNX-20) SUPPRESSES FOOD INTAKE AND PROMOTES GLYCOLYSIS IN ZEBRAFISH

Rajeswari JJ (1), Blanco AM (1,2), Unniappan S (1)

(1) Western College of Veterinary Medicine, University of Saskatchewan, Saskatchewan, Saskatoon, Canada

(2) Facultade de Bioloxía and Centro de Investigación Mariña, Universidade de Vigo, Vigo, Spain

Phoenixin is a 20 amino acid peptide (PNX-20) cleaved from the precursor named small integral membrane protein 20 (SMIM20). PNX20 has multiple biological functions in mammals. However, the biology of PNX-20 in non-mammalian vertebrates is unclear. This research aimed to determine whether PNX-20 has any role in feeding and metabolism in zebrafish. The qPCR based mRNA expression analysis suggests that both SMIM20 and its putative receptor, super conserved receptor expressed in brain 3 (SREB3), are expressed in both central and peripheral tissues of zebrafish. Our immunohistochemical analysis showed the presence of PNX and SREB3-like immunoreactivity in the gut and zebrafish liver (ZFL) cell line, which suggests its possible role in feeding and metabolism. Short-term fasting (7 days) resulted in significantly decreased smim20 mRNA expression in the brain, gut, liver, gonad, and muscle, pointing to a possible role for PNX-20 in food intake regulation. Indeed, single intraperitoneal injection of 1000 ng/g body weight PNX-20 reduced feeding in both male and female zebrafish. qPCR analysis suggests that the appetite suppressive effect is likely in part by enhancing hypothalamic cart and reducing hypothalamic/gut preproghrelin mRNA expression. Furthermore, our in vivo results using ZFL cells demonstrated that PNX-20 modulates the expression of genes involved in glucose transport and metabolism in zebrafish. We observed upregulation of glycolytic genes and downregulation of gluconeogenic genes in ZFL cells treated with PNX-20. In addition, PNX-20-treated ZFL cells exhibited significantly higher ATP production rate associated to glycolysis than control cells, confirming a positive role for PNX-20 on glycolysis. Together, these results indicate that PNX-20 is an anorexigen and has important roles in the regulation of metabolism in zebrafish.

Acknowledgements: Supported by NSERC Discovery Grant and the Centennial Enhancement Chair in Comparative Endocrinology (SU), and the WCVM-CGPS Dean's scholarship to (JJ).

#14-1: FIBROBLAST GROWTH FACTOR 8-DEFICIENT MICE EXHIBIT NORMAL NEONATAL GONADAL MORPHOLOGY AND FUNCTION

Jewell K, Tsai PS

Department of Integrative Physiology, University of Colorado, Boulder, Colorado, USA

Fibroblast growth factor 8 (FGF8) is a signaling molecule implicated in the development and maintenance of the hypothalamic-pituitary-gonadal axis. FGF8 signaling deficiency disrupts the gonadotropin-releasing hormone (GnRH) system in both mice and humans, leading to reduced GnRH, aberrant puberty, and infertility. In addition to a role in GnRH neuron ontogeny, limited evidence suggests FGF8 may also participate in the prenatal organization of ovaries and testes, although this notion has not been rigorously tested. The goal of the present study was to determine if neonatal mice harboring a FGF8 hypomorphic allele (FGF8 Het mice) exhibited testicular and ovarian morphological and functional defects. The gonads of FGF8 Het mice and their wildtype (WT) littermates were collected on postnatal days (PN) 1 and 3. Neonatal ovarian follicular function and oocyte reserve were analyzed by immunohistochemistry (IHC) of proliferating cell nuclear antigen (PCNA), a marker present in mitotic cells and neonatal oocytes, coupled with hematoxylin counterstain. The number of PCNA-positive cells per unit area was scored for PN1 and PN3 ovaries. PN3 ovaries were further analyzed to quantify the number of oocytes and primary follicles per unit area. Neonatal testes were processed to analyze steroidogenic capacity and seminiferous tubule (ST) morphology. One subset of PN1 and PN3 testicular sections was stained by IHC for Cyp17a1, an enzyme involved in the androgen synthesis pathway in Leydig cells. A second subset of testicular sections was stained with hematoxylin and eosin for the morphometric analysis of ST area, perimeter, and diameter. Our results revealed that FGF8 hypomorphs exhibited normal neonatal morphology and functional parameters

in both ovaries and testes. Collectively, our data suggest that the developmental impact of FGF8 signaling deficiency responsible for reproductive deficits in humans and mice is primarily upon the hypothalamus, not the gonad.

Acknowledgements: Supported by NIH Grant R01 HD083260.

#14-2: THE ROLE OF THE SOMATOTROPIC AXIS IN SOCIAL STATUS-DEPENDENT GROWTH AND METABOLISM IN RAINBOW TROUT

Gilmour KM (1), Best C (1), Magnan J (1), Jennings K (1), Touma K (1), Bernier NJ (2), Mennigen JA (1)

(1) Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

(2) Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

Juvenile rainbow trout (Oncorhynchus mykiss) confined in pairs form social hierarchies, with subordinate fish showing both anorexia and prolonged elevation of the stress hormone cortisol. This combination of low food intake and elevated cortisol levels induces a catabolic state in subordinate fish, including mobilization of lipid reserves and decreased rates of muscle protein synthesis. By contrast, dominant fish monopolize and defend food resources, entering an anabolic state with increases in de novo lipogenesis. These differences provide a useful system in which to study the somatotropic axis and in particular, context-dependent actions of growth hormone (GH) in promoting lipolysis versus protein accretion. Synteny analysis supported the existence of four GH receptor paralogues in trout, ghra1 (formerly ghr1), ghra2, ghrb1 (formerly ghr2a) and ghrb2 (formerly ghr2b). After 4 d of social interaction, subordinate trout exhibited elevated transcript abundances of ghrb1 and ghrb2 in red and white muscle together with elevated hsl1 (hormone-sensitive lipase) transcript abundance. Transcript abundances of ghra1 and ghra2 did not differ from control values in white muscle of subordinates, but were reduced in red muscle, and correspondingly, transcript abundances of igf1 and igf2 (insulin-like growth factor) also were lowered in red muscle of subordinates. These results are consistent with a role for ghra in stimulating somatic growth via igf1 versus a role for ghrb in promoting lipolysis via hsl.

Acknowledgements: Supported by NSERC Discovery Grant funding to KMG, NJB and JAM, and NSERC and OGS graduate student awards to CB.

#14-3: CAN FROGS GET ANXIOUS? THE INFLUENCE OF SIMULTANEOUS EXPOSURE TO A PREDATOR AND FOOD ON FORAGING-AVOIDANCE BEHAVIOR AND GENE EXPRESSION IN THE OPTIC TECTUM OF AFRICAN-CLAWED FROGS (XENOPUS LAEVIS).

Menon NM, Carr JA

Department of Biological Sciences, Texas Tech University, Lubbock, Texas, USA

One of the long-term goals of our laboratory is to better understand the role of subcortical visual areas in fear and anxiety using frogs as a model system, as they rely entirely on subcortical visual processing. We predicted that the presence of a visual predator would decrease food intake, increase anxiety-like behavior, and increase the abundance of anxiety related transcripts in the optic tecta of African clawed frogs (Xenopus laevis). We used a total of 19 (n=9-10 in each group) adult frogs and a modified ecological tradeoff model in which we presented frogs with a repeating momentary threat (bird) as well as a food stimulus to create an ecologically relevant tradeoff where frogs must balance the persistent risk of predation with the need to forage. We measured several behavioral endpoints as well as the transcript abundance in the tectum for several anxiety-related genes. In contrast to our previous studies in juvenile frogs, food consumption was not significantly decreased in predator-exposed animals (n=10) compared to the control group (n=9). However, arm sweeping, a behavior used to gather olfactory cues of food was significantly decreased in the predator-exposed animals compared to controls. Predator-exposed animals spent significantly more time stationary and significantly reduced their entrance into the side of the tank with the predator stimulus compared to the control group. There were no changes in relative abundance of the following transcripts: CRF, serotonin 1A receptor, dopamine D1 receptor, and dopamine D2 receptor in optic tecta between the treatment groups. Collectively, this novel foraging tradeoff test was able to induce anxiety-like behavior, however its effect on feeding and anxiety-related transcript abundance requires further study.ext

Acknowledgements: Supported by National Science Foundation IOS #1656734, Texas Tech University Department of Biological Sciences and the Texas Tech University Association of Biologists.

#14-4: CHARACTERIZATION OF A LEPTIN RECEPTOR ORTHOLOG IN A JAWLESS VERTEBRATE (AGNATHAN)

Gong N (1), McCormick SD (2), Sheridan MA (1)

(1) Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA

(2) US Geological Survey, S.O. Conte Anadromous Fish Research Laboratory, Turners Falls, MA 01376, USA

Leptin has been identified in all jawed vertebrates (Gnathostomes) and plays a key role in appetite regulation and energy homeostasis. However, the origins of the leptin system are unknown and the question remains whether the system is present in jawless vertebrates (Agnathans) or it arose after the divergence of Gnathostomes and Agnathans. In this study, we identified leptin receptor (LepR) orthologs from lampreys and in-shore hagfish, extant representatives of the two Agnathan lineages, through genomic database searches. The identified LepR ortholog from the sea lamprey was further cloned and sequenced, yielding a full-length LepR-like mRNA, encoding a deduced protein of 105 kDa, designated LepRl. The lamprey LepRl is composed of a single cytokine receptor homology domain, which is in contrast to this domain being duplicated in gnathostome LepRs. In juvenile sea lamprey, the LepRl mRNA was more abundant in gill and kidney than in the brain. In up-migrating adult lamprey, LepRl mRNA was more abundant in the pituitary gland than in the brain. Specific antisera to lamprey LepRl were prepared and specifically detected the recombinant LepRl expressed by HEK293 cells, and thus were used for immunohistochemistry. LepRl-immunoreactive (LepRl-ir) cells were barely detected in the hypothalamic region of juvenile lamprey brain; however, LepRl-ir staining was seen in the ionocytes of gill. Moreover, staining was intensified in gill tissues after juvenile lamprey were acclimated in seawater (SW) for 2 weeks. The SW-induced increase in LepRl immunoreactivity corresponded to increased abundance of LepRl mRNA in gill tissue of SW-acclimated juvenile lamprey. The localization of LepRl in the Na^+/K^+-ATPase-bearing ionocytes suggests a role for leptin in SW adaption of juvenile sea lamprey, possibly involving cellular energy utilization. The potential role of LepRl in appetite regulation and energy homoeostasis in the central nervous system remains to be elucidated in adult sea lamprey during the active-feeding stage of their life cycle.

Acknowledgements: Supported by the National Science Foundation (no. 1558037).

#14-5: A REV-ERBA AGONIST ELICITS STRONG ANORECTIC RESPONSES IN FISH

Saiz N, Herrera-Castillo L, Cebrián A, Villar V, Isorna E, Delgado MJ, de Pedro N

Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Madrid, Spain

REV-ERBα is a nuclear receptor with transcriptional repressing activity, which modulates the circadian system and energy balance in mammals. However, its role in fish is much less known. To investigate whether REV-ERBα plays a role in fish energy homeostasis and feeding behavior, we administered a well-known REV-ERBα agonist, SR9009 (100 mg/kg, intraperitoneal injection) for 7 days to goldfish (Carassius auratus). Food intake, body weight and standard length were registered daily, as well as general and feeding-related locomotor activity. After the treatment, fish were sacrificed, and liver, intestine, and hypothalamus were sampled to analyze expression of feeding regulators (ghrelin, npy, leptin, pomc, orexin) by qPCR. Sub-chronic treatment with SR9009 caused a pronounced decrease in food intake, with no short-term tolerance observed. In agreement, several biometric parameters related to growth (weight and length gain, and standard growth rate) were also significantly decreased in SR9009-treated fish. The expression of hypothalamic central intake regulators (npy, pomc, orexin) was not modified by the treatment, while gut ghrelin was induced and hepatic leptin was repressed. These results suggest that none of the studied feeding regulators would be mediating the anorectic effect of REV-ERBα and other underlying mechanisms must be considered. Moreover, it should be noted that the drive to eat is regulated by both the homeostatic and hedonic brain pathways. Sub-chronic SR9009 administration did not significantly modify general locomotor activity in goldfish, although a reduction trend in food anticipatory activity was observed. These results suggest possible actions of SR9009 in the initial phases of alert and food localization, as in the consummatory phase of feeding. In summary, our study supports the involvement of REV-ERBα in the regulation of feeding and energy balance in goldfish.

Acknowledgements: Supported by the Spanish MICIU (PID2019-103969RB-C32). N.S. is a predoctoral fellow from Universidad Complutense de Madrid-Banco Santander (CT42/18-CT43-18).

#14-6: INTERACTIONS OF GLUCOREGULATORY HORMONES AND LEPTIN IN THE CONTROL OF GLUCOSE HOMEOSTASIS IN THE MOZAMBIQUE TILAPIA

Russell J. Borski, Courtney A. Deck, and Jamie Mankiewicz

Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina, USA

Leptin, insulin, and glucagon are involved in regulating glycaemia in vertebrates, yet little is known about the interactions of these hormones in regulating glucose homeostasis in poikilotherms. Through a series of studies, we found that glucagon increases hepatic lepa in vitro and in vivo, with the latter being 18-fold higher than saline injected controls. However, the effects of recombinant tilapia LepA (rtLepA) on glub were more variable. Prior work in our lab showed that glucose reduces lepa in vitro, while a bolus glucose injection caused a 14-fold increase in hepatic lepa. This paradoxical result may be due to glucose stimulating the release of insulin in vivo. Indeed, we found that insulin increased hepatic lepa by 2.5-fold in primary hepatocyte incubations, while a bolus injection of insulin stimulated hepatic lepa by 70-fold. As leptin and insulin have opposing actions on carbohydrate metabolism in tilapia, we hypothesised that there may be a negative feedback loop that exists between the liver and Brockmann bodies (islet tissue). Results show rtLepA reduces insa levels in isolated Brockmann bodies under basal glucose conditions, while it stimulates insa at high glucose concentrations. These data support the idea of a leptin-insulin axis in tilapia and possibly other poikilotherms. This feedback loop, in which insulin stimulates hepatic leptin and leptin in turn inhibits further insulin production, likely serves to maintain blood glucose levels and prevent futile cycling of insulin-induced glycogenesis and leptin-induced glycogenolysis in the liver. This is the first evidence for such an axis in a non-mammalian vertebrate and may indicate conservation of this feedback loop throughout vertebrate evolution.

Acknowledgements: Funding provided by the National Science Foundation (IOS-1457040).

#14-7: IDENTIFICATION AND CHARACTERIZATION OF THE HALLOWEEN AND ECDYSONE-RESPONSIVE GENES IN THE OVARIES OF RHODNIUS PROLIXUS

Samiha Benrabaa, Ian Orchard, Angela B. Lange

Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, ON, L5L 1C6, Canada

Rhodnius prolixus is blood-gorging insect, which is medically important since it causes Chagas disease by transmitting the Trypanosomes cruzi parasite via its feces and urine after a blood meal. In the adult females, the blood meal and ecdysteroid hormone (20-hydroxyecdysone) are involved in the growth of the ovary and development of eggs. Halloween genes are necessary for ecdysteroid synthesis since they code for cytochrome P450 enzymes in the ecdysteroidogenic pathway. The ecdysteroid receptor (EcR/USP) binds 20-hydroxyecdysone, resulting in serial activation of ecdysone-responsive genes. We identified and characterized the Halloween genes, Neverland, CYP18a1, and eight ecdysone-responsive genes in the R. prolixus ovary using transcriptomic data. We used BLAST to compare transcriptome sequences with other arthropod sequences to identify similar transcripts. Our results indicate the Halloween genes and ecdysone-responsive genes are present in the ovary of R. prolixus. Future work will quantify Halloween gene expression and ecdysone-responsive gene expression in the ovary following a blood meal.

Acknowledgements: Supported by NSERC Discovery Grants to IO and ABL.

#14-8: THE SYNTHESIS AND RELEASE OF PITUITARY GROWTH HORMONE IS DIFFERENTIALLY REGULATED BY SEVERAL NEUROPEPTIDES AMONG VERTEBRATES

Urban-Sosa VA, Ávila-Mendoza J, Carranza M, Martínez-Moreno CG, Luna M, Arámburo C

Department of Cellular and Molecular Neurobiology, Institute of Neurobiology, Universidad Nacional Autónoma de México, Querétaro, México.

The synthesis and release of pituitary growth hormone (GH) are controlled by complex neuroendocrine mechanisms that involve several neuropeptides. In mammals, GHRH and SST are the main regulators of GH. However, in non-mammalian organisms this regulation seems to be more complex and involves other neuropeptides, including PACAP, TRH, GnRH,

Ghrelin, among others. To test the hypothesis that these neuropeptides have a differential impact on the regulation of GH between vertebrates we studied the ability of these peptides to control the expression and secretion of GH in pituitary cultures of three vertebrate models: rat (mammals), chicken (birds) and iguana (reptiles). Our results showed that GHRH significantly stimulated both GH mRNA expression and GH secretion in rat and iguana, whereas in chicken only increased GH secretion. TRH increased GH secretion only in iguana, but stimulated GH mRNA expression in chicken and iguana. PACAP stimulated GH secretion in chicken and iguana, and the GH mRNA expression in the three species. Ghrelin increased the GH secretion in chicken and iguana but decreased it in rats, while it decreased the GH mRNA levels in rats and iguana. GnRH stimulated both GH mRNA expression and GH release in chicken pituitary cultures, while in iguana only GH secretion was significantly increased. SST directly inhibited GH mRNA expression in iguana, while in the other species it only inhibited GHRH-stimulated secretion. To further validate whether the differential response that we observed could be explained by structural differences in the GH gene, we analyzed the GH promoter activity of each species and, using transfection-reporter assay, we found that GHRH and TRH-stimulated promoter activity was higher in the chicken GH gene in comparison with rat and iguana. Our results support that variations in the GH responsiveness to neuropeptides could be mediated, in part, by differences in the structure of the GH gene promoter between species.

Acknowledgements: We thank Gerardo Courtois and Adriana González for technical assistance. Partially supported by PAPIIT-UNAM (IN201817, IN208419, IN227020), PILGRIM´S México and CONACYT (285004). VAUS received a graduate fellowship from CONACYT (787975/612881).

#14-9: COMPARISON OF THE PITUITARY AND GONADOTROPINS CELL LOCALIZATION IN TURBOT AND MOUSE

Jia YD (1), Gao YH (1), Hong L (1), Lin JX (2)

(1) Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China

(2) Shanghai Laboratory Animal Research Center, Shanghai, China

Pituitary gland is the center of the hypothalamic-pituitary-gonad axis. Morphology and cell lines of turbot and mouse pituitary were evaluated via histological and immunohistochemical analyses. Results showed that turbot pituitary is chicken heart-shaped, dorsoventral, located on the ventral surface of the diencephalon. And, the mouse pituitary is oval, located in the pituitary fossa of the sella turcica at the skull base. Two well-distinguished areas adenohypophysis (AH) and neurohypophysis (NH) in pituitary were identified. Turbot AH comprised the rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI). NH was not pronounced and with finger-like protrusions into PPD. However, mouse AH only comprised the pars distalis (PD) and PI. NH distribution was semicircular. Three main types of cells (acidophilic, basophilic, and chromophobic cells) were distributed in the mouse PD region, whereas in the turbot PPD, RPD, and PI. Moreover, the percentage of mouse chromophobic and basophilic cells was higher and lower than that of turbot, respectively. The diameter of the three aforementioned cells in the mouse was significantly higher than that of the turbot. fshβ- and lhβ-immunoreactive signals were identified in turbot-distinct pituitary cells that primarily occupied the peripheral and central regions of AH. However, mouse fsh and lh-immunoreactive cells were expressed in the same cells and present in the PD. In conclusion, the pituitary morphology, cell lines, and gonadotropins (fshβ and lhβ) expression in turbot and mouse significantly differed. These differences reflected species differences and help for fully understand the evolution of pituitary networks in vertebrates.

Acknowledgements: Supported by National Natural Science Foundation of China (31972811) and Special Fund for Basic Scientific Research of Central Public Research Institutes (20603022019014).

#14-10: THYROTROPIN-RELEASING HORMONE (TRH) AND SOMATOSTATIN (SST), BUT NOT GROWTH HORMONE-RELEASING HORMONE (GHRH) NOR GHRELIN (GHRL), REGULATE EXPRESSION AND RELEASE OF IMMUNE GROWTH HORMONE (GH) IN CHICK BURSAL B-LYMPHOCYTE CULTURES

Santiago M Pech-Pool (1 ,2), Laura C Berumen (2), Carlos G Martínez-Moreno (1), Guadalupe García-Alcocer (2), Martha Carranza (1), Carlos Arámburo (1), Maricela Luna (1)

(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro 76230, México

(2)Posgrado en Ciencias Químico-Biológicas, Facultad de Química, Universidad Autónoma de Querétaro, Centro

Universitario, Querétaro 76010, México

Growth hormone (GH) is expressed in immune cells where it exerts immunomodulatory effects. However, the mechanisms that regulate expression and release of GH in the immune system remain unclear. Since the expression of classic GH secretagogues, as well as their corresponding receptors (-R), has been described in B-lymphocytes of several species, we analyzed the effect of growth hormone-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH), ghrelin (GHRL), and somatostatin (SST) upon GH mRNA expression, intracellular and released GH, Ser133-phosphorylation of CREB (pCREBS133), intracellular Ca2+ levels, as well as B-cell activating factor (BAFF) mRNA expression in bursal B-lymphocytes (BBLs) cell cultures. The expression of TRH/TRH-R, ghrelin/GHS-R1a, and SST/SST-Rs (Subtypes 1 to 5) was observed in BBLs by RT-PCR and immunocytochemistry (ICC); interestingly GHRH/GHRH-R were not observed in these cells. We found that TRH significantly stimulated local GH mRNA expression and CREB phosphorylation. Conversely, SST directly decreased GH mRNA expression, and also prevented TRH-induced GH mRNA expression, although no changes were observed in pCREBS133 levels. Furthermore, TRH stimulated GH release to the culture media, while SST increased the intracellular content of this hormone. Additionally, SST inhibited TRH-induced GH release in a dose-dependent manner. The co-administration of TRH and SST decreased the intracellular content of GH. After 10 min of incubation with either TRH or SST, the intracellular calcium levels significantly decreased, but they were increased at 60 min, whereas the combined treatment with both peptides maintained the Ca2+ levels reduced up to 60-min of incubation. Also, we observed that BAFF cytokine mRNA expression was significantly increased by TRH. Altogether, these results suggest that TRH and SST are implicated in the regulation of local GH expression and release in BBLs cultures, which also involve changes in pCREBS133 and intracellular Ca2+ concentration. It is likely that TRH, SST, and GH exert autocrine/paracrine immunomodulatory actions and participate in the development of chicken BBLs.

Acknowledgements: The authors thank Pilgrim's Pride, Querétaro, México, who donated fertilized broiler eggs used during this research. Supported by PAPIIT-DGAPA UNAM (IN201817, IN209621, IN208419) and CONACYT (285004). SMPP received a PhD fellowship (475619) from CONACYT.

#15-1: CORTICOSTEROID DEFICIENT 21-HYDROXYLASE (CYP21A2) KNOCKOUT TADPOLES SURVIVE THROUGH METAMORPHOSIS

Paul B (1), Shewade LH (2), Buchholz DR (2)

(1,2) Department of Biological Sciences, University of Cincinnati, Ohio, USA

Corticosteroids (glucocorticoids and mineralocorticoids) are critical for vertebrate organogenesis. In utero corticosteroid deficiency or excess can alter developmental outcomes starting from mortality in infants to debilitating diseases in adults. Tadpoles are widely used for studying the mechanism of corticosteroid signaling during development because tadpoles are free of maternal hormonal influence and hormonal control of development is conserved between humans and frogs. To understand the role of corticosteroid signaling during development, we created 21-hydroxylase (CYP21A2) (21-OHKO) knockout frogs using CRISPR-Cas9 gene disruption technology. The enzyme 21-hydroxylase(21-OH) catalyzes corticosteroid biosynthesis from progesterone. CYP21A2 mutations in mammals cause neonatal mortality from corticosteroid deficiency, and surviving individuals suffer from infertility. Corticosteroid deficiency inhibits negative feedback to the hypothalamic-pituitary-adrenal (HPA) axis causing overproduction of crh, acth and corticosteroid precursors (e.g. progesterone) causing infertility in surviving individuals. 21-OHKO tadpoles were thus predicted to represent a classic corticosteroid deficient vertebrate model. We found that 21-OHKO tadpoles have very low plasma glucocorticoid levels, low mRNA expression of glucocorticoid-response gene klf9 and exhibited delayed development. Abrogated negative feedback to the HPA axis in mutant tadpoles was demonstrated through high levels of progesterone and higher mRNA expression of crh, acth, star and aldo synthase. Two surprising phenotypes of 21-OHKOs were: 1) survival through metamorphosis unlike mammalian 21-OHKO models which die at birth and 2) similar mineralocorticoid levels as sibling wild-types. We found that progesterone can transactivate glucocorticoid receptor in tail tip culture, which may possibly be sufficient for survival through metamorphosis in vivo. Also, we found higher mRNA expression of aldo synthase which may explain the wild-type mineralocorticoid levels in 21-OHKO tadpoles. Thus, 21-OHKO frogs exhibit impaired HPA negative feedback as in mammals, but compensatory signaling mechanisms likely enable them to survive where mammalian 21-OH mutants die.

Acknowledgements: Supported by Department of Biological Sciences, Weiman-Benedict Graduate Student Award and

University of Cincinnati, Graduate Student Government Research Fellowship Award to B. Paul.

#15-2: CORTICOSTEROIDS MEDIATE GLUCOCORTICOID ACTIONS IN THE LESSER SPOTTED CATSHARK (SCYLIORHINUS CANICULA)

Cabrera-Busto J (1), Mancera JM (1) and Ruiz-Jarabo I (1,2)

(1) Departament of Biology, Faculty of Marine and Environmental Sciences, University of Cadiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Puerto Real, Cádiz, Spain.

(2) Department of Physiology, Faculty of Biological Sciences, University Complutense, Madrid, Spain.

Corticosteroids, hormones produced in vertebrates, are involved in gluco- and mineralocorticoid actions (GC and MC) mediated by specific receptors (GR and MR, respectively). These hormones are involved in a wide range of physiological processes, such as the endocrine stress response and carbohydrate metabolism. The 1α-hydroxycorticosterone (1α-OHB) is the major circulating corticosteroid in elasmobranchs. This hormone act as a MC, but to the date its role as a GC is not entirely clear. Through the employment of a set of in vivo and ex vivo approaches, the present study tried to test GC actions of corticosteroids, mediated by GR, in the lesser spotted catshark (Scyliorhinus canicula). Dexamethasone (DEX, a synthetic corticosteroid) slow-release implants were used together with an ex vivo culture of liver and white muscle explants to analyze GC actions. DEX and 1α-OHB were analyzed in blood plasma through UPLC-QTOF-MS. The results showed that 7 days of DEX slow-release implants decreased plasma 1α-OHB concentrations, as well as modified carbohydrate metabolism in liver and white muscle. Moreover, ex vivo explants of liver and white muscle were stimulated by DEX and cortisol, increasing glucose secretion (a GC response). In addition, dose-response curves, altogether with the use of specific GR inhibitor mifepristone (or RU-486), confirmed the involvement of GR mediating glucose secretion induced by both cortisol and DEX. In conclusion, this study provides for the first time strong evidences supporting the influence of corticosteroids in the glucose balance of S. canicula, but the role of 1α-OHB as a GC hormone in sharks should be further confirmed.

#15-3: DIRECT ACTIVATION OF TRNA METHYLTRANSFERASE-LIKE 1 (METTL1) GENE BY THYROID HORMONE RECEPTOR IMPLICATES A ROLE IN ADULT INTESTINAL STEM CELL DEVELOPMENT AND PROLIFERATION DURING XENOPUS TROPICALIS METAMORPHOSIS

Wonho Na, Liezhen Fu, Nga Luu, Yun-Bo Shi

Section on Molecular Morphogenesis, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

Thyroid hormone (T3) plays an important role in vertebrate development. Compared to the postembryonic development of uterus-enclosed mammalian embryos, T3-dependent amphibian metamorphosis is advantageous for studying the function of T3 and T3 receptors (TRs) during vertebrate development. The effects of T3 on the metamorphosis of anurans such as Xenopus tropicalis is known to be mediated by TRs. Many putative TR target genes have been identified previously. Among them is the tRNA methyltransferase Mettl1. We studied the regulation of Mettl1 gene by T3 during intestinal metamorphosis, a process involves near complete degeneration of the larval epithelial cells via apoptosis and de novo formation of adult epithelial stem cells and their subsequent proliferation and differentiation. We observed that Mettl1 was activated by T3 in the intestine during both natural and T3-induced metamorphosis and that its mRNA level peaks at the climax of intestinal remodeling. We further showed that Mettl1 promoter could be activated by liganded TR via a T3 response element upstream of the transcription start site in vivo. More importantly, we found that TR binding to the TRE region correlated with the increase in the level of H3K79 methylation, a transcription activation histone mark, and the recruitment of RNA polymerase II by T3 during metamorphosis. Our findings suggest that Mettl1 is activated by liganded TR directly at the transcriptional level via the TRE in the promoter region in the intestine during metamorphosis. Mettl1 in turn regulate target tRNAs to affect translation, thus facilitating stem cell formation and/or proliferation during intestinal remodeling.

Acknowledgements: Supported by the intramural Research Program of NICHD, NIH.

#15-4: TISSUE-SPECIFIC HISTONE VARIATIONS IN PREMETAMORPHIC RANA (LITHOBATES) CATESBEIANA

Kuecks-Winger HN, Thambirajah AA, Helbing CC

Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

Thyroid hormone (TH) is essential for development in all vertebrate species, including the metamorphosis of a tadpole into a frog. The metamorphic process encompasses a diversity of tissue-specific changes in gene expression which must be tightly coordinated. The current dogma of TH-signaling does not sufficiently explain how TH is able to elicit these tissue- and developmental stage-specific changes in gene expression required for metamorphosis. It has been established that histone post-translational modifications (PTMs), and the incorporation of histone variants are involved in mediating gene expression during developmental periods. Thus, such changes in histone composition may be involved in tissue-specific TH-dependent gene expression during tadpole metamorphosis. To address this, we first described the histone composition of premetamorphic American bullfrog, Rana (Lithobates) catesbeiana, tadpole tissues. Blood, liver, and tailfin were sampled from premetamorphic R. catesbeiana tadpoles and the nuclei were isolated. Protein extracted from the nuclei were resolved by 15% SDS-PAGE, 15% Acid Urea Triton (AUT)-PAGE, and 15% AU-PAGE. These PAGE analyses revealed tissue-specific banding patterns, suggesting differential histone PTMs, variants, or other isoforms. To our knowledge, this is the first attempt to describe the global histone composition in amphibian tissues. This work builds a foundation for future research that will expand our understanding of the intersections of TH-signaling and variable histone composition in a tissue- and development-specific manner, with broad implications for vertebrate species.

Acknowledgements: Supported by NSERC Discovery Grant (CCH), a University of Victoria Fellowship (HNK), and a University of Victoria Graduate Award (HNK).

#15-5: MITIGATION OF GLYPHOSATE EXPOSURE-INDUCED DEVELOPMENTAL DEFECTS BY VITAMIN-C CO-TREATMENT

Weidman D, Mathewson AS, Killian D, Bhandari RK

Department of Biology, University of North Carolina at Greensboro, NC 27412, USA

Glyphosate-based herbicide use is increasing yearly to keep up with the growing demand of the agriculture world. Although glyphosate-based herbicides target the enzymatic pathway in plants, the effects on endocrine systems of vertebrate organisms, particularly fish, are widely unknown. The present study examined the effects of glyphosate exposure on embryo development and the thyroid system of the Japanese Medaka fish (Oryzias Latipes). The Hd-rR medaka embryos were exposed to RoundUp® containing 0.05, 0.5, 5, 10, and 20 mg/L glyphosate (glyphosate acid equivalent) from the 8 hours post-fertilization stage through 14 days post-fertilization stage. Phenotypes observed include delayed hatching, increased developmental deformities, growth, and embryo mortality. The lowest concentration (0.05 mg/L) and the highest concentration (20 mg/L) of glyphosate induced similar phenotypes in embryos and juveniles. Toxic effects of exposure on embryos induced by LC50 concentration of glyphosate were ameliorated by co-treatment of vitamin-C (10 mg/L). The present results demonstrate that glyphosate via exposure to RoundUp® affects the early development of medaka in a non-monotonic dose-response manner and that glyphosate-induced toxic effects can be ameliorated by vitamin-C cotreatment. Future studies will determine mechanisms underlying the role of vitamin-C in neutralizing the toxic effects of glyphosate.

Acknowledgements: UNCG Biology Department.

#15-6: MITIGATION OF GLYPHOSATE-INDUCED DEVELOPMENTAL DEFECTS IN MEDAKA FISH

Bhandari R (1), Weidman D (2), Matthewson A (3)

(1) Assistant Professor @ University of North Carolina at Greensboro

(2) Post-bac of University of North Carolina at Greensboro

(3) Post-bac of University of North Carolina at Greensboro

Glyphosate-based herbicides are the most universally used herbicides worldwide. Glyphosate, an active ingredient in RoundUp®, has been known to induce oxidative stress in animal cells, including fish. Antioxidants, such as ascorbic acid

(vitamin C) can reduce oxidative stress by donating an electron to stabilize the reactive oxygen species. Our previous studies have demonstrated that environmentally relevant concentrations of glyphosate can affect developing medaka fish embryos, but less is known about potential methods to reduce the negative effects of glyphosate exposure on embryo development. In this study, medaka fish, Oryzias latipes, embryos were exposed to 15 mg/L glyphosate, 10 mg/L ascorbic acid (vitamin C, vit-C), and a combination of 15 mg/L glyphosate and 10 mg/L vit-C for 15 days and their development was monitored under a microscope. Each treatment was run in triplicate to validate the collected empirical data and observed results. Embryos were examined 6-8 days post-fertilization (dpf) stages, as well as at 15-dpf stage to determine if their development was affected by exposure. During the two weeks of monitoring, mortality rate and phenotypic changes, mainly hatching rate, spinal curvature, enlarged yolk sac, uninflated swim bladder, and greying of yolk sac were recorded and analyzed. Hatching success rate was reduced by glyphosate exposure and vit-C co treatment mitigated these defects. Similarly, a higher mortality rate was found in the glyphosate group, which was mitigated by vit-C co-treatment. Similarly, vit-C co-treatment tended to decrease yolk sac graying and spinal curvature defects in embryos ($p<0.06$-~<0.09). The study demonstrates that vitamin C, an antioxidant, can have an ameliorative effect on developing fish embryos exposed to glyphosate.

Acknowledgements: UNCG Biology Department, our principal investigator and mentor Dr. Ramji Bhandari, Chelsea Smith, whose previous research served as a baseline for this study, and fellow lab members of the UNCG Bhandari Lab who assisted us in the completion of this project.

#15-7: TRANSGENERATIONAL DIFFERENCES IN MEDAKA GUT MICROBIOTA POPULATION INDUCED BY ANCESTRAL BPA EXPOSURE

Guzman E (1), Wang X (1,2), Feng Y (1), Bhandari, RK (1)

(1) Department of Biology, University of North Carolina at Greensboro, NC 27412, USA

(2) College of Life Sciences, South China Normal University, Guangzhou 510631, China

The Intestinal microbiome plays a critical role in the health of their host organism. While the intestinal microbiome is typically acquired environmentally from birth, composition of the microbiota is found to be modulated by intestinal epithelial cells via expression of key regulatory genes. Endocrine disrupting chemicals found as pollutants in the environment may thus perturb composition of the core microbiome via alteration of the interactions between host gene expression and microbiota. Bisphenol-A, a ubiquitous endocrine disrupting pollutant, has previously been shown to induce microbial dysbiosis via direct exposure; however, research on its transgenerational effects is limited. In this study, developing medaka embryos were continuously exposed to bisphenol A (BPA, 10 ug /L) from 8 hours post-fertilization to 12 days post-fertilization stages and never thereafter. They were allowed to grow in water without chemical exposure for the rest of their life and spawn for three generations. Whole gut samples were collected from the fish in F0 (grandparents) and F2 (grandchildren) generation. DNA from the water samples were collected at the time of sampling to determine environmental microbiota population. DNA was extracted from all samples and 16S rRNA was sequenced. A dysbiosis of the gut microbiome was found in the medaka fish whose grandparents were developmentally exposed to BPA. BPA exposure was associated with decreased number of overall OTUs in F2 intestine. A decrease in normal genus Vibrio and a higher abundance of pathogenic Aeromonas and Shewanella was observed. Presence of genera typically absent in medaka gut microbiome, such as Acidovorax, was also observed in the BPA lineage fish. Current mechanisms by which intestinal dysbiosis occurs is currently unknown; however, we are investigating epigenetic inheritance of BPA established memories in the germline and their interactions with host intestinal epigenome and transcriptome as well as their relationship with metabolic diseases that we have observed in the grandchildren.

Acknowledgements: Supported by University of North Carolina Greensboro Graduate School and the Department of Biology.

#15-8: EFFECTS OF GRANDPARENTAL EMBRYONIC BISPHENOL A EXPOSURE ON THE LIVER OF GRANDCHILDREN OF MEDAKA FISH

Godwin J (1), Chakraborty S (1), Bhandari RK

Department of Biology, University of North Carolina at Greensboro, NC 27412, USA

Bisphenol A (BPA) is a component of mass-produced plastics found in everything from plastic bottles to the coating inside metal cans. It has been linked to a multitude of adverse endocrine-disrupting health effects, as it can interact with estrogen, androgen, and thyroid hormone receptors. It has also been linked to the modification of genes that affect lipid synthesis and accumulation, including de novo fatty acid synthesis. Accumulating evidence suggests a role of bisphenol A (BPA) in the development of adverse liver outcomes, including non-alcoholic fatty liver disease (NAFLD). NAFLD is a condition of excess fat accumulation in the liver of animals and humans. Moreover, all observed adverse liver health outcomes are based on only the direct exposure of animals and humans to BPA. Thus, there is a research gap in determining whether grandparental BPA exposure can lead to NAFLD in grandchildren who are not exposed to it. To address this question, the livers of the medaka (Oryzias latipes) fish whose grandparents were exposed to an environmentally relevant concentration of BPA (10 ug/L) during their first 12 days of embryonic life were histologically examined along with the gene expression of genes significant to lipid metabolism.

Acknowledgements: Department of Biology.

#15-9: INCREASED DE-DIFFERENTIATION OF GONADOTROPIN-RELEASING HORMONE NEURONS IN A FGF SIGNALING-DEFICIENT MOUSE

Andersen ND, Tsai P-S

Department of Integrative Physiology, University of Colorado, Boulder, CO, USA

Gonadotropin-releasing hormone (GnRH) neurons drive the activation of the reproductive axis. Abnormal GnRH neuron development, survival, or function can lead to severe reproductive impairment in all vertebrates. Our and other laboratories have shown that fibroblast growth factor (FGF) signaling is critical for the formation and function of the GnRH system in human and mouse. We previously generated a mouse model (dnFGFR mouse) expressing a dominant-negative FGF receptor (FGFR) in GnRH neurons and found that this mouse exhibited a progressive loss of GnRH neurons after birth and suffered significant reproductive deficits. Since the only marker of GnRH neurons is GnRH, it was unclear if the loss of GnRH neurons in dnFGFR mice was due to neuronal death or a failure to produce the hallmark GnRH peptide associated with neuronal de-differentiation. The goal of this study was to answer this question using a lineage-tracing approach. Towards this goal, we generated control and dnFGFR mice that also expressed a fluorescent marker, TDTomato (TDT), in GnRH neurons. In these mice, all neurons fated to become GnRH neurons were TDT+, and all differentiated GnRH neuron expressing GnRH neurons were GnRH+. Brains from 200-day-old control and dnFGFR mice were harvested, processed for immunohistochemistry of GnRH, and the number of neurons positive for TDT, GnRH, or both were scored in brain regions housing GnRH neurons. Our results revealed that both control and dnFGFR mice had equivalent numbers of neurons fated to become GnRH neurons, suggesting no significant cell death occurred when FGF signaling was deficient. Surprisingly, only 84.8±3.3% of neurons fated to become GnRH neurons expressed GnRH in control animals. This number was further reduced to 47.5±2.4% in dnFGFR mice. Our results showed that the FGF signaling deficiency induced a loss of GnRH neurons, in part, by increasing GnRH neuronal de-differentiation but not death. In addition, GnRH neuron de-differentiation occurred naturally in control animals, albeit less frequently than dnFGFR mice. Our study suggests that GnRH neuronal loss in diseased conditions may not be due to cell death, and that pharmacological interventions may be able to stimulate these de-differentiated neurons to re-enter a differentiated state capable of producing and secreting GnRH.

Acknowledgements: Supported by NIH Grant R01 HD083260 to PST.

#16-1: THE INFLUENCE OF CRF ON EVOKED CALCIUM INFLUX IN THE OPTIC TECTUM OF THE SOUTH AFRICAN CLAWED FROG XENOPUS LAEVIS

Wang C, Keyel PA, Carr JA

Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA

Feeding and predator avoidance are modulated in part by CRFR1 receptors in the optic tectum, the primary visual center of the non-mammalian vertebrate brain. Tectal CRF neurons respond to stressors and food deprivation, using an intrinsic

CRF signaling system, but the signaling pathway is poorly understood. Since gamma-aminobutyric acid (GABA) is one major inhibitory neurotransmitter in the optic tectum, GABAergic neurons might act downstream of CRFR1. This study tested the hypothesis that CRF acts on tectal CRFR1 to inhibit basal and K+-evoked calcium flux in tectal neurons via GABAergic signaling. We performed three sets of experiments using in-vitro tectal slices from Xenopus laevis adults. We tested the following questions: 1) Does CRF dose-dependently inhibit evoked calcium influx in tectal neurons? 2) Do any inhibitory effects of CRF on evoked calcium influx require CRFR1? 3) Does GABA inhibit evoked calcium influx in tectal neurons? These questions were tested using 300 µm brain slices perfused with artificial cerebrospinal fluid containing 2.5 mM K+ (basal) or 30-60 mM K+ (depolarization). Peak evoked calcium influx, the latency to peak influx, and the decay in evoked influx were measured as dependent variables. We found subtle but unreproducible effects of CRF at single doses on peak and latency to peak evoked calcium. The water-soluble and selective CRFR1 antagonist NBI 35965 hydrochloride did not change evoked calcium influx. Importantly, there were no substantial inhibitory effects of GABA on calcium influx evoked with either 30 or 60 mM K+. These findings suggest that peak calcium influx evoked with high K+ primarily reflects the rapid opening of voltage-gated calcium channels in presynaptic nerve endings upstream of tectal GABA-responsive cells. Whether these findings reflect a lack of CRF-mediated presynaptic inhibition needs to be confirmed using electrophysiological methods.

Acknowledgements: Supported by Texas Tech University, National Science Foundation IOS #1656734 and Texas Tech University Association of Biologists.

#16-2: OSMOTIC REGULATION OF TRANSCRIPTION FACTOR MRNA LEVELS IN PROLACTIN CELLS OF MOZAMBIQUE TILAPIA

Malintha GHT, Celino-Brady FT, and Seale AP

Department of Human Nutrition, Food and Animal Sciences, University of Hawai'i at Mānoa, 1955 East-West Road, Honolulu, HI 96822, USA

In euryhaline fish, prolactin (Prl) plays a key role in freshwater acclimation by promoting ion retention and absorptive processes in osmoregulatory tissues. Consistent with its osmoregulatory function, Prl release is directly stimulated by a fall in extracellular osmolality. Recently, we identified multiple putative transcription factors (TFs) that are predicted to bind the promoter region of the two prl genes, prl177 and prl188, in the euryhaline Mozambique tilapia, Oreochromis mossambicus. Moreover, we confirmed the mRNA expression of these TFs in tilapia Prl cells and found that several were sensitive to environmental salinity. It is unknown, however, whether these TFs are directly regulated by extracellular osmolality. To investigate the possible link between changes in extracellular osmolality and the transcriptional regulation of prl genes, we characterized the responses of the most abundant TF transcripts to variations in medium osmolality, ranging from 280 to 420 mOsm/ kg, in dispersed Prl cells, in vitro. By 6 h, gene expression of pituitary transcription factor 1 (pit1), and signal transducer and activator of transcription 3 (stat3) was inversely related to extracellular osmolality. Other highly expressed TFs, pre- β-cell leukemia homeobox (pbx) and stat1 were unresponsive to changes in extracellular osmolality. These results indicate that multiple TFs predicted to bind prl177 and prl188 promoter regions are differentially responsive to extracellular osmolality and may play a role in the osmotic regulation of prl transcription.

Acknowledgements: Supported by HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01) and NSF (IOS-1755016) to A.P.S.

#16-3: MOLECULAR CHARACTERIZATION OF CCHAMIDE2 AND DEORPHANIZATION OF ITS RECEPTOR IN THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI

Tan J, Paluzzi JP

Department of Biology, York University, Toronto, Ontario, Canada

The neuropeptide CCHamide 2 (CCHa2) and its corresponding G-protein coupled receptor (CCHa2R) were detected across insects. A mammalian homolog of CCHa2R, Bombesin receptor subtype-3, is expressed within the insulin-producing beta cells of the endocrine pancreas. The expression profile of CCHa2 in Drosophila melanogaster was demonstrated that was highly expressed in the gut and only a small amount detected in the brain. In contrast, the expression profile of CCHa2R is exactly opposite with high expression in the nervous system including insulin-producing cells in the fly brain. In the human

disease vector, Aedes aegypti, expression patterns of CCHa2 and CCHa2R remain elusive as well as their physiological roles. The aim of this research is to identify, localize and quantify expression of CCHa2 and CCHa2R and functionally deorphanize them in the yellow fever mosquito. To date, RT-qPCR revealed CCHa2 transcript undergoes changes over developmental stages with highest abundance in pupa and one day old adults. Similar methods are being used to quantitate the tissue and organ-specific expression in adults. Further, immunohistochemistry and in situ hybridization will be utilized to localize cell-specific expression of CCHa2 and its receptor CCHa2R. A heterologous system will be used to confirm specificity and sensitivity of CCHa2R to CCHa2 in a dose-dependent fashion. Previous studies indicated CCHa2 may play essential roles in D. melanogaster feeding behaviour and development due to its regulation of the insulin-like peptide signalling pathway. CCHa2 was also demonstrated to have a biphasic activity on primary urine production of Rhodnius prolixus. Based on preliminary data of peptide and receptor expression pattern, various bioassays and reverse genetic approaches will be employed to elucidate physiological roles of CCHa2 in this important human-disease vector.

#16-4: MECHANISMS OF NEUROPEPTIDERGIC REGULATION OF REPRODUCTIVE PHYSIOLOGY IN STARFISH

Piñon Gonzalez V, Elphick MR

School of Biological & Chemical Sciences, Queen Mary University of London, UK

Neuropeptides are intercellular signalling molecules secreted by neurons that act as neurotransmitters, neuromodulators or hormones. They are derived from larger precursor proteins and typically exert effects on target cells by binding to specific G-protein coupled receptors. One of the most important physiological processes regulated by neuropeptides is reproduction. Although much is known about the pathways that promote gamete maturation and gonadal function in vertebrates, less is known about the mechanisms of neuropeptidergic control of reproduction in invertebrates. However, insights into the mechanisms of neural control of reproduction have been obtained in starfish (phylum Echinodermata), where a relaxin-type neuropeptide triggers the resumption of meiosis in immature eggs and spawning. Building upon this knowledge, the aim here was to identify other neuropeptides and receptors involved in the regulation of the reproductive physiology in starfish, using Asterias rubens as a model system. Analysis of A. rubens transcriptome sequence data revealed gonadal expression of transcripts encoding twenty-one candidate neuropeptide precursors and fourteen neuropeptide receptors. Furthermore, an ongoing immunohistochemical study is investigating neuropeptide expression in the reproductive system of A. rubens at different stages of gonadal maturation, employing use of specific antibodies to neuropeptides derived from eight precursor proteins. For example, during early stages of gamete development (late September), a calcitonin-type neuropeptide (ArCT) was detected in the gonoduct of both males and females, whereas a pedal peptide-type neuropeptide (ArPPLN1b) was only detected in the gonoduct of females. These preliminary findings indicate that there may be sex-specific differences in mechanisms of neuropeptidergic regulation of reproductive function in starfish. Furthermore, these findings provide a basis for experimental investigation of the physiological roles of neuropeptides expressed in the gonoduct as regulators of reproductive function in starfish. Discovery of the roles of neuropeptides as regulators of reproduction in starfish would provide new insights into the evolution of neural mechanisms controlling reproductive physiology and behaviour in the animal kingdom.

Acknowledgements: Victor Piñon Gonzalez supported by Queen Mary University of London and a scholarship awarded by CONACyT

#16-5: ALTERNATION OF HEPATIC GLYCOLYSIS, LIPOGENESIS, AND BLOOD BIOCHEMISTRY IN TIGER PUFFER (TAKIFUGU RUBRIPES) UNDER TWO DIFFERENT CULTURE SYSTEMS

Jia YD (1), Wang ZY (2), Li MY (1), Jing QQ (1), Zhai, JM (3), Guan CT (1)

(1) Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China

(2) College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Taian China

(3) Ming Bo Aquatic Co. Ltd., Laizhou, China

Tiger puffer (Takifugu rubripes) is one of the most commercially important fish species cultivated and consumed in East Asian countries, including Japan, South Korea and China. In this study, the hepatic glycolysis, and lipogenesis, and serum biochemistry of tiger puffers subjected to short-term culture in offshore sea cage aquaculture system (OSCS) and recirculating aquaculture system (RAS) were evaluated. Results showed that the activity of hepatic phosphofructokinase,

and lipoprotein lipase, ATP level, and serum cortisol content of the male tiger puffers reared in OSCS were significantly higher than those of female tiger puffers reared in OSCS and those of fish reared in RAS. The lowest hepatic fatty acid synthetase activity, lipid droplet number, serum aspartate aminotransferase activity, Na+ and Cl- concentrations were observed in male tiger puffers reared in OSCS. However, serum glucose, triglyceride, total cholesterol, K+ concentrations and alanine aminotransferase activity of tiger puffers reared in RAS and OSCS showed no significant differences. Hepatic glycolysis and lipogenesis were profoundly affected by rearing system and gender. The interaction effect of gender and rearing system on hepatic lipoprotein lipase activity, lipid droplet number and serum Na+ level was observed. These results indicate male tiger puffers exhibited different metabolic status in hepatic glycolysis, lipogenesis, and serum biochemical parameters when reared under two different culture systems. These findings will provide important information for improving tiger puffer (Takifugu rubripes) farming and profitable production using the RAS and OSCS aquaculture systems in the north coast of China.

Acknowledgements: Supported by National Key R&D Program of China (2019YFD0900904), Shandong Major Science and Technology Innovation Projects (2018SDKJ0303-4), Central Public-interest Scientific Institution Basal Research Fund, CAFS (NO.2020TD51)

#16-6: NESFATIN-1 AND NESFATIN-1-LIKE PEPTIDE STIMULATE PROOPIOMELANOCORTIN SYNTHESIS IN MURINE ATT-20 CORTICOTROPHS THROUGH THE AMP/PKA/CREB SIGNALING PATHWAY

Nasri A, Unniappan S

Laboratory of Integrative Neuroendocrinology, Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7V 1H2, Canada

Nucleobindin (NUCB) - derived peptides, nesfatin-1 (NES-1) and nesfatin-1-like peptide (NLP) have various physiological roles in vertebrates. Due to the fact that NES-1 is implicated in stress, we hypothesized that both NES-1 and NLP stimulate the adrenocorticotropic hormone (ACTH) precursor, proopiomelanocortin (POMC). We tested this using mouse pituitary corticotrophs (AtT-20 cells). First, immunocytochemical colocalization of ACTH and nucleobindin-derived peptides was assessed in AtT-20 cells. Moreover, the ability of nucleobindin-derived peptides to modulate POMC mRNA and protein in AtT-20 cells was assessed. The cell-signaling molecules mediating the effect of nucleobindin-derived peptides on POMC synthesis in mouse corticotrophs were studied using pharmacological PKA and cAMP blockers. Our results showed that AtT-20 cells colocalize NUCBs and ACTH. Both NES-1 and NLP increased POMC mRNA and protein expression in AtT-20 cells. Pharmacological studies revealed that NES-1 and NLP act through the cAMP/PKA/CREB cellular pathway to stimulate POMC synthesis. Collectively, the outcomes support our hypothesis. Corticotrophs are a source of NUCBs, and the encoded peptides NES-1 and NLP elicit a direct action to stimulate ACTH, the pituitary stress hormone synthesis. This stimulatory effect is mediated by an uncharacterized GPCR that utilizes the cAMP/PKA/CREB pathway.

Acknowledgements: Supported by the Ferring Innovation Grant, Canada Foundation of Innovation (CFI) and Saskatchewan Foundation of Health Research (SHRF) to SU, and a University of Saskatchewan Dean's scholarship and the WCVM graduate student travel award to AN.

#16-7: LIGAND-BIAS IN GNRH TRANSDUCTION NETWORKS: ROLES OF RECEPTOR-INTERACTING EFFECTORS IN THE CONTROL OF GOLDFISH PITUITARY HORMONE SECRETION

Khalid E, Chang JP

Department of Biological Sciences, University of Alberta, Edmonton, Canada

In the goldfish pituitary, two gonadotropin-releasing hormone (GnRH) isoforms, GnRH2 and GnRH3, stimulate luteinizing hormone (LH) and growth hormone (GH) release by binding to a shared population of G protein-coupled GnRH receptors (GnRHRs; GPCRs) on pituitary gonadotrophs (LH-secreting cells) and somatotrophs (GH-secreting cells). In addition to the use of shared conserved signalling modules, GnRH-isoform-, cell-type-, and function- selective engagement of signalling effectors also transpires. Although ligand-selective recruitment of intracellular effectors following GnRHR activation is clear, the proximal receptor-interacting elements underlying this "GnRHR biased signalling" are not well understood. In general, immediate effectors such as G proteins, G protein subunits, β-arrestins, and G protein-coupled receptor kinases (GRKs) play substantial roles in facilitating GPCR ligand bias. Goldfish GnRHRs are hypothesized to chiefly recruit Gαq/11

subunits to drive hormone release and we have previously shown that Gβγ subunits and β-arrestins underlie some of the observed GnRHR ligand bias in goldfish pituitary. However, whether additional G protein-related elements are involved is unknown. To address this question, we investigated the effects of pharmacologically inhibiting Gαq/11-selective and pan-Gα protein subunits, and GRK2/3, on acute GnRH2/3-stimulated LH and GH secretion from dispersed goldfish pituitary cells in column perifusion. Results reveal cell-type-specific differences in the use of G proteins; while GnRH-induced LH release relied solely on Gαq/11, additional inhibitory G-protein elements are involved in GH secretion. Additionally, GRK2/3 facilitate LH release from gonadotrophs, whereas they mediate ligand-selective desensitization in somatotrophs. Further study is needed to definitively link the functional selectivity in downstream GnRH signalling networks to differential participation within the suite of receptor-interacting effectors.

Acknowledgements: Supported by a NSERC Discovery Grant (JPC) and an Alberta Graduate Excellence Scholarship (EK).

#16-8: DOES LEPTIN SIGNALING REGULATE MUCUS SECRETION IN XENOPUS LAEVIS EMBRYONIC EPIDERMIS, A MODEL FOR RESPIRATORY EPITHELIUM?

Kourtnie Whitfield, Robyn Reeve, Grace Curtis, Erica Crespi

School of Biological Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA 99164-4236

Mucus hypersecretion in the mucociliary epithelia of the respiratory tract is a major contributor to the severity of respiratory diseases, including COVID-19. Leptin, a cytokine hormone, promotes mucus production in other mucociliary epithelial types, such as the gut. To test whether leptin signaling promotes mucus secretion or production in mucociliary epithelia, we conducted experiments using the embryonic epidermis of the frog Xenopus laevis, an in vivo model for mucociliary epithelia. First, fluorescent immunohistochemistry showed that leptin protein is localized in small secretory cells (SSCs) and in the cement gland cells, both of which secrete mucins. Second, we chronically exposed X. laevis embryos to leptin protein in media (100 ng/mL) from fertilization to Nieuwkoop-Faber stage 42, and imaged embryos with scanning electron microscopy. Leptin-treated embryos displayed fewer SSCs and reduced mucus secretion from SSCs and goblet cells, the major mucus-secreting cell types. We hypothesize that chronic leptin exposure induced mucus hypersecretion such that cells were unable to replenish mucins and underwent apoptosis. To confirm this, we will measure mucus secretion in response to acute leptin treatment. These findings link leptin expression and signaling to mucus secretion in the embryonic epidermis, but we need to further characterize leptin's role in the regulation of mucus secretion by localizing leptin receptor expression and characterizing the effects of leptin knockdown using both transient immunoneutralization experiments and the X. tropicalis CRISPR/CAS9 leptin knockdown model.

#16-9: STARVATION INFLUENCES NOCICEPTION AND SENSITIZATION IN AN INSECT MODEL

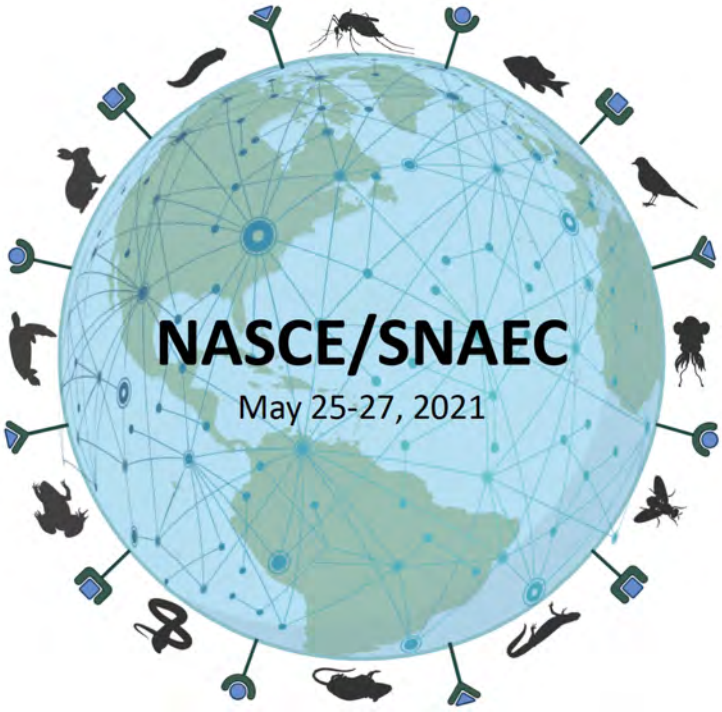
Crawford N, Fuse M

Department of Biology, San Francisco State University, San Francisco, California, USA

Hunger and pain/injury are two very powerful conditions that an organism must monitor, and even prioritize during its lifetime. These have been shown to be conflicting needs states in some animals, where one condition may be prioritized over the other. We assessed this in the larval hornworm, Manduca sexta. We allowed early 5th stage larvae to feed ad libitum or provided water crystals in lieu of food over a 24 hr period, which resulted in a significant difference in weight gain between the groups. Using our up-down behavioral assay, we assessed the threshold force required to elicit a defensive strike - as a gauge of the pain-like state of the insect - in well-fed and starved conditions. We noted that larvae had a higher nociceptive threshold (higher threshold to strike) when starved, and this was relieved with the injection of octopamine, in a dose-dependent manner. In contrast, larvae were equally sensitized to injury in fed and starved conditions, resulting in a highly reduced threshold to strike after a pinch to the body wall. Presentation of food to starved, injured larvae blocked the sensitization response, increasing the threshold to strike to baseline levels. This was rapidly reversed when food was removed. Injection of octopamine had no effect on either the sensitization or the feeding response, even though it significantly reduced feeding in starved, non-injured animals. We suggest that starvation may result in depleted circulating octopamine levels, which alters feeding and nociceptive behaviors, but plays a minimal role, if any, in sensitization. The competing effects of feeding (mechanical chewing) in a starved state appears to override sensitization to injury, but the mechanism for this is still unknown. It will be imperative to assess octopamine levels in M.

sexta in future experiments and verify whether the blocking effects of feeding on sensitization are due to changes in nutritional status or the mechanical act of chewing.

Acknowledgements: Supported by NIH SC3 grant 1SC3GM118218-01A1 (MF), Genentech Foundation MS Dissertation Scholarship (NC), NIH MBRS-RISE fellowship R25-GM059298 (NC), and IRA funds from San Francisco State University (NC).



thank you for attending the

6th Biennial Conference of the North American Society for Comparative Endocrinology (NASCE)