5th Biennial North American Society for Comparative Endocrinology

Gainesville, Florida
May 24-28, 2019

held jointly with

5th Biennial Conference of the North American Society for Comparative Endocrinology (NASCE)

and

10th International Symposium on Amphibian and Reptilian Endocrinology and Neurobiology (ISAREN)

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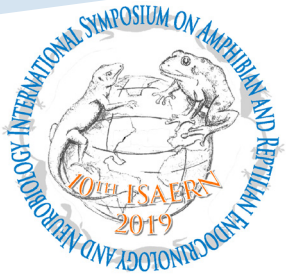

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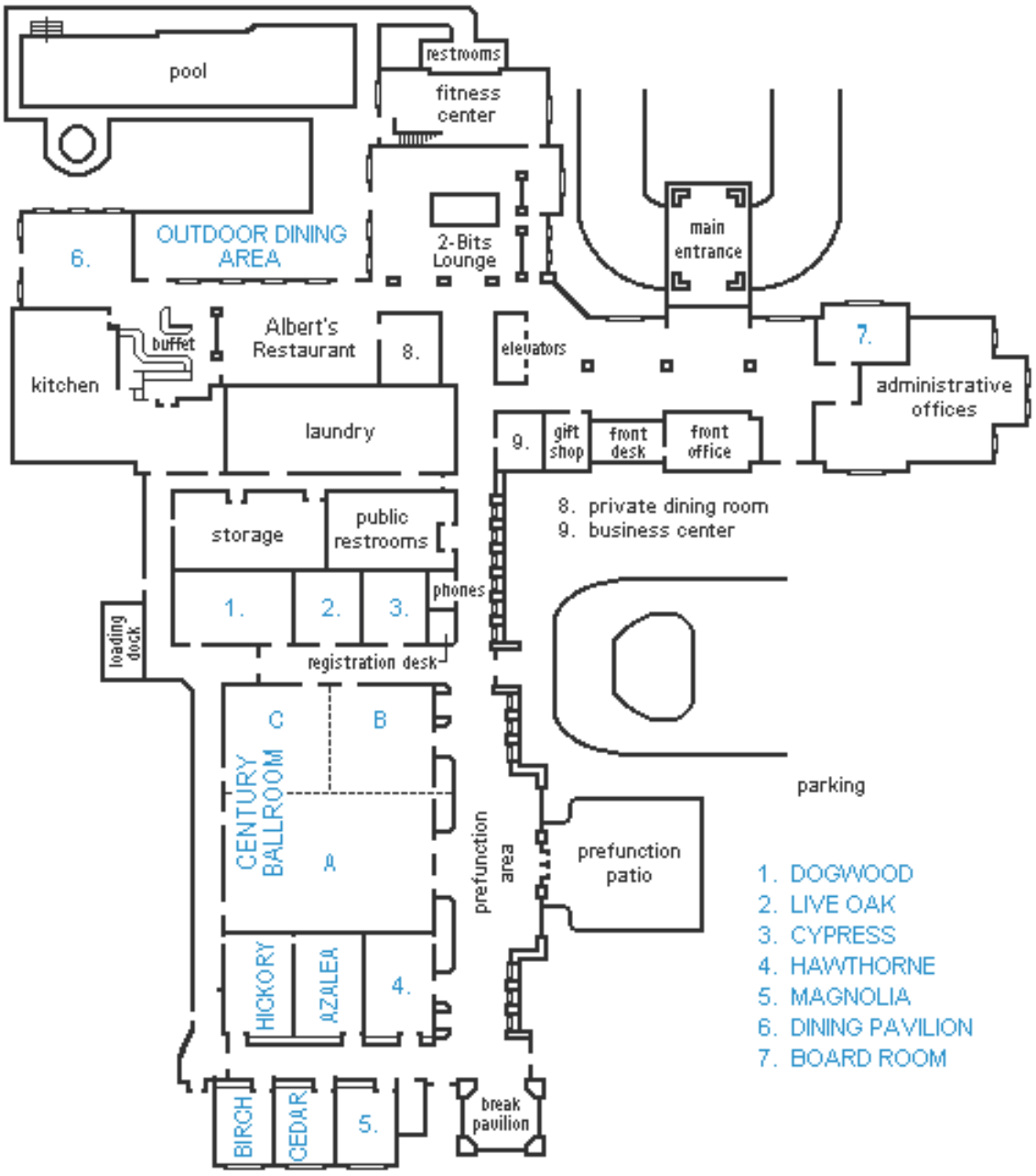

Agenda

Friday, May 24, 2019			
14:00-16:30	NASCE Council Meeting 1- Hawthorne		
18:00-18:15	Welcome - Harn Museum of Art		
18:15-19:15	Dr. Peter Thomas- Gorbman Bern Lecture		
19:15-21:00	Opening Reception		
Saturday, May 25, 2019			
08:00-09:00	Registration		
Plenary 09:00-10:00	Dr. Sheue-Yann Cheng- Century Ballroom ABC		
10:00-10:30	Coffee Break		
	Dogwood	Century Ballroom	Azalea
10:30-12:30 Session Chair	Thyroid Hormones and Development Daniel Buchholz and Aurea Orozco	Neuropeptide Signaling Pathways in Arthropods Angela Lange and Ian Orchard	Stress Axis Function: From Mechanisms to Consequences 1 Bob Dores, James Carr, Kathleen Gilmour, and Matt Vijayan
12:20-14:00	Lunch		
14:00-16:00 Session Chair	Topics in Comparative Endocrinology Christopher Martyniuk and Nancy Denslow	Endocrinology of Domestic and Wild Fauna Marta Romano	Stress Axis Function: From Mechanisms to Consequences 2 Bob Dores, James Carr, Kathleen Gilmour, and Matt Vijayan
16:00-16:30	Coffee Break		
16:30-17:30 Plenary	Dr. Michael Romero- Century Ballroom ABC		
17:30-19:30	Poster Session 1- Odd Number Pre-Function Space		
Sunday, May 26, 2019			
Plenary 09:00-10:00	Dr. Ian Orchard- Century Ballroom ABC		
10:00-10:30	Coffee Break		
	Dogwood	Century Ballroom	Azalea
10:30-12:30 Session Chairs	Metabolism Regulation Suraj Unniappan and Peggy Biga	Non-Coding RNA in Cell Signaling Chun Peng	Omics: Analysis of Genomes, Proteomes, Transcriptomes, and Metabolomes in Comparative Endocrinology John Chang and Hamid Habibi

12:30-14:00	Lunch		
14:00-16:00 Session Chairs	Neuroendocrinology of Feeding Nick Bernier and Helene Volkoff	Novel Hormones and Hormonal Control David Lovejoy	Advancement of Gene Editing and Their Applications Yong Zhu and Yun-bo Shi
16:00-16:30	Coffee Break		
Plenary 16:30-17:30	Gorbman-Bern New Investigator Jason Breves		
17:30-19:30	Poster Session 2- Even Number Pre-Function Space		
Monday, May 27, 2019			
8:00-13:30	Silver Springs State Park Excursion *Shuttles Buses will leave hotel at 8:30*		
	Dogwood	Century Ballroom	Azalea
14:00-16:00 Session Chairs	**Moved to Azalea**- Hormonal Control of Germinal Stem Cell Development and Gametogenesis Hamid Habibi	Comparative Endocrinology of Osmoregulation Steve McCormick and Jason Breves	**Moved to Dogwood**- ISAREN: Epigenetic Analysis in Amphibian and Reptile Endocrinology and Neurobiology Satomi Kohno and Daniel Buchholz
Plenary 16:00-17:00	Dr. Carlos Aramburo- Century Ballroom ABC		
Tuesday, May 28, 2019			
ISAREN Plenary 09:00-10:00	Dr. Bob Denver- Century Ballroom ABC		
10:00-10:30	Coffee Break		
	Dogwood	Century Ballroom	Azalea
10:30-12:30 Session Chairs	Growth and Growth Factors Maricela Luna	Aspects of Reproductive Endocrinology & Neuroendocrinology 1 Natalia Garcia-Reyero and Vance Trudeau	Neuroendocrine Disruption of Animal Vocalizations and Socio-Sexual Behaviors Cheryl Rosenfeld and Frauke Hoffmann
12:30-14:00	Lunch		
14:00-16:00 Session Chairs	Advances in Endocrine Disruption Science Valerie Langlois and Jan Mennigan	Aspects of Reproductive Endocrinology & Neuroendocrinology 2 Vance Trudeau and Natalia Garcia-Reyero	GnRH-related Peptides in Metazoa: Recent Progress and Discoveries Jean-Paul Paluzzi and Pei-San tsai
16:00-18:00	NASCE Council Meeting 2- Hawthorne		
18:00-19:00 19:00-10:00	Closing Ceremony and Student Awards Presentation + Closing Banquet (separate ticket required)		

Plenary Speakers

DR. PETER THOMAS

GORBMAN-BERN LECTURE

Dr. Peter Thomas is a Professor in the Marine Science and the Ecology, Evolution and Behavior Departments at the University of Texas at Austin and holds the H.E.B. Endowed Chair in Marine Science. He has published over 280 papers in peer reviewed scientific journals and 30 chapters on a wide range of topics in vertebrate reproductive endocrinology and has received several honors in recognition of his contributions to the field, including an honorary doctorate and distinguished lectures in endocrinology. Dr. Thomas has pioneered the identification of novel receptors on the cell surface that mediate the rapid actions of steroid hormones. There is widespread interest in rapid steroid actions because they have been implicated in human diseases such as breast and prostate cancers, hypertension, metabolic syndrome, and neurodegenerative disorders. However, lack of knowledge on the identities of the membrane receptors mediating these steroid actions had slowed progress in this emerging field of endocrinology for over 25 years. In 2003 Thomas and coworkers reported the discovery of the gene encoding the progesterone membrane receptor (mPR) in fish ovaries and the homologous mPRs in other vertebrates, including humans. The mPRs were the first steroid receptors identified that were unrelated to nuclear steroid receptors and naturally generated great interest because they provided an explanation for results showing rapid progesterone effects in many cell types in which nuclear steroid receptors were absent. The two papers describing these findings have been highly cited (779 and 690 times) and have stimulated studies by many research groups on the functions of mPRs in numerous animal models and tissues, including their roles in human reproductive health. Thomas' group have studied mPR's roles in human birth, sperm motility, and malignancy (breast and endometrial cancers) and non-reproductive functions (cardiovascular protection, neurodegeneration). In 2005 Thomas reported that an orphan receptor GPR30 (now known as GPER), is a membrane estrogen receptor in human breast cancer cells (cited 1291 times to date), which was confirmed independently shortly afterwards by another research group. This discovery has stimulated intensive research of the functions of GPER, especially in breast cancer, diabetes, and in cardiovascular system and has resulted in over a thousand publications. Recently, Thomas and coworkers reported the discovery of a novel androgen membrane receptor, ZIP9, in fish ovaries, and that human ZIP9 also functions as an androgen receptor. Importantly, androgens induce apoptosis through ZIP9 in human breast and prostate cancer cells, suggesting it is a potential therapeutic target for cancer treatment. The discovery by the Thomas research group of the identities of all three sex steroid membrane receptors in vertebrates, the mPRs, GPER, and ZIP9, has opened up an entirely new avenue for research on rapid steroid hormone actions and functions in health and disease in humans, as well as providing new potential targets for treating major causes of mortality such as cardiovascular disease, breast and prostate cancers, and neurodegeneration.

RAPID SEX STEROID ACTIONS MEDIATED THROUGH NOVEL TRANSMEMBRANE RECEPTORS: LESSONS FROM STUDIES ON FISH GONADS

Thomas P

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

In addition to the classic genomic mechanism of steroid action through activation of intracellular nuclear receptors which results in a slow hormonal response (hours to days), steroids also exert rapid actions through binding to receptors unrelated to nuclear receptors on the cell surface to activate intracellular signaling pathways within seconds to minutes

resulting in hormonal responses that are often nongenomic. However, progress in this emerging field of rapid steroid signaling was severely hampered for over 20 years by the failure to identify the membrane receptors mediating these steroid actions. In 2003 we reported the first identification of a membrane steroid receptor in vertebrates, membrane progestin receptor alpha (mPRα) in spotted seatrout ovaries. The mPRα is a member of the progesterone and adipoQ receptor (PAQR) family and is the intermediary in progestin induction of oocyte meiotic maturation and follicle cell anti-apoptosis in seatrout ovaries. Human mPRα was also found to have the characteristics of a membrane progestin receptor and mediate anti-apoptotic effects of progestins in cancer cells. Two years later we used a membrane estrogen binding receptor assay developed in Atlantic croaker ovaries to demonstrate that the orphan receptor, GPR30 (now known as G protein-coupled estrogen receptor, GPER), is a membrane estrogen receptor in breast cancer cells and croaker oocytes. Recently, we identified the membrane androgen receptor, ZIP9, which is a member of the ZIP (SLC39A) zinc transporter family, in croaker ovaries using the same approach we had used to identify seatrout mPRα. Human ZIP9 is expressed in cancer cells where it also functions as a membrane androgen receptor and has the same apoptotic functions as croaker ZIP9. Comparative studies of the ligand binding characteristics, G protein coupling, intracellular signaling pathways, and functions of these three membrane receptors in fish ovarian and human cancer cells have shown they are remarkably similar in these two distantly related vertebrate groups. These findings suggest the steroid signaling and cellular functions of these novel membrane receptors are conserved in vertebrates and probably their primary physiological roles.

JASON P. BREVES
GORBMAN-BERN NEW INVESTIGATOR

Dr. Jason Breves obtained his PhD in 2010 from the University of Hawaii at Manoa under the co-supervision of Drs. Gordon Grau and Tesuya Hirano. Following post-doctoral studies at the Department of Biology and Center for Neuroendocrine Studies, University of Massachusetts Amherst, and Conte Anadromous Fish Research Center, U.S. Geological Survey, Turners Falls, MA, he took up his current position as Assistant Professor, Department of Biology, Skidmore College, NY in 2014 and Associate Chair as of 2018. Dr. Breves' research focuses on the comparative endocrinology of osmoregulation and ion transporting epithelium, as well as metabolism and development in fish models. He received the Scholander Award and the Research Recognition Award from the American Physiological Society in 2013 and is the recipient of the 2019 NASCE Gorbman-Bern New Investigator Award.

Jason is an Assistant Professor in the Biology Department at Skidmore College (NY, USA). Following the completion of a M.S. (University of Rhode Island) with Dr. Jennifer Specker where he investigated the stress physiology of flounder, Jason completed his Ph.D. (University of Hawaii) with Drs. Gordon Grau and Tetsuya Hirano. His dissertation centered on the endocrine control of branchial ionocytes in tilapia. He then joined the University of Massachusetts as a National Institutes of Health NRSA post-doctoral fellow in the Center for Neuroendocrine Studies where he studied the developmental physiology of zebrafish. At the inaugural NASCE meeting in 2011, Jason received the Best Postdoctoral Poster Award. In 2013, Jason received both the Scholander and Research Recognition Awards from the Comparative and Evolutionary Physiology Section (CEPS) of the American Physiological Society. More recently, Jason was named as the recipient of the New Investigator Award from CEPS. Jason's current research program employs a variety of fish models (tilapia, zebrafish, mummichog, and Atlantic salmon) to identify molecular targets of prolactin signaling in addition to resolving the roles of insulin-like growth-factor binding proteins in the regulation of growth and development. As an organismal physiologist, Jason's work seeks to connect observations at the cellular and molecular levels with the physiology, development, and natural histories of the organisms he studies. Jason's laboratory is currently supported by the National Science Foundation (IOS-1755131).

PROLACTIN SUPPORTS ION UPTAKE BY TELEOST IONOCYTES

Breves JP

Department of Biology, Skidmore College, Saratoga Springs, New York, USA

Teleost fishes inhabiting freshwater environments sustain hydromineral balance via the activities of a suite of tissues that counteract diffusive ion losses to the external environment. For nearly sixty years, the pituitary hormone prolactin (Prl) has been widely recognized as a key stimulator of ion-conserving processes; nonetheless, a detailed understanding of the molecular and cellular mechanisms of these actions has proven elusive. This presentation will describe how various experimental approaches (e.g., hypophysectomy, hormone replacement, morpholinos, in vitro gill incubation, Prl receptor blockade) were leveraged to define how Prl controls 'freshwater-type' branchial ionocytes. These ionocytes directly absorb Na^+ and Cl^- from dilute environments. A series of investigations employing euryhaline (tilapia) and stenohaline (zebrafish) models revealed novel targets of Prl that support discrete solute and water-handling processes. Prl-signaling underlies the salinity-dependent gene and/or protein expression of branchial Na^+/Cl^- cotransporter 2 (Ncc2), Clc family Cl^- channel 2c (Clc-2c), and aquaporin 3 (Aqp3). In addition to providing mechanistic insight into how Prl underlies hydromineral balance in teleosts, these investigations have supported the identification of Prl-regulated ion transport pathways in the mammalian kidney.

Acknowledgements: Supported by the National Science Foundation (IOS-1755131).

DR. SHEUE-YANN CHENG

Sheue-yann Cheng, PhD, is the Section Chief of the Gene Regulation, Laboratory of Molecular Biology, NCI, NIH. Dr. Cheng's lifetime interest has been to unravel the complex molecular actions of thyroid hormone. She pioneered the development of mouse models to understand the molecular basis of diseases caused by mutations of thyroid hormone nuclear receptors and developed preclinical mouse models of metastatic follicular thyroid cancer. For her outstanding research accomplishments, Dr. Cheng has received many awards including the prestigious Sidney H. Ingbar Distinguished Lectureship Award of the American Thyroid Association (ATA), Distinguished Service Award and the John B. Stanbury Thyroid Pathophysiology Medal of ATA, the Pitt-Rivers Lectureship Award of The British Thyroid Association, the Charles Harkin Award of NCI, the Merit Awards of the NIH, and the Abbott Thyroid Research Clinical Fellowship Mentor Award of the Endocrine Society. She is an Associate Editor of Thyroid and an Associate Editor-in-Chief of the American Journal of Cancer Research. She serves on the Editorial Board of many journals. She holds patents, and licenses of her inventions and has published more than 265 papers in peer-reviewed journals.

MOLECULAR ACTIONS OF THYROID HORMONE RECEPTOR MUTANTS: WHAT THE ZEBRFISH TELLS US ABOUT A HUMAN DISEASE

Han CR, Holmsen E and Cheng SY

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892

Thyroid hormone receptors (TR) mediate the diverse biological activities of thyroid hormone (T3) in growth, development, and differentiation. Mutations of the THRA gene cause a debilitating disease known as resistance to thyroid hormone (RTHα). Patients exhibit severe growth retardation, delayed bone development, cognitive defects and skin disorders. That patients are heterozygotes indicates the pathogenesis is mediated by the dominant negative actions of TRα1 mutants. However, the molecular basis underlying these deleterious defects is yet to be fully elucidated, especially during development. We created mutant zebrafish to model RTHα by expressing mutated thra genes (gene duplication in zebrafish: thraa and thrab) by CRISPR/Cas-9 mediated targeted mutagenesis. We obtained two fish lines expressing mutated thraa (8-bp insertion) or thrab (1-bp insertion) to encode C-terminal mutated TRα1 (L405EfsX6 and E394X mutants, respectively). These two mutants exhibited dominant negative activity, similar to those found in patients. Zebrafish expressing E394X mutant displayed more severe and persistent impaired growth than fish expressing L405EfsX6 mutant, with male predominance. We elucidated that the impaired growth was mediated by suppression of the expression of the growth-related genes (gh1, smtla and smtlb) and attenuation of the GH/IGF1 signaling. Further analysis indicated that E394X mutant could act as early as day 7 to delay bone development to contribute to impaired growth. Moreover, E394X mutant fish, but not L405EfsX6 mutant fish, presented the striking hypoplastic epidermis phenotype in 30-day juveniles and adults, due to the suppression of the expression of several T3-regulated keratin genes at both the mRNA and protein levels by the E394X mutant. Thus, we have generated mutant zebrafish faithfully reproduce RTHα in patients. That RTHα is caused by TRα mutations is conserved in humans, mice and zebrafish. The novel zebrafish model not only is useful to understand the in vivo pathogenic actions of TRa1 mutants in a human disease, but also to provide a platform for rapid screening of drugs to treat abnormalities at early development.

DR. MICHAEL ROMERO

L. Michael Romero, Professor of Biology at Tufts University, has studied stress for over 30 years. He earned his PhD from Stanford under the direction of Robert Sapolsky where he focused on the hypothalamic regulation of the pituitary during psychological stress. He then shifted gears from biomedical research to ecological research during his postdoc with John Wingfield at the University of Washington. The unifying theme to his research continues to be the concept of stress. He blends his graduate experience studying stress in white lab rats with his postdoctoral experience studying stress responses in many different wild animals in their natural habitats. He has been trying to answer three main questions: what causes stress in a wild animal; what physiological, endocrinological, and behavioral mechanisms are turned on in response to those stressors; and how do those mechanisms help wild animals live in their natural environments. Throughout his career, the study system has been less important than the questions that could be addressed. Consequently, Professor Romero has work completed or in progress with 16 avian species (focusing on European starlings and house sparrows), 7 reptile species (focusing on Galapagos marine iguanas), 5 wild mammalian species (focusing on brown lemmings and degus), and 2 amphibians (focusing on spotted salamanders). His research takes an integrative approach, utilizing neuroendocrinology, endocrinology, and ecology in both the lab and the field, all with the goal of increasing our comprehension of the causes and effects of stress in wild animals. He has also recently summarized the work in this field in a book he co-wrote with John Wingfield entitled: “Tempests, Poxes, Predators, and People: Stress in Wild Animals and How They Cope.”

REACTIVE SCOPE AS A POTENTIAL PATH FORWARD TO UNDERSTANDING STRESS

Traditionally, our understanding of stress has focused on three main features: (1) stressors that created lack of predictability or controllability; (2) an acute physiological and/or behavioral stress response to those stressors; and (3) chronic stress when acute responses were too frequent or lasted too long. This conceptualization of stress was derived from biomedical and clinical studies, often in laboratory settings with domesticated laboratory species, and has helped us understand many phenomena related to an animal’s responses to stressors. However, data from the last 2+ decades indicates that this conceptualization of stress often lacks explanatory or predictive power, especially when applied to free-living or captive wild animals. Examples include an inability to explain why seasonal variation exists in stress responses, an inability to explain why there is a lack of correlation of responses across different tissues, and an inability to predict physiological responses to chronic stress. Allostasis, with it’s focus on an animal’s energy balance, was a major step forward. For the first time, energy budgets could be used to predict an individual animal’s responses to stress. However, allostasis does not incorporate many important behavioral or physiological responses to stressors, especially acute responses. Reactive scope, with a focus on wear-and-tear, melded these two approaches. Recent data, such as predicting survival of marine iguanas to famine and understanding wound healing rates during stress, suggest that reactive scope may provide better explanatory and predictive power than either of the other approaches.

DR. IAN ORCHARD

Ian Orchard is a Professor Emeritus, University of Toronto. The central theme of his research program is the functioning of the nervous system, using insects as experimental models. His research examines the mechanisms by which the nervous system communicates information; defining hormonal, synaptic, and modulatory mechanisms, using neurophysiological, neurochemical, endocrinological, and molecular biological techniques. The questions he is asking are fundamentally important for all nervous systems. In particular he is interested in the role of peptides and amines as neurohormones, released into the blood to co-ordinate activities of diverse groups of tissues, and as neuromodulators, modulating the ongoing activities of distinct pathways. He has published more than 200 refereed articles and trained more than 150 highly qualified research personnel at the undergraduate, graduate and postdoctoral level, 17 of whom have obtained university faculty positions. He earned a Doctor of Science degree (1988), a PhD (1975), and a B.Sc. (1972), all from the University of Birmingham, UK. Along with this successful research career, he also served as a Vice-President Academic and Provost, University of Waterloo (2014-2017), Vice-President of the University of Toronto, and Principal of the University of Toronto Mississauga (2002-2010), Vice-Provost Students, University of Toronto (1998-2002), and Associate Dean, Sciences, Faculty of Arts and Science, University of Toronto (1993-1998).

THE KISSING BUG RHODNIUS PROLIXUS: A MODEL FOR ENDOCRINOLOGICAL AND PHYSIOLOGICAL STUDIES

Ian Orchard

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

All instars and adults of the kissing bug, *Rhodnius prolixus*, are obligatory blood-feeders and all are vectors for the parasite *Trypanosoma cruzi* that results in human Chagas disease. Given access to a host, unfed *R. prolixus* take a massive blood meal over 15 - 20 mins, which triggers long-term endocrinological and physiological events associated with growth, development and reproduction. Short-term events are also initiated to jettison the excess water and salts imbibed in the blood meal. *T. cruzi* is passed to the human during this diuresis.

The ability to precisely time the onset of both short and long term events (initiated by blood gorging) has made this insect an ideal model for research into physiology and endocrinology, as originally shown by Sir Vincent Wigglesworth in the 1930's and beyond. Here we examine the neurohormones that control diuresis.

Serotonin is a diuretic hormone (DH) in *R. prolixus* and acts in concert with a member of the corticotropin-releasing factor (CRF)-related family of insect neuropeptides (Rhopr-CRF/DH), via their respective G-protein-coupled receptors (GPCRs). Serotonin and Rhopr-CRF/DH have potent biological activity on anterior midgut and Malpighian tubules, and work synergistically, via cAMP, to stimulate diuresis following gorging.

An anti-diuretic hormone (ADH), a member of the CAPA family, Rhopr-CAPA-2, and its cognate GPCR, terminate this diuresis, inhibiting serotonin-stimulated secretion by Malpighian tubules. Rhopr-CAPA-2 eliminates the synergism between serotonin and Rhopr-CRF/DH, thereby leading to a quick cessation of diuresis.

The interplay between the DHs and ADH in R. prolixus results in a remarkable diuresis; a diuresis that has evolved to eliminate excess water and salts from a massive blood meal in a very rapid way. The parasite, *T. cruzi*, is transmitted via the excreted fluid during this diuresis, and therefore these neurohormones control the transmission of Chagas disease.

Supported by an NSERC Discovery Grant.

DR. CARLOS ARAMBURO

He was born on October 7, 1953 in Teziutlán, Pue., Mexico. He completed his undergraduate studies of Pharmacobiological Chemist (1972-76), Master (1978-80) and Doctorate in Chemical Sciences (Biochemistry) (1981-83) in the Faculty of Chemistry, at the National Autonomous University of Mexico (UNAM), and made several research stays (Comparative Endocrinology) at Rutgers-The State University of New Jersey, USA (1985-1993) in the laboratory of Dr. Colin G. Scanes. His research work has been carried out in the National Institute of Nutrition "Salvador Zubirán" (1975-83); and then in the Institute for Biomedical Research (1983-93) and in the Institute of Neurobiology (1993-to date), both at UNAM, where he is a tenured Full Professor.

His area of specialty is the biochemistry of proteins, particularly the molecular and functional characterization of peptide hormones and neuroendocrine messengers. His research lines have always been involved with comparative endocrinology. In the last decades he has focused on studies on the heterogeneity of growth hormone (GH) and the changes it undergoes during the evolution of vertebrates, showing that it is a family of proteins with functional and molecular diversity. Also, his group has studied the relevance of extrapituitary expression of GH in diverse organs and tissues of the nervous, immune, and reproductive systems, among others, and has shown that this hormone plays an important role through autocrine, paracrine and/or intracrine mechanisms, which modulate cell proliferation, differentiation and survival/protection effects. Some of his studies include the description of local expression of GH and GH receptor (GHR) mRNAs and proteins in various neural tissues of several vertebrate models (mammals, birds, reptiles), as well as the neurotrophic and neuroprotective actions of GH in response to neural damage provoked by different insults, in the cerebellum (hypoxia/ischemia) and in the neuroretina (excitotoxic damage). These studies have shown that a complex cascade of neurotrophins and growth factors, which have been classically related to damage prevention and neural tissue repair, likely mediates GH neuroprotective actions. Another research interest has been the evolution of the mechanisms involved in the regulation of the somatotropic axis in vertebrates.

He was founder and served as the first Academic Secretary of the Centre of Neurobiology (1993-2002) and as the first Director of the Institute of Neurobiology (2002-2007) at UNAM's Campus Juriquilla in Queretaro. He served, for eight years, as Vice-President of Scientific Research at UNAM, and as Chairman of the Technical Council of Scientific Research of the University (2007-2015). Currently, he serves as Director General of Academic Affairs (DGAPA) at UNAM.

He is a member of 12 scientific societies, and has served as member of the Executive Board of the Mexican Society of Physiological Sciences (1994-95) and of the International Council of the International Society of Avian Endocrinology (1997-2004, 2012-2020). He was one of the three co-founders of the North American Society for Comparative Endocrinology, NASCE (2010-2011), where he has been Vice-President (2011-2013), President (2013-2015), ex-officio member of the Executive Board (2015-2017), and member of the International Council (2017-2019).

ENDOCRINE/PARACRINE/AUTOCRINE NEUROPROTECTIVE ACTIONS OF GROWTH HORMONE

Arámburo C (1), Ávila-Mendoza J (2), Baltazar-Lara MR (1), Fleming T (1,2), Alba-Betancourt C (1), Carranza M (1), Balderas-Márquez JE (1) Epardo D (1), Harvey S (2), Luna M (1), Martínez-Moreno C (1).

[1]Dept. Cellular and Molecular Neurobiology, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México, and [2]Dept. Physiology, University of Alberta, Edmonton, T6G 2H7, Canada.

It is now accepted that, besides the pituitary somatotrophs, growth hormone (GH) can be expressed in several extrapituitary locations, such as the nervous, immune and reproductive systems, among others. The brain is a GH target site and GH receptors are expressed widely throughout the central nervous system (CNS). The presence of GH and GH mRNA in neural tissues is well established. There is increasing evidence that GH may be involved in neurotrophic, neuroprotective and neuro-regenerative actions. Although systemic (endocrine) GH may exert some of these effects, evidence indicates that locally expressed neural GH, acting through autocrine and/or paracrine mechanisms may also participate in these actions. We described the local expression of GH and GH receptor (GHR) mRNAs and proteins in various neural tissues of several vertebrate models (mammals, birds, reptiles), and studied the neurotrophic, neuroprotective and neuroregenerative actions of GH in response to neural damage provoked by

different insults, in the cerebellum (hypoxia/ischemia) and in the neuroretina (excitotoxic damage). We also analyzed the participation of canonical and non-canonical pathways and some of the mechanisms involved in these actions, which include a complex network of several neurotrophins, cytokines and neural growth factors that have been related to neural damage prevention, which likely mediate GH protective roles in neural tissues.

This work was partially supported by PAPIIT-DGAPA-UNAM (IN201817, IN206115, IA200717, IN207018) and CONACYT (178335, 285004).

DR. ROBERT J. DENVER

ISAREN LECTURE

Dr. Robert J. Denver is Professor and Chair of the Department of Molecular, Cellular and Developmental Biology (MCDB), and Professor of Ecology and Evolutionary Biology (EEB) at the University of Michigan, Ann Arbor. He earned his B.S. in Physiology from Rutgers University, and the Ph.D. in Zoology from the University of California at Berkeley. He is a developmental neuroendocrinologist with expertise in gene regulation by nuclear hormone receptors; development and evolution of the neuroendocrine stress axis; mechanisms of developmental plasticity; endocrinology, ecology, and molecular biology of amphibian metamorphosis; and mechanisms of action of Krüppel-like factors in nervous system development and regeneration. He is an elected fellow of the American Association for the Advancement of Science (AAAS), he was co-founder and first president of the North American Society for Comparative Endocrinology (NASCE), and he served as president of the International Federation of Comparative Endocrine Societies (IFCES). He was a regular member of the Integrative and Clinical Endocrinology and Reproduction (ICER) Study Section for the National Institutes of Health, a member of the Annual Meeting Steering Committee for the Endocrine Society, and he has served on seven scientific advisory panels for the US Environmental Protection Agency, including the Endocrine Disrupter Screening Program, and four grant review panels for the US National Science Foundation. He serves as associate editor for General and Comparative Endocrinology.

THYROID HORMONE ACTION IN XENOPUS TADPOLE BRAIN DURING METAMORPHOSIS

Robert J. Denver

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During metamorphosis the tadpole brain undergoes dramatic changes such as cell expansion, cell migration, apoptosis, differentiation and maturation. These cellular and tissue processes are regulated by thyroid hormone (TH), which induces gene regulation programs that drive the diverse morphogenetic processes. Thyroid hormone acts via two evolutionarily conserved nuclear receptors, TRα and TRβ. The gene that codes for TRα (thra) is expressed immediately after the tadpole hatches, and is maintained throughout tadpole development and metamorphosis. By contrast, expression of the gene that codes for TRβ (thrb) coincides with the development of the tadpole's thyroid gland, increasing during metamorphosis and peaking at metamorphic climax. This expression pattern depends on TH; i.e., thrb is autoinduced. The TRα is the major TR subtype expressed in tadpole brain, and it is required for the majority of gene regulation responses to TH, and for neurogenesis. Tadpoles with the thra gene inactivated have proportionally smaller brains compared with wild type. The TRβ mediates TH actions on cell differentiation and apoptosis. Thyroid hormone receptors function as epigenetic switches to modify chromatin structure, thereby influencing gene transcription. Earlier work showed that TH induces posttranslational modifications of histones. We found that TH also influences chromatin structure by modulating DNA methylation. While TH modulates both DNA methylation and demethylation in tadpole brain, DNA demethylation predominates during metamorphosis. The action of TH on DNA methylation is mediated, in part, by direct TH regulation of the DNA methyltransferase 3a gene. The action of TH on DNA demethylation is more complex, with TH directly and indirectly regulating transcription of genes that code for enzymes involved in the DNA demethylation pathway. We also found that liganded TRs recruit ten eleven transferase (TET) enzymes to chromatin, which leads to localized DNA demethylation. The TET enzymes are dioxygenases that convert 5-methylcytosine to several DNA demethylation intermediates. Thyroid hormone has complex actions on developing tadpole brain, and studies in mammals support that many of these actions are evolutionarily conserved.

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Program

Friday, May 24, 2019			
14:00-16:30	NASCE Council Meeting 1- Hawthorne		
18:00-18:15	Welcome and Opening Reception- Harn Museum of Art		
18:15-19:15	Dr. Peter Thomas- Gorbman Bern Lecture		
19:15-21:00	Opening Reception		
Saturday, May 25, 2019			
08:00-09:00	Registration		
Plenary 09:00-10:00	Dr. Sheue-Yann Cheng- Century Ballroom ABC		
10:00-10:30	Coffee Break		
	Dogwood	**Century Ballroom**	**Azalea**
10:30-12:30 **Session** **Chairs**	**Thyroid Hormones and Development** **Daniel Buchholz and Aurea Orozco**	**Neuropeptide Signaling Pathways in Arthropods** **Angela Lange and Ian Orchard**	**Stress Axis Function: From Mechanisms to Consequences 1** **Bob Dores, James Carr, Kathleen Gilmour, and Matt Vijayan**
10:30-10:50	**Darras, Veerle** AGE-DEPENDENT CHANGES IN GLUCOSE METABOLISM IN DEIODINASE TYPE 2 KNOCKOUT ZEBRAFISH *Houbrechts A.M., Darras V.M.*	**Mykles, Donald** SIGNALING PATHWAYS CONTROLLING PHASE TRANSITIONS IN THE CRUSTACEAN MOLTING GLAND *Mykles DL, Chang ES, Chang SA, Durica DS, Tomanek L, Ventura T , Zhou W*	**Faught, Erin** LOSS OF THE GLUCOCORTCOID RECEPTOR IN ZEBRAFISH IMPROVES MUSCLE GLUCOSE AVAILABILITY AND INCREASES GROWTH *Faught E, Vijayan M*
10:50-11:10	**Orozco, Aurea** KNOCK-DOWN OF SPECIFIC THYROID HORMONE RECEPTOR ISOFORMS IMPAIRS BODY PLAN DEVELOPMENT IN ZEBRAFISH *Lazcano I, Rodríguez-Ortiz R, Villalobos P, Solís-Saínz JC, Orozco A*	**Park, Yoonseong** DISRUPTION OF NEUROPEPTIDERGIC SYSTEM IN ARTHROPOD PEST CONTROL *Park Y*	**Deviche, Pierre** GLUCOCORTICOIDS AND GLYCEMIA DURING STRESS IN BIRDS *Deviche P, Griffith S, Buchanan K*
11:10-11:30	**Shibata, Yuki** THYROID HORMONE RECEPTOR DEFFICIENCY ACCELERATES CONNECTIVE TISSUE DEVELOPMENT BUT PREVENTS EPITHELIAL TRANSFORMATION DURING METAMORPHOSIS IN XENOPUS TROPICALIS. *Shibata Y, Okada M, Wen L, Shi YB.*	**Zandawala, Meet** MODULATION OF DROSOPHILA POST-FEEDING PHYSIOLOGY AND BEHAVIOR BY THE NEUROPEPTIDE LEUCOKININ *Zandawala M, Yurgel M, Texada M, Liao S, Rewitz K, Keene A, Nässel D*	**Hoglin, Brianne** CHARACTERIZATION OF WHALE SHARK MELANOCORTIN-2 RECEPTOR REVEALS DISTINCT PATTERNS OF MC2R ACTIVATION FOR HOLOCEPHALAN AND ELASMOBRANCH CARTILAGINOUS FISHES *Hoglin B, Dores R*

11:30-11:50	**Suzuki, Ken-ichi** INVOLVEMENT OF THYROID HORMONE RECEPTORS IN THE HEMOGLOBIN SWITCH DURING FROG METAMORPHOSIS *Suzuki KT, Buchholz D*	**Rocco, David** EXPRESSION PROFILING, DOWNSTREAM SIGNALING AND INTER-SUBUNIT FUNCTIONAL CHARACTERIZATION OF AN EVOLUTIONARY ANCIENT GLYCOPROTEIN HORMONE SYSTEM (GPA2/GPB5) IN THE MOSQUITO, AEDES AEGYPTI *Rocco D, Paluzzi JP*	**Dores, Robert** EVALUATING THE ROLE OF THE MELANOCORTIN-5 RECEPTOR IN THE HPA/HPI AXIS: A PHYLOGENETIC STUDY *Dores RM, Oberer N, Hoglin B*
11:50-12:10	**Taylor, Elias** NON-GENOMIC THYROID HORMONE SIGNALING IN INVERTEBRATES: T4 REGULATES SKELETOGENESIS IN THE PURPLE SEA URCHIN VIA AN INTEGRIN-MEDIATED MAPK CASCADE *Taylor E, Heyland A*	**Leyria, Jimena** THE INVOLVEMENT OF INSULIN-LIKE PEPTIDE SIGNALING IN THE REPRODUCTIVE SUCCESS OF RHODNIUS PROLIXUS, A VECTOR OF CHAGAŚ DISEASE *Leyria J, Orchard I, Lange AB*	**Bernier, Nicholas** CORTICOTROPIN-RELEASING FACTOR EXERTS NEUROPROTECTIVE EFFECTS AGAINST AMMONIA NEUROTOXICITY IN ISOLATED LARVAL ZEBRAFISH BRAINS *Bernier NJ, Williams TA*
12:10-12:30		**Ayub, Mahnoor** THE ROLE OF SIFAMIDE AS A NEUROHORMONE IN THE BLOOD-GORGING INSECT, RHODNIUS PROLIXUS. *Ayub M, Lange AB, Orchard I*	**Schaaf, Marcel** GLUCOCORTICOID MODULATION OF THE IMMUNE RESPONSE IN ZEBRAFISH *Xie Y, Schaaf MJM*
12:30-14:00	**Lunch**		
14:00-16:00 **Session** **Chair**	**Topics in Comparative Endocrinology** **Christopher Martyniuk and Nancy Denslow**	**Endocrinology of Domestic and Wild Fauna** **Marta Romano**	**Stress Axis Function: From Mechanisms to Consequences 2** **Bob Dores, James Carr, Kathleen Gilmour, and Matt Vijayan**
14:00-14:20	**Denslow, Nancy** PROTEOGENOMICS OF FATHEAD MINNOW (PIMEPHALES PROMELAS) AS A FIRST STEP TO IDENTIFY TRANSCRIPT VARIANTS OF IMPORTANCE TO NEUROENDOCRINOLOGY *Denslow ND, Lavelle C, Smith LC, Bisesi JH, Sanchez CS, Buerger AN, Garcia-Reyero N, Sabo-Attwood T*	**Hamlin, Heather** INFLUENCE OF TEMPERATURE REGIME AND EPIZOOTIC SHELL DISEASE ON ECDYSTERONE CONCENTRATIONS IN AMERICAN LOBSTERS, HOMARUS AMERICANUS *Hamlin HJ, Tudor MS, Bouchard DA*	**Lutterschmidt, Deborah** MECHANISMS OF LIFE-HISTORY TRANSITIONS: INTERACTIONS AMONG GLUCOCORTICOIDS, NEUROPEPTIDES, AND METABOLIC FACTORS REGULATE THE SEASONAL SWITCH FROM REPRODUCTION TO FORAGING BEHAVIOR IN GARTER SNAKES *Lutterschmidt DI, Dayger CA, Lucas AR, Wilson, RC*
14:20-14:40	**Sangha, Vishal** PHYSIOLOGICAL EFFECTS OF STRUCTURAL ANALOGS OF KININS AND CAPA IN RHODNIUS PROLIXUS *Sangha V, Nachman RJ, Orchard, Lange AB*	**González-de-la-Vara, Marcela** HOW TO GROUP PRIMIPAROUS DAIRY COWS: BEHAVIOR, CORTISOL IN SERUM AND HAIR AND PRODUCTION PERFORMANCE. *González-de-la-Vara M, De Anda F, Vázquez-Ch JC, Romano MC*	**Gilmour, Kathleen** TOO STRESSED TO EAT OR GROW: THE METABOLIC COST OF CHRONIC SOCIAL STRESS IN RAINBOW TROUT (Oncorhynchus mykiss) *Gilmour KM, Best C, Culbert BM, Jennings K, Kostyniuk DJ, Saulnier RJ, Lamarre SG, Mennigen J, Moon TW*
14:40-15:00	**Wu, Xinjun** INVOLVEMENT OF MULTIPLE PROGESTERONE RECEPTORS IN OVARY MAINTENANCE IN ZEBRAFISH *XinJun W, Andrew L, Marcus W, Pujan P, Tyler O, Yong Z*	**Romano, Marta** SEXUAL MATURATION OF AFRICAN ELEPHANTS RAISED IN CAPTIVITY *García-Delgado SD, Martínez G, Pedernera M, Olloqui E, Valdez RA, Romano MC*	**Edwards, Thea** PHYSIOLOGICAL COSTS OF CHRONIC SEASONAL HYPOXIA IN OKAVANGO TILAPIA *Edwards TM, Mosie IJ, Moore BC, Lobjoit G, Bachman RE, Murray-Hudson M*

15:00-15:20	**Thomson, Paisley** DOES CHRONIC EXPOSURE TO AGRICULTURAL RETENTION POND WATER INDUCE ENDOCRINE DISRUPTION IN THE AMERICAN TOAD? *Thomson P, Labranche P-A, Patey G, Robinson SA, Gruyer N, Thériault G, and Langlois VS*	**Rodas-Martinez, Alba Zulema** SERUM GLUCOCORTICOID PROFILES IN THREE SPECIES OF MEXICAN PRIMATES: RESPONSE TO CAPTURE-RESTRAINT *Rodas-Martínez AZ*	**Harris, Breanna** OF MICE AND MEN: RELATIONSHIP AMONG STRESS, GLUCOCORTICOIDS, AND COGNITIVE FUNCTION *Harris BN*
15:20-15:40	**Zhang, Hugh** CAGE ENRICHMENTS NEGATIVELY IMPACT THE REPRODUCTIVE BRAIN IN MALE MICE *Zhang HS, Tsai PS*	**Kohno, Satomi** TIMING OF A GONADAL COMMITMENT TO THE TESTICULAR DIFFERENTIATION BEYOND ESTROGEN-SIGNAL PRODUCING OVARY IN THE TEMPERATURE-DEPENDENT SEX DETERMINATION OF THE AMERICAN ALLIGATOR *Vang D, Ang E, Schoenfuss HL, Kohno S*	**Gorissen, Marnix** A NOVEL ROLE FOR LEPTIN IN FISH: BRANCHIAL LEPTIN IS INVOLVED IN SHORT-TERM SEAWATER ACCLIMATION IN ATLANTIC SALMON (SALMO SALAR, L.) *Nilsen TO, Ebbesson LOE, van den Akker M, McCormick SD, Bernier NJ, Flik G, Gorissen M*
15:40-16:00	**Sajadi, Farwa** CAPA NEUROPEPTIDES: ANTI-DIURETIC HORMONE ACTIVITY AND SIGNALING CASCADE IN THE DISEASE-VECTOR MOSQUITO, AEDES AEGYPTI *Sajadi F, Paluzzi JP*		**Carr, James** POTENTIAL NEW ROLES FOR CRF AND NPY IN MIDBRAIN DEFENSE *Carr JA, Prater CM, Islam R, Harris BN*
16:00-16:30	**Coffee Break**		
Plenary 16:30-17:30	**Dr. Michael Romero- Century Ballroom ABC**		
17:30-19:30	**Poster Session 1- Odd Number** **Pre-Function Space**		
Sunday, May 26, 2019			
Plenary 09:00-10:00	**Dr. Ian Orchard- Century Ballroom ABC**		
10:00-10:30	**Coffee Break**		
	Dogwood	**Century Ballroom**	**Azalea**
10:30-12:30 **Session** **Chairs**	**Metabolism Regulation** **Suraj Unniappan and Peggy Biga**	**Non-Coding RNA in Cell Signaling** **Chun Peng**	**Omics: Analysis of Genomes, Proteomes, Transcriptomes, and Metabolomes in Comparative Endocrinology** **John Chang and Hamid Habibi**
10:30-10:50	**Mennigen, Jan** ACUTE AND LONG-TERM METABOLIC CONSEQUENCES OF EMBRYONIC ZEBRAFISH EXPOSURE TO AQUATIC CONTAMINANTS *ALLAIRE-LEUNG M, TRAHAN A , HUM C, TU W, MENNIGEN JA*	**Peng, Chun** MICRORNAS AS KEY PLAYERS IN ENDOCRINOLOGY *Peng C*	**Weljie, Aalim** THE RHYTHMS OF METABOLISM: TRANSLATIONAL CHRONOBIOLOGY DECOUPLES TRANSCRIPTION FROM METABOLISM *Krishanaiah S, Sengupta A, Malik D, Botallico L, Altman B, Dang CV, Hogenesch J, Weljie AM*
10:50-11:10	**Chung, J. Sook** EVOLUTIONARY AND ECOLOGICAL ENDOCRINOLOGY OF INVERTEBRATE CARBOHYDRATE METABOLISM *Chung JS*	**Li, Julang** THE ROLE OF MICRORNA IN THE REGULATION OF OOCYTE MATURATION *Li J, Pan B, Toms D*	**Martyniuk, Chris** TWENTY YEARS? OMICS, ESTROGENS, AND FISH. *Martyniuk CJ, Feswick A, Munkittrick KR, Dreier DA, Denslow ND*

11:10-11:30	**Charli, Jean-Louis** THYROTROPIN-RELEASING HORMONE-DEGRADING ECTOENZYME CONTROLS THYROTROPIN SECRETION AND BODY WEIGHT IN MALE RODENTS *Charli JL, Cote-Vélez A, Rodríguez-Rodríguez A, Hernández-Ortega K, Uribe MR, Anaya-Vergara M, Pérez-Estrada JR, Matziari M, Joseph-Bravo P*	**Yang, Burton** YAP IS ANTAGONIZED BY ITS CIRCULAR RNA VIA SUPPRESSING THE ASSEMBLY OF THE TRANSLATION INITIATION MACHINERY *Wu N, Yuan Z, Du WW, Fang L, Lyu J, Zhang C, He A, Eshaghi E, Ma J, Yang BB*	**Vijayan, Matt** MINERALOCORTICOID RECEPTOR SIGNALLING IN ZEBRAFISH LARVAE *Faught E, Vijayan M*
11:30-11:50	**Deck, Courtney** EVIDENCE FOR A LEPTIN-INSULIN AXIS IN THE MOZAMBIQUE TILAPIA (OREOCHROMIS MOSSAMBICUS) *Deck CA, Honeycutt JL, Severance ME, and Borski RJ*	**O'Brien, Jacob** MIR-218-5P MODULATES NEUROPEPTIDE Y SIGNALING IN TROPHOBLASTS *O'Brien J, Hayder H, Brkic J, Dunk C, Lye S, and Peng C*	**Ladisa, Claudia** METABOLIC PROFILING OF MALE GOLDFISH LIVER REVEALS PATTERNS OF ENERGY ALLOCATION IN SUPPORT OF GROWTH AND REPRODUCTION *Ladisa C, Ma Y, Habibi HR*
11:50-12:10			**Bonett, Ronald** GENOMIC AND TRANSCRIPTOMIC CONSEQUENCES OF THYROID HORMONE SENSITIVITY EVOLUTION IN SALAMANDERS *Bonett RM, Herrboldt MA, Clay TA, Ledbetter NM,Torres CD*
12:10-12:30			**Reynolds, Hannah** FISHING FOR PHYSIOLOGY IN BIG DATA: A MACHINE LEARNING ROADMAP FROM TRANSCRIPTOME TO PHYSIOLOGY IN THE TILAPIA *Reynolds HM, Baltzegar DA, Douros JD, Reading BJ, Borski RJ*
12:30-14:00	**Lunch**		
14:00-16:00 **Session** **Chairs**	**Neuroendocrinology of Feeding** **Nick Bernier and Helene Volkoff**	**Novel Hormones and Hormonal Control** **David Lovejoy**	**Advancement of Gene Editing and Their Applications** **Yong Zhu and Yun-bo Shi**
14:00-14:20	**Schneider, Jill** INGESTIVE OR REPRODUCTIVE BEHAVIOR? HORMONE-NEUROPEPTIDE INTERACTIONS THAT ORCHESTRATE THE TRADEOFF *Schneider J. E., Kriegsfeld L.*	**Lovejoy, David** DISCOVERY AND FUNCTION OF THE TENEURINS AND THEIR INTERACTION WITH LATROPHILINS IN VERTEBRATES: A PHYLOGENETICALLY ANCIENT MECHANISM OF RECEPTOR-LIGAND INTERACTIONS IN THE CENTRAL NERVOUS SYSTEM *Tucker R, Lovejoy D*	**Ge, Wei** FUNCTIONAL ANALYSIS OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN THE ZEBRAFISH – A GENETIC APPROACH WITH GENOME EDITING TECHNOLOGY *Ge W*
14:20-14:40	**Volkoff, Helene** THE ENDOCRINE REGULATION OF FEEDING IN SELECTED FRESHWATER TELEOST FISH *Volkoff, H*	**Biga, Peggy** INCREASED METABOLIC RATE BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-3: A COMPARATIVE ANALYSIS ACROSS ZEBRAFISH LIFE STAGES. *Reid R, D'Aquila A, Lovejoy D, Biga PR*	**Chen, Liangbiao** STUDIES ON MOLECULAR ADAPTION OF HORMONAL PEPTIDES FROM ANTARCTIC FISHES USING THE CRISPR-CAS9 TECHNOLOGY IN MODEL FISHES *Mingli L, Yan W, Chen L*

14:40-15:00	**Takahashi, Akiyoshi** EFFECTS OF CHROMATIC LIGHT ON SOMATIC GROWTH AND ENDOCRINE FUNCTIONS OF FLATFISHES *Takahashi A, Shimizu D, Kasagi S, Mizusawa K*	**Hogg, David** CORTICOTROPIN-RELEASING FACTOR (CRF) SIGNALING IS ANTAGONIZED BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP-1): INSIGHTS INTO THE INTERACTION OF TCAP-1 AND CRF IN NEURONS *Hogg D, Lovejoy D*	**Buchholz, Daniel** THYROID HORMONE SIGNALING IS NOT NECESSARY NOR SUFFICIENT FOR FROG METAMORPHOSIS *Shewade LH, Sterner ZR, Buchholz DR*
15:00-15:20	**Butt, Robyn** GOLDFISH (CARASSIUS AURATUS) GUT MICROBIOTA COMPOSITION AND THE EXPRESSION OF GENES RELATED TO APPETITE AND DIGESTION *Butt RL, Volkoff H*	**D'Aquila, Andrea** USING COMPARATIVE MODELS OF MUSCULAR DYSTROPHY TO ASSESS TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-1 AS A NOVEL THERAPEUTIC APPROACH. *Andrea L. D'Aquila, Rylie M. Hightower, David A. Lovejoy, Matthew S. Alexander, Peggy R. Biga*	**Wang, Deshou** ADVANCEMENT IN GENE EDITING AND THEIR APPLICATION IN TILAPIA SEX DETERMINATION *Wang DS, Li MH, Dai SF*
15:20-15:40	**Unniappan, Suraj** BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IS A MEAL-RESPONSIVE OREXIGEN IN ZEBRAFISH *Blanco AM, Bertucci JI, Unniappan S*	**Peng, Di** DISTRIBUTION OF THE NOVEL REPRODUCTIVE PEPTIDE SECRETONEURIN IN THE BRAIN AND PITUITARY OF THE ZEBRAFISH *Peng D*	**Carter, Nichole** OVARIAN DEVELOPMENT IN ZEBRAFISH REQUIRES ADAMTS9 *Carter N, Roach Z, Yong Z*
15:40-16:00			**Hui, Zhao** DELINEATE THE REGULATORY NETWORK FOR BMP SIGNALING DURING embryonic development *Wang C, Liu Z, Zhao H*
16:00-16:30	**Coffee Break**		
Plenary 16:30-17:30	**Gorbman-Bern New Investigator** **Jason Breves**		
17:30-19:30	**Poster Session 2- Even Number** **Pre-Function Space**		
Monday, May 27, 2019			
8:00-13:30	**Silver Springs State Park Excursion** *Shuttles Buses will leave hotel at 8:30*		
	Dogwood	**Century Ballroom**	**Azalea**
14:00-16:00 **Session** **Chairs**	**Moved to Azalea-Hormonal Control of Germinal Stem Cell Development and Gametogenesis** **Hamid Habibi**	**Comparative Endocrinology of Osmoregulation** **Steve McCormick and Jason Breves**	**Moved to Dogwood- ISAREN: Epigenetic Analysis in Amphibian and Reptile Endocrinology and Neurobiology** **Satomi Kohno and Daniel Buhholz**
14:00-14:20	**Dobrinski, Ina** MODELS TO STUDY CELL-CELL INTERACTIONS IN THE MAMMALIAN TESTIS *Sakib S, Goldsmith T, Valenzuela-Leon P, Dobrinski I*	**Seale, Andre** ACCLIMATION OF FISH TO DYNAMICALLY CHANGING SALINITIES: INSIGHTS FROM THE EURYHALINE MOZAMBIQUE TILAPIA *Seale AP*	**Akashi, Hiroshi** ELUCIDATION OF MOLECULAR MECHANISM UNDERLYING TEMPERATURE-SENSING DURING SEX DETERMINATION IN ALLIGATOR AND TURTLES *Hiroshi Akashi, Kenji Toyota, Satomi Kohno S, Taisen Iguchi , Shinichi Miyagawa*

14:20-14:40	**Nobrega, Rafael** CORTISOL AND THYROID HORMONES: "NEW" PLAYERS OF ZEBRAFISH SPERMATOGENESIS *Nobrega RH, Tovo-Neto A, Rodrigues MS, Habibi HR*	**Crespi, Erica** RELATIONSHIPS BETWEEN OSMOREGULATION AND IMMUNITY IN AMPHIBIANS *Crespi EJ, Hall EM, Schock DM*	**Parrott, Ben** PRECOCIOUS ESTROGEN SIGNALING DURING EMBRYONIC DEVELOPMENT UNDERLIES PERSISTENT ALTERATIONS OF OVARIAN TRANSCRIPTIONAL NETWORKS IN AN ENVIRONMENTAL MODEL OF ENDOCRINE DISRUPTION *Hale M,Parrott B*
14:40-15:00	**Pourmohammadi fallah, Hamideh** Paracrine control of germinal stem cell development and spermatogenesis by GnIH in zebrafish (Danio rerio). *Fallah HP, Rodrigues MS, Nóbrega RH, Habibi HR*	**McCormick, Stephen** EVIDENCE FOR A ROLE OF THYROID STIMULATING HORMONE, DEIODINASE AND THYROID HORMONE IN THE PHOTOPERIOD-DRIVEN SEASONAL CLOCK OF FISH *McCormick SD, Irachi S, Fleming M, Maugars G, Björnsson BT, Dufour S*	**Ishihara, Akinori** EPIGENETIC CHANGES CAUSED BY FASTING AND LOW TEMPERATURE IN AMPHIBIANS *Ishihara A, Yamauchi K*
15:00-15:20	**Martinez Bengochea, Anabel Lee** GONADAL TRANSCRIPTOME OF HYBRIDS DERIVED FROM CLOSELY RELATED SPECIES WITH THE SAME SEX DETERMINING GENE: O. latipes and O. curvinotus. *Martinez-Bengochea, A., Adolfi, M.C., Kneitz, S., Herpin, A., Nóbrega, RH. Schartl, M.*	**Gong, Ningping** DIVERGENT RECEPTORS FOR GROWTH HORMONE AND PROLACTIN DISCOVERED IN AGNATHANS: GENE SEQUENCES AND TISSUEEXPRESSION PATTERNS AT DIFFERENT LIFE STAGES OF SEA LAMPREY *Gong N (1), Sheridan MA (1), Ferreira-Martins D (2), McCormick SD (3)*	**Shi, Yun-Bo** EPIGENETIC MODIFICATIONS IN THE DEVELOPMENT OF INTESTINAL STEM CELLS *Shi YB*
15:20-15:40	**da Silva Rodrigues, Maira** ROLE OF THYROID HORMONES IN ZEBRAFISH SPERMATOGENESIS *Rodrigues MS, Tovo-Neto A, Nobrega RH, Habibi HR*		**Buisine, Nicolas** DNA METHYLATION LANDSCAPE CHANGES DURING THYROID HORMONE AND GLUCOCORTICOID CROSSTALKS AT XENOPUS METAMORPHOSIS *Jonchere, Blugeon, Pouch, Sachs, Buisine*
15:40-16:00			**Helbing, Caren** LOOKING FOR A SILVER LINING: THE IMPACT OF NANOSILVER ON THYROID HORMONE SIGNALING IN FROG TADPOLE METAMORPHOSIS *Helbing CC*
Plenary 16:00-17:00	**Dr. Carlos Aramburo- Century Ballroom ABC**		
Tuesday, May 28, 2019			
ISAREN Plenary 09:00-10:00	**Dr. Bob Denver- Century Ballroom ABC**		
10:00-10:30	**Coffee Break**		
	Dogwood	**Century Ballroom**	**Azalea**
10:30-12:30 **Session** **Chairs**	**Growth and Growth Factors** **Maricela Luna**	**Aspects of Reproductive Endocrinology & Neuroendocrinology 1** **Natalia Garcia-Reyero and Vance Trudeau**	**Neuroendocrine Disruption of Animal Vocalizations and Socio-Sexual Behaviors** **Cheryl Rosenfeld and Frauke Hoffmann**

10:30-10:50	**Riesgo-Escovar, Juan** CHARACTERIZATION OF INSULIN PATHWAY MUTANTS IN DROSOPHILA *Álvarez-Rendón J, Riesgo-Escovar JR*	**Lutterschmidt, Deborah** MECHANISMS UNDERLYING TEMPERATURE-INDUCED REPRODUCTIVE BEHAVIOR: ARE OVERWINTERING ECTOTHERMS REALLY "DORMANT"? *Lutterschmidt DI, Lucas AR, Stratton K, Winters TJ*	**Rhodes, Justin** FEMINIZATION OF BEHAVIOR, PLASMA SEX HORMONE PROFILE, GONADAL HISTOLOGY AND BRAIN GENE EXPRESSION FROM ENDOCRINE DISRUPTION IN SEXUALLY LABILE ANEMONEFISH *Rhodes JS, Gonzalez JA, Lange DA, Bhuvanagiri SA, Kaur A, Parker CG, Rosenfeld CS, Martyniuk CJ, Denslow ND*
10:50-11:10	**Rotwein, Peter** INSULIN-LIKE GROWTH FACTOR 2 THROUGH THE AGES: LOCUS AND GENE CONSERVATION AND DIVERSIFICATION DURING VERTEBRATE SPECIATION *Rotwein P*	**Cheung, Eugene** A POSSIBLE ROLE FOR LEPTIN IN SEXUAL MATURATION AND REPRODUCTIVE FUNCTION OF THE MOZAMBIQUE TILAPIA OREOCHROMIS MOSSAMBICUS *Cheung E, Tada MD, Borski RJ*	**Rosenfeld, Cheryl** DEVELOPMENTAL EXPOSURE OF CALIFORNIA MICE (PEROMYSCUS CALIFORNICUS) TO BISPHENOL A OR GENISTEIN AND EFFECTS ON THE GUT MICROBIOME, AND METABOLOME AND SOCIO-COMMUNICATIVE BEHAVIORS *Marshall BL, Liu Y, Farrington MJ, Mao J, Helferich WG, Schenk AK, Hoffmann F, Bivens NJ, Sarma SJ, Lei Z, Sumner LW, Joshi T, Rosenfeld CS*
11:10-11:30	**Vélez, Emilio** SOMATOTROPE REGULATION: A NOVEL FUNCTION OF NUCLEOBINDIN ENCODED PEPTIDES? *Vélez EJ, Unniappan S*	**Khalid, Enezi** LIGAND-BIAS IN GOLDFISH PITUITARY GNRH RECEPTOR ACTIVATION: INVOLVEMENT OF BETA-ARRESTINS *Khalid E, Chang JP*	**Zhang, Wo Su** NAPHTHENIC ACIDS DISRUPT COURTSHIP BEHAVIOURS IN THE WESTERN CLAWED FROG (SILURANA (XENOPUS) TROPICALIS) *Zhang WS, Farmer EJ, Trudeau VL*
11:30-11:50	**Björnsson, Thrandur** HOW OCEAN-WARMING COULD AFFECT GROWTH OF COLD-WATER MARINE TELEOSTS: GH-INDUCED STIMULATION OF ATLANTIC WOLFFISH GOWTH AT TEMPERATURES APPROACHING THE UPPER THERMAL TOLERANCE LIMITS *Björnsson BT, Gunnarsson A, Steinarsson A, Danielsdottir AK, Arnason T*	**Zmora, Nilli** CHALLENGING THE PARADIGM OF GNRH CONTROL OF REPRODUCTION: THE CASE OF GNRH3 IN ZEBRAFISH *Zmora N, Tanaka S, Marvel MM , Zohar Y*	**Hoffmann, Frauke** (NEURO)ENDOCRINE DISRUPTION OF AMPHIBIAN REPRODUCTIVE PHYSIOLOGY AND BEHAVIORS *Hoffmann F, Kloas W*
11:50-12:10	**Epardo, David** NEUROREGENERATIVE EFFECT OF GROWTH HORMONE (GH) IN THE CHICKEN NEURAL RETINA *Epardo D, Balderas-Márquez JE, Fleming T, Carranza M, Luna M, Harvey S, Arámburo C, Martínez-Moreno CG*	**Tanaka, Sakura** EXAMINING VASOACTIVE INTESTINAL PEPTIDE AS A POTENTIAL REPRODUCTIVE COMPENSATOR FOR HYPOPHYSIOTROPIC GONADOTROPIN-RELEASING HORMONE LOSS-OF-FUNCTION IN ZEBRAFISH *Tanaka S, Zmora N, Marvel M, Zohar Y*	
12:10-12:30		**Trudeau, Vance** SECRETONEURIN IS A PEPTIDE HORMONE THAT RESCUES IMPAIRED SPAWNING IN ZEBRAFISH LACKING THE PRECURSOR PROTEIN SECRETOGRANIN-II *Trudeau VL, Mitchell K, Lu C, Hu W*	
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14:00-16:00 Session Chairs	Advances in Endocrine Disruption Science Valerie Langlois and Jan Mennigan	Aspects of Reproductive Endocrinology & Neuroendocrinology 2 Vance Trudeau and Natalia Garcia-Reyero	GnRH-related Peptides in Metazoa: Recent Progress and Discoveries Jean-Paul Paluzzi and Pei-San Tsai
14:00-14:20	**Wiseman, Steve** INTRA-GENERATIONAL EFFECTS OF EARLY LIFE-STAGE EXPOSURE TO TEBUCONAZOLE ON REPRODUCTIVE CAPACITY OF ZEBRAFISH (DANIO RERIO) *Miller C, Ilnytskyy Y, Kovalchuk I, Wiseman S*	**Parker, Coltan** ACTIVE FEMINIZATION OF THE PREOPTIC AREA OCCURS INDEPENDENTLY OF THE GONADS IN AMPHIPRION OCELLARIS Dodd L, Nowak E, Lange D, Parker CG, DeAngelis RS, Rhodes JS	**Zandawala, Meet** CORAZONIN NEUROENDOCRINE PATHWAY ORCHESTRATES STRESS-ASSOCIATED PHYSIOLOGY IN DROSOPHILA *Zandawala M, Nguyen T, Johard H, Amcoff M, Paluzzi JP, Nässel D*
14:20-14:40	**Martínez López, Rubén Francisco** DEVELOPMENTAL EXPOSURE TO FLUOXETINE REDUCES OFFSPRING BASAL CORTISOL CONCENTRATION VIA LIFE STAGE-DEPENDENT MATERNAL TRANSMISSION IN ZEBRAFISH *Martínez R, Vera-Chang MN, Haddad M, Zon J, Navarro-Martin L, Trudeau VL, Mennigen JA*	**Ai, Nana** EVIDENCE FOR ROLES OF ANGIOGENESIS IN FOLLICULOGENESIS OF ZEBRAFISH *Ai N, Zhu B, Ge W*	**Jones, Christopher** FROM ECHINODERMS TO HUMANS – EXPLORING THE EVOLUTION OF METAL-BINDING TO GNRH PEPTIDES. *Tran KK, Jayawardena BM, Peacey L, Elphick MR, Jones CE*
14:40-15:00	**Langlois, Valerie** MOVING THE LAST DECADES OF ENDOCRINE DISRUPTION WORK INTO A PROVINCIAL ROUTINE SCREENING PROGRAM FOR COMPLEX EFFLUENTS *Langlois VS, Robitaille J*	**Jia, Yudong** INVOLVEMENT AND EXPRESSION OF GH/IGF SYSTEMS GENE IN THE OVARIAN DEVELOPMENT OF TURBOT *JIA YD, Meng Z, Huang B, Lei JL*	**Tsai, Pei-San** ADIPOKINETIC HORMONE IN A GASTROPOD: INSIGHT FROM LOCALIZATION AND FUNCTIONAL STUDIES *Tsai PS, Martillotti AW, Kavanaugh SI*
15:00-15:20	**Bhandari, Ramji** EPIGENETIC REPROGRAMMING AND TRANSGENERATIONAL INHERITANCE OF EPIMUTATIONS IN MEDAKA *Bhandari RK, Wang X, Bhandari P, vom Saal FS, Tillitt DE*	**Williams, Marcus** GPER IN FEMALE ZEBRAFISH REPRODUCTION *Williams M, Wu X, Zhu Y*	**Paluzzi, Jean-Paul** INSIGHT INTO GNRH-RELATED NEUROPEPTIDE RECEPTOR SPECIFICITY REVEALED THROUGH ANALYSIS OF NATURALLY OCCURRING AND SYNTHETIC ANALOGS OF THIS NEUROPEPTIDE FAMILY
15:20-15:40	**Tubbs, Christopher** GUT MICROBIOTA AND PHYTOESTROGEN-ASSOCIATED INFERTILITY IN SOUTHERN WHITE RHINOCEROS *Williams CL, Ybarra AR, Meredith AN, Durrant BS, Tubbs CW*	**Robitaille, Julie** STEROID-5ALPHA-REDUCTASE TYPE 2 KNOCK-OUT IN SILURANA TROPICALIS *Robitaille Julie, Langlois Valerie S.*	
15:40-16:00	**Heyland, Andreas** TRANSGENERATIONAL REPRODUCTIVE EFFECTS OF TWO SEROTONIN REUPTAKE INHIBITORS AFTER ACUTE EXPOSURE IN DAPHNIA MAGNA EMBRYOS *Heyland A, Bastien T, Halliwushka K*		
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#1 Fu, Liezhen
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#2 Koide, Emily
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Koide EM, Helbing CC

#3 Holloway, Nick
LOCALIZATION OF THE SODIUM-IODIDE SYMPORTER (NIS) IN THE BRAINS OF TELEOST FISH, AND ITS PROPOSED FUNCTION IN MAINTENANCE AND DEVELOPMENT

Nick Holloway, Duncan MacKenzie

#4 Dodsworth, Thomas
RECEPTOR IDENTIFICATION OF THE CRF-RELATED PEPTIDE, TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP); RECEPTOR KNOCKDOWN BY SIRNA AND CRISPR/CAS9 METHODS

Dodsworth, T.L., Barsyte-Lovejoy, D., Hogg, D.W., Jurado Soria, F., Lovejoy, D.A.

#5 Lazcano, Iván
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Lazcano I, Villalobos P, Orozco A

#6 Pech Pool, Santiago
EFFECT OF HYPOTHALAMIC HORMONES UPON THE EXPRESSION AND RELEASE OF GROWTH HORMONE IN B LYMPHOCYTES FROM THE CHICKEN BURSA OF FABRICIUS

Pech SM, Berumen LC, Martínez-Moreno CG, García G, Carranza ME, Arámburo C, Luna M

#7 Nakajima, Ami
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Ami Nakajima, Kiyoshi Yamauchi

#8 Read, Casey
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#13 Lajevardi, Aryan
FUNCTIONAL CHARACTERIZATION AND TISSUE-SPECIFIC EXPRESSION PROFILING OF TWO PYROKININ RECEPTORS IN ADULT AEDES AEGYPTI MOSQUITO

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#14 Li, Minghui
INSULIN LIKE GROWTH FACTOR 3, A TARGET OF ANDROGEN, IS REQUIRED FOR SPERMATOGENESIS IN TILAPIA

Li MH, Dai SF, Xiao HS, Liu XY, Zheng QY, Qi SS, Wang DS

#15 Carr, James
DO CATEGORICALLY DISTINCT STRESSORS AFFECT VISUAL ATTENTION TO FOOD IN HUMANS?

Li S, Keene JR, Harris BN, Carr JA

#16 Dai, Shengfai
AMHY DETERMINES MALE SEX VIA SIMULTANEOUS ACTIVATION OF GSDF AND REPRESSION OF FOXL2/CYP19A1A EXPRESSION IN TILAPIA

Dai SF, Liu XY, Xiao HS, Qi SS, Zhang XB, Li MH, Wang DS

#17 Honeycutt, Jamie
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Jamie L. Honeycutt, Courtney A. Deck, Jordan D. Taylor, Jonathan D. Douros, Russell J. Borski

#18 Brady, Fritzie
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#19 Moleón, María Soledad
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#20 Bottalico, Lisa
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#21 Moore, Brandon
ANDROGEN RECEPTOR IMMUNOREACTIVITY LOCALIZATION IN HATCHLING AMERICAN ALLIGATOR CLITERO-PENIS

Brandon C Moore, Katie Wayne, Matt Hale, and Ben Parrott

#22 Lambert, Faith
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#23 Felton, Rachel
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Felton R, Owen C, Cossaboon J, Steiner C, Tubbs C

#24 Lewis, Kelsey
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#25 Bhandari, Ramji
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#26 Mayasich, Sally
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Sally Mayasich, Jeff Denny, Joseph Korte, Patricia Kosian, Jennifer Olker, Philip Degoey, Joseph O'Flanagan, Sigmund Degitz and Michael Hornung

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#30 Shahadur, Shohag
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#31 Miyagawa, Shinichi
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#32 Kurita Oyamada, Hajime
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#33 Balderas, Jerusa
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#37 CARDENAS, RODOLFO
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#38 Bruno, Renato
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#39 Vélez, Emilio
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#43 Reid, Ross
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#44 Hall, Breanna
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#45 Gomez Pacheco, Liliana
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#46 Herrboldt, Madison
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#47 Colli-Dula, Reyna C
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#48 Jia, Yudong
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#52 Souders, Chris
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#54 Garcia-Reyero, Natalia
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Garcia-Reyero N, Burgoon L, Woodley C, Mylroie J, Wilbanks M, Urbanczyk A, Lemasson B, Smith D, Nouri Z, Kroll K, Denslow N, Perkins E

#55 Wei, Chi
A COMPREHENSIVE, GENOME-WIDE ANALYSIS OF BRAIN AND GONAD TRANSCRIPTS REVEALS EXPRESSION CHANGES OF KEY SEX REVERSAL-RELATED GENES IN THREE STAGES OF MONOPTERUS ALBUS.

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#56 Veloz Contreras, Arlet
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AEROBIC METABOLISM IN NEURONS AND ASTROCYTES UNDERGOING CELLULAR STRESS: MODULATION BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE-1 (TCAP-1)

David Wosnick, Ola Michalec, Aishwarya Chand, Gina Trubiani, Tiffany Ng, David W. Hogg, David A. Lovejoy

Abstracts

Saturday, May 25 - Thyroid Hormones and Development

AGE-DEPENDENT CHANGES IN GLUCOSE METABOLISM IN DEIODINASE TYPE 2 KNOCKOUT ZEBRAFISH.

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Thyroid hormones (TH) influence many aspects of energy balance and hypothyroidism is known to induce changes in carbohydrate, lipid and protein metabolism. As such, activity reducing polymorphisms in deiodinase type 2 (DIO2), an essential enzyme in the peripheral activation of TH, were found to be associated with an increased risk for type 2 diabetes mellitus and insulin resistance. We used male Dio2 knockout (Dio2KO) zebrafish to study the link between deficiency in this enzyme and insulin dependent glucose metabolism in more detail. Dio2KO zebrafish had severely reduced levels of 3,5,3'-triiodothyronine in all tissues tested and this hypothyroidism was accompanied by a reduced metabolic rate as tested at intervals from 2-15 months (M) of age. Measurements at 6 M showed strongly increased blood glucose levels in mutant fish compared to wild type. This was accompanied by increased pancreatic mRNA expression of both insulin (*ins*) and glucagon (*gcga/b*). Results from measurements at 9 M showed the same pattern: increased blood glucose levels and increased expression of *ins* and *gcga/b*. Immuno-histochemical staining at that stage revealed no differences in pancreatic islet size or number of insulin-positive cells while the number of glucagon-positive cells was slightly increased. Analysis of Dio2KO and wild type zebrafish at 18 and 24 M surprisingly showed that blood glucose levels in the mutants were normalized while *insa* and *gcga/b* expression remained higher. To further investigate the underlying mechanisms we also measured the expression of insulin and glucagon receptors (*insra/b*, *gcgra/b*) and glucose transporters (*glut2=slc2a2, glut12=slc2a12*). *Insra/b* levels in muscle tissue were downregulated at all stages tested (6, 9, 18 and 24 M) while *gcgra/b* levels were only lowered at 18 and 24 M. *Glut2* and *glut12* expression were decreased in gastrointestinal tract at all stages except 6 M, while their expression in muscle tissue was only decreased at 9 M. In summary, young Dio2KO zebrafish show signs of insulin resistance but unlike the typical progress of type 2 diabetes in humans, there is a spontaneous normalization of blood glucose levels in older fish. Further investigation of this animal model should provide valuable insights into the mechanisms governing this age-dependent recovery from hyperglycemia.

KNOCK-DOWN OF SPECIFIC THYROID HORMONE RECEPTOR ISOFORMS IMPAIRS BODY PLAN DEVELOPMENT IN ZEBRAFISH

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The role of thyroid hormones (THs) in development has been extensively studied; however, the specific molecular mechanisms involved are far from being clear. THs act by binding to TH nuclear receptors (TR) that act as ligand-dependent transcription factors to regulate TH-dependent gene expression. Like vertebrates, zebrafish express different isoforms of functional Tr alpha and beta, some of which can bind alternative ligands like 3,5-T2. In this study, we first analyzed the effects of exogenous T3 and 3,5-T2 exposure during embryogenesis. The percentage of affected embryos was similar to those vehicle-injected, suggesting that the early exposure to low THs is not sufficient to elicit effects upon the phenotype of the embryo. We then generated crispants for four isoforms of *thr* to learn more about the role of these receptors in early development. We found that crispant larvae from *thraa* and a newly identified *l-thrb+*, but not *thrab* and canonical *thrb1* showed profound deleterious effects upon symmetry and laterality, suggesting early novel roles for these Tr isoforms in the body plan developmental program. Since critical events that determine cell fate start in the late gastrula, we tested if some genes that are expressed during early developmental stages could indeed be TH targets. We identify early development genes, like *sox10* and *eve*, that were specifically over-expressed in *thraa* and *l-thrb+* crispants, suggesting that these specific *thr* isoforms function as transcription repressors for these genes, while transcription of *zic* and *ets* appear to be *thraa* and *l-thrb+*-mediated, respectively. Overall, present results show that TH signaling participates in early zebrafish development and identify Tr isoform-mediated specific regulation of early gene expression.

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THYROID HORMONE RECEPTOR DEFFICIENCY ACCELERATES CONNECTIVE TISSUE DEVELOPMENT BUT PREVENTS EPITHELIAL TRANSFORMATION DURING METAMORPHOSIS IN XENOPUS TROPICALIS.

Shibata Y (1), Okada M (1), Wen L (1), Shi YB (1)

(1) Eunice Kennedy Shriver National Institute Child Health and Human Development (NICHD), National Institute of Health, Bethesda (NIH), Maryland, USA

Thyroid hormone (TH) is essential for the development throughout vertebrates including humans and amphibians. During metamorphosis of the tadpole intestine in *Xenopus tropicalis*, *de novo* formation of adult stem cells via dedifferentiation of some larval cells occurs in a process that is controlled by TH via gene regulation by TH receptor (TR). This remodeling process also involves larval epithelial cell death under the induction of TH during early metamorphosis. Like other vertebrates, there are two types of TRs, *TRα* and *TRβ* in Xenopus tropicalis. To determine the role of endogenous TRs during development, we knocked out *TRβ* gene by using CRISPR/Cas9 genome editing technology in *TRα* knockout animals and generated homozygous TR knockout [$TR\alpha^{(-/-)}TR\beta^{(-/-)}$] animals.

Analyses of the wild type and knockout animals showed that TH target gene expression were upregulated in the $TR\alpha^{(-/-)}TR\beta^{(-/-)}$ premetamorphic tadpoles. However, such tadpoles were resistant to TH treatment with no change in target gene expression. Developmentally, homozygous TR double-knockout led to tadpole lethality at stage 61, the climax of metamorphosis, with little tail resorption and gill repression took place, suggesting that these processes are completely dependent on the TH-TR pathway. The tadpole intestine had abnormal morphology with a multiple folded structure, resembling that at the end of metamorphosis in wild-type animals, whereas no proliferating cells were detected in the $TR\alpha^{(-/-)}TR\beta^{(-/-)}$ tadpoles at the climax of metamorphosis. Furthermore, RNA-seq analysis revealed that the extracellular matrix organization related genes were up-regulated at the pre-metamorphic stage tadpoles, likely due to the depression of TH target genes in the absence of TR. However, genes related to stem cell, cell proliferation and the apoptosis, which are known to be important for intestinal remodeling, were repressed in the $TR\alpha^{(-/-)}TR\beta^{(-/-)}$ tadpoles.

Our data suggest that TR is not required for the initiation of metamorphosis but is essential for the completion of metamorphosis. Furthermore, the differential effects of TR knockout on different organs/tissues indicate tissue specific rules for TR to control temporal progression of metamorphosis in various organs.

INVOLVEMENT OF THYROID HORMONE RECEPTORS IN THE HEMOGLOBIN SWITCH DURING FROG METAMORPHOSIS

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Globin switching from the larval- to adult-type occurs under the control of thyroid hormone (TH) signaling during amphibian metamorphosis. However, it still remains unclear how TH regulates the globin switching. To address this issue, we examined the involvement of TH receptors (TRs) in the globin switching in the model frog *Xenopus tropicalis*. We first identified a set of globin genes from RNA-seq data of larval and adult liver, the main hematopoietic organs in amphibians. We also found that major adult-type globin genes were induced in premetamorphic tadpoles by administration of T3 and GC-1, agonists for TRs. In addition, TH-treated frog embryos do not express adult-type globin genes presumably due to lack of tissue competence to respond to TH, but we observed adult-type globin gene expression in response to TH in embryos overexpressing TR and RXR. In the absence of TH, tissues that express TRs actively repress TH response genes, such that lack of TRs may result in reduced repression, and thus progression, of TH-dependent processes. Accordingly, TRalpha knock-out tadpoles exhibited precocious expression of the adult-type globin genes. These results indicate that the action of TRalpha regulates the timing of TH-dependent globin switching during metamorphosis.

NON-GENOMIC THYROID HORMONE SIGNALING IN INVERTEBRATES: T4 REGULATES SKELETOGENESIS IN THE PURPLE SEA URCHIN VIA AN INTEGRIN-MEDIATED MAPK CASCADE

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Thyroid hormones (THs) are crucial for the physiology, development, and metamorphosis of chordate deuterostomes. Recent evidence suggests that THs are also responsible for regulating metamorphosis in some invertebrates, including sea urchins, mollusks, and annelids. We have found that THs regulate skeletogenesis and development in the sea urchin *Strongylocentrotus purpuratus* by means of a potentially conserved signaling pathway through a membrane-bound integrin receptor. THs, principally 3,5,3',5'-Tetraiodo-L-thyronine (T4), bind to the membrane or extracellular matrix of primary mesenchyme cells, triggering a mitogen-activated protein kinase (MAPK) cascade, phosphorylating key transcription factors in the gene regulatory network controlling skeletogenesis (ETS1, ALX1). Pre-incubating sea urchin gastrulae with RGD peptide, a competitive inhibitor of TH binding to integrins, prevented the effect of T4 on phosphorylation and skeletogenesis. The effect of THs on skeletogenesis was also inhibited by the ERK 1/2 MAPK inhibitor, PD98059. Also, imaging gastrula with fluorescently labeled THs revealed a binding location on the membranes of primary mesenchyme cells – the cells responsible for producing the larval skeleton. Binding kinetics performed on membrane extract from sea urchin gastrulae confirmed that T4 binds to the cell membrane. These results suggest THs may regulate sea urchin development via an integrin-mediated MAPK cascade. Future investigation into the role of non-genomic TH signaling in invertebrates may elucidate the function of THs in organisms lacking a nuclear TH receptor ortholog, such as cnidarians or sponges. We propose that non-genomic signaling may have evolved first, later followed by the canonical TH pathway in bilaterians.

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Saturday, May 25 - Neuropeptide Signaling Pathways in Arthropods

SIGNALING PATHWAYS CONTROLLING PHASE TRANSITIONS IN THE CRUSTACEAN MOLTING GLAND

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Ecdysteroids, which are synthesized by a pair of molting glands or Y-organs (YOs), initiate and coordinate the diverse physiological processes necessary for successful molting and growth. Recent advances using transcriptomic and proteomic tools have revealed a complex interaction of signaling pathway genes that mediate YO phase transitions over the molt cycle. Molt-inhibiting hormone (MIH)/cyclic nucleotide-dependent signaling maintains the YO in the basal state during intermolt. As proposed, MIH signaling is composed of a rapid cAMP/Ca2+ triggering phase followed by a prolonged NO/cGMP summation phase. Reduction in MIH release from the eyestalk neurosecretory center activates the YO and the animal enters premolt. Activation requires mTOR-dependent protein synthesis, as rapamycin inhibits YO ecdysteroid synthesis in vivo and in vitro. A major decision point occurs in mid premolt, when the animal becomes committed to molt. The committed YO becomes insensitive to MIH and reaches maximum ecdysteroid synthetic capacity in late premolt. The YO transition from the activated state to the committed state is mediated by TGFbeta/activin-dependent signaling. Finally, at the end of premolt and in postmolt, the YO is in the repressed state, in which the levels of contigs encoding signaling and hormone synthesis genes are at their lowest and ecdysteroid synthesis is suppressed. It is hypothesized that this prevents molting until the exosketeton is fully formed and calcified. Transcriptomic analysis suggests that a dozen or more signaling pathways (e.g., Wnt, Hedgehog, MAP kinase, and Notch) are involved in integrating a variety of internal and environmental cues. mTOR activity controls the levels of thousands of contigs, including a positive feedback of mTOR signaling genes, up-regulation of ecdysteroidogenic enzymes, and down-regulation of MIH signaling genes. Proteomic analysis identified numerous oxidative stress, cytoskeletal, and energy metabolism proteins associated with YO hypertrophy and increased ecdysteroidogenic capacity during premolt. As tools for discovery, transcriptomic and proteomic approaches have given us new insights into molt regulation and have revealed that the YO is potentially capable of responding to a variety of trophic and static factors.

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DISRUPTION OF NEUROPEPTIDERGIC SYSTEM IN ARTHROPOD PEST CONTROL

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Neuropeptides are controllers of vital biological processes in development, physiology, and behavior of multicellular organisms. The neuropeptidergic signaling systems hasvebeen considered potential targets for the development of novel pesticide because of their essential functions in various life stages. Practical application of the concept in arthropod pest control has been advanced by understanding the basic biology including deorphanization of G protein-coupled receptors (GPCR), the major class of neuropeptide receptors. To enhance the capacity in biorational approaches to implement this strategy, we have characterized pharmacological properties of a number of GPCRs. We have been working on one of potential pesticidal target ecdysis triggering hormone (ETH) signaling system that is a neuropeptide highly conserved for the essential functions in insect ecdysis. Another target neuropeptidergic system that we have been working on is proctolin and its receptor that specifically lack in honeybee, providing an opportunity to develop bee-safe acaricide. Development of efficient toxic compounds acting on neuropeptide receptors with optimal levels of target spectra against arthropod pests may be possible in the near future.

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MODULATION OF DROSOPHILA POST-FEEDING PHYSIOLOGY AND BEHAVIOR BY THE NEUROPEPTIDE LEUCOKININ

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Behavior and physiology are orchestrated by neuropeptides acting as central neuromodulators and/or circulating hormones. An outstanding question is how these neuropeptides function to coordinate complex and competing behaviors. In *Drosophila*, the neuropeptide leucokinin (LK) modulates diverse functions, but mechanisms underlying these complex interactions remain poorly understood. As a first step towards understanding these mechanisms, we delineated the LK circuitry that governs various aspects of post-feeding physiology and behavior. We found that impaired LK signaling in *Lk* and *Lk receptor* (*Lkr)* mutants affects diverse but coordinated processes, including regulation of stress, water homeostasis, feeding, locomotor activity, and metabolic rate. Next, we sought to define the populations of LK neurons that contribute to the different aspects of this physiology. We find that the calcium activity in abdominal ganglia LK neurons (ABLKs), but not in the two sets of brain neurons, increases specifically following water consumption, suggesting that ABLKs regulate water homeostasis and its associated physiology. To identify targets of LK peptide, we mapped the distribution of *Lkr* expression, mined a brain single-cell transcriptome dataset for genes coexpressed with *Lkr,* and identified synaptic partners of LK neurons. *Lkr* expression in the brain insulin-producing cells (IPCs), gut, renal tubules and chemosensory cells, correlates well with regulatory roles detected in the *Lk* and *Lkr* mutants. Furthermore, these mutants and flies with targeted knockdown of *Lkr* in IPCs displayed altered expression of insulin-like peptides (DILPs) and transcripts in IPCs and increased starvation resistance. Thus, some effects of LK signaling appear to occur via DILP action. Collectively, our data suggest that the three sets of LK neurons have different targets, but modulate the establishment of post-prandial homeostasis by regulating distinct physiological processes and behaviors such as diuresis, metabolism, organismal activity and insulin signaling. These findings provide a platform for investigating feeding-related neuroendocrine regulation of vital behavior and physiology.

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EXPRESSION PROFILING, DOWNSTREAM SIGNALING AND INTER-SUBUNIT FUNCTIONAL CHARACTERIZATION OF AN EVOLUTIONARY ANCIENT GLYCOPROTEIN HORMONE SYSTEM (GPA2/GPB5) IN THE MOSQUITO, AEDES AEGYPTI

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GPA2/GPB5 and its receptor (LGR1) represent an ancient neuroendocrine glycoprotein hormone-signaling system identified in the genomes of both vertebrates and invertebrates, however its function remains elusive. To determine the function of GPA2/GPB5 in the mosquito *Aedes aegypti*, we aimed to characterize the expression profile of GPA2/GPB5 and LGR1 in the adult stage, elucidate downstream signaling pathways and utilize reverse genetics (i.e. RNA interference). Immunohistochemical, RT-qPCR and *in situ* hybridization data have revealed GPA2 and GPB5 subunit expression localizes to bilateral pairs of neuroendocrine cells situated within the first five abdominal ganglia of adult mosquitoes. To study dimerization patterns of the *A. aegypti* GPA2 and GPB5 hormone subunits, immunoblotting experiments were performed using protein collected from HEK293T cells transiently expressing *A. aegypti* GPA2/GPB5. Moreover, the functionality of individual subunits and GPA2/GPB5 in LGR1 activation using bioluminescent reporter assays was examined. To our surprise, unlike human GPA2/GPB5 that demonstrated strong heterodimerization between the subunits, our results confirm *A. aegypti* GPA2 and GPB5 subunits do not heterodimerize, yet both subunits are required for LGR1 activation. LGR1 transcript expression and regionalized immunoreactivity in reproductive tissues suggest GPA2/GPB5 signaling could be involved in aiding gamete development in adult mosquitoes. Subcellular immunolocalization analyses of LGR1 throughout spermatogenesis in adult testes determined LGR1 localized to the plasma membrane of spermatids and is associated with the centriole adjunct, which is a region responsible for coordinating the proper development of flagella. LGR1 knockdown using RNAi resulted in significant spermatozoa defects, such as shortened flagella, in adult males. Moreover, LGR1 knockdown mosquitoes possessed an average ~60% less sperm and were less fertile than controls. Taken together, this data supports the notion that GPA2/GPB5 and LGR1 signaling regulates male reproductive biology, a significantly understudied research area in mosquitoes and other insects.

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THE INVOLVEMENT OF INSULIN-LIKE PEPTIDE SIGNALING IN THE REPRODUCTIVE SUCCESS OF RHODNIUS PROLIXUS, A VECTOR OF CHAGAS´ DISEASE

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Insulin-like peptides (ILPs), such as insulin, insulin-like growth factor, and relaxin are peptide hormones that mediate metabolism, growth, lifespan and reproduction in vertebrates and invertebrates. In this context, it was demonstrated that in some insects, ILP signaling has vital roles as nutritional sensors promoting yolk deposition (vitellogenesis) of lipids and proteins by oocytes that ultimately leads to the laying of mature eggs. The relationship between ILPs and reproductive success in triatomines, however, has never been studied. The triatomine *Rhodnius prolixus* is a blood-feeding insect and a primary vector of the etiological agent of Chagas´ disease. Over the past century, *R. prolixus* has been the subject of intense investigations, which have contributed to our understanding of important aspects of metabolism and physiology in insects. The aim of the current work was to study events involved in egg formation, focusing on ILPs. Using *R. prolixus* as a model, we have performed experiments on non-vitellogenic (unfed condition) and vitellogenic (fed condition) females. Using qPCR, RNA-seq and transcriptome profiling of the central nervous system (CNS), fat bodies and ovaries of vitellogenic females we have preliminary evidence that the genes involved with ILPs are upregulated compared with those from non-vitellogenic tissues. These results were confirmed using western blot assays. Using immunohistochemistry, we identified cells in the CNS and showed that the insulin receptor (InR) in oocytes is differentially expressed according to the stage of development. Using RNA interference for ILP and InR, we tested the involvement of ILPs in synthesis of yolk precursor proteins, oocyte development and consequently, in quality of eggs laid. This is the first description of the network of regulatory pathways of the different tissues implicated in the vitellogenic process. As reproduction is an event responsible for propagation of insect populations, the functional characterization of factors that drive successful reproductive biology of triatomines is important in answering fundamental biological questions, but also as leads to the development of innovative biocontrol methods.

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THE ROLE OF SIFAMIDE AS A NEUROHORMONE IN THE BLOOD-GORGING INSECT, RHODNIUS PROLIXUS.

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SIFamides are a family of neuropeptides that are highly conserved among arthropods. In insects, this peptide is mainly expressed in four medial neurons in the pars intercerebralis of the brain, with projections throughout the central nervous system (CNS). Although SIFamide has been shown to influence sexual behavior, feeding and sleep regulation in holometabolous insects such as *Drosophila melanogaster*, little is known about its role in hemimetabolous insects. A possible neurohormonal function for SIFamide in insects remains unclear. *Rhodnius prolixus* is a blood-gorging insect and a vector for human Chagas disease. Consumption of a blood meal initiates important developmental events for this hemimetabolous hemipteran. In this study, we use molecular and physiological techniques to explore the role of SIFamide in *R. prolixus* (Rhopr-SIFa), specifically in relation to feeding. Immunohistochemistry shows characteristic expression of SIFamide in four cells of the pars intercerebralis in *R. prolixus*. A novel discovery is that processes also project into the corpus cardiacum and along the dorsal vessel, indicating for the first time in insects, that SIFamide may be a neurohormone. In addition, application of Rhopr-SIFa to the dorsal vessel shows a dose-dependent increase in heart beats/minute. We also observed enhanced feeding behavior (size of meal) in insects injected with Rhopr-SIFa, in comparison to those injected with saline. Immunohistochemistry of the CNS showed diminished SIFamide-like staining in the neurons in the brain two hours following feeding, and restocking of those cells twenty-four hours later, indicating Rhopr-SIFa stores in this regions are involved in feeding. The results of temporal qPCR analysis were consistent with the immunohistochemical findings, showing an increase in Rhopr-SIFa transcript expression in the brain two hours after feeding, suggesting the restocking of Rhopr-SIFa in the medial neurons following release of the peptide during feeding. This data suggests that the four SIFamidergic neurons and associated arborizations may play an important function in the neuronal circuitry controlling *R. prolixus* feeding behavior, as a central and peripheral neuromodulator/neurohormone.

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LOSS OF THE GLUCOCORTCOID RECEPTOR IN ZEBRAFISH IMPROVES MUSCLE GLUCOSE AVAILABILITY AND INCREASES GROWTH

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Chronic stress and the associated elevation in corticosteroid levels increases muscle protein catabolism. We hypothesized that GR-regulated restriction of muscle glucose availability may play a role in the increased protein catabolism during chronic stress. To test this, we generated a ubiquitous GR knockout (GRKO) zebrafish to determine the physiological consequences of glucocorticoid stimulation on muscle metabolism and growth. Cortisol treated larvae were half the size of the wildtype (WT) at 15 days post-fertilization, and this growth reduction was abolished in GRKO larvae. The GRKO larvae had significantly more protein compared to WT, and the larval metabolome analysis supported an enrichment of glucose-derived metabolites for protein synthesis. As adults, the GRKO were larger than the wildtype supporting a faster growth trajectory. The higher body mass in the GRKO fish corresponded with an increased protein and lipid, but not carbohydrate content. Also, the stressor-induced increase in plasma glucose level observed in the wildtype was completely abolished in the GRKO fish. The muscle, but not liver, capacity for glucose uptake was enhanced in the GRKO fish, and this corresponded with a higher hexokinase activity in the mutants. The higher protein content of the GR fish corresponded with a higher capacity for protein synthesis, including increased phosphorylation of eIF4B, higher expression of heat shock protein cognate 70. Altogether, loss of GR increases growth in fish, and our results indicate that GR signalling limits muscle glucose uptake. This may a play a role in protein catabolism, leading to the growth suppression during chronic stress in fish.

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GLUCOCORTICOIDS AND GLYCEMIA DURING STRESS IN BIRDS

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Stress in mammals generally stimulates the secretion of glucocorticoids and these hormones increase glycemia, thereby making more glucose (GLU) available to metabolically active tissues. Birds maintain blood glucose at levels 2-3 times those in size-matched mammals, respond poorly to insulin, and do not express insulin-dependent GLUT4 cell membrane transporters, suggesting that they use different plasma GLU-regulating mechanisms. We used free-ranging House Sparrows, *Passer domesticus*, to test whether capture and restraint stress is associated with elevated glycemia and if so, whether this elevation is related to plasma corticosterone (CORT). We sampled adult males and females during a three week-long period at three Australian locations (coastal: 38^0 N; temperate: 34^0 N; interior desert: 32^0 N), and measured plasma CORT and GLU within three minutes of capture (baseline) and again 30 minutes later (stress-induced). Neither plasma CORT nor plasma GLU was sex-dependent. Baseline CORT but not GLU differed between locations, but during stress both increased in a location-unrelated manner. Neither baseline CORT nor baseline GLU changed during the day. However, the amplitude of the stress-induced increase in plasma CORT, but not GLU, decreased between morning and afternoon. Thus, acute stress in House Sparrows increased plasma CORT and GLU concentrations. However, comparisons of concentrations either between locations or as a function of sampling time of day, revealed no support to the hypothesis that baseline CORT is related to glycemia or that CORT mediates stress-related increases in glycemia. Whether and the extent to which CORT in birds normally controls plasma GLU remains an open question and requires further investigation.

CHARACTERIZATION OF WHALE SHARK MC2R REVEALING A POTENTIAL PATTERN IN ACTIVATION SCHEME AMONG THE MC2R OF CARTILAGINOUS FISH

Hoglin, Brianne

The melanocortin-2 receptor (MC2R) is distinct among the family of five melanocortin receptors due to its ligand selectivity for ACTH alone. This trait for activation is consistent among all bony vertebrate MC2Rs analyzed, but a difference is seen from the MC2R of the elephant shark and red stingray which can also be functionally activated by MSH-sized ligands. Previous studies utilized alanine-substituted analogs involving the H6F7R8W9 and K15K16R17R18P19 motifs of hACTH(1-24) to analyze the activation characteristics of elephant shark (Callorhincus milli) and red stingray (Dasyatis akajei) compared to a representative bony fish species, the spotted gar (Lepisosteus oculatus). The present study sought to analyze the activation characteristics of the whale shark (Rhincodon typus) MC2R utilizing the same series of alanine-substituted analogs of ACTH. Alanine substitutions to either motif blocked activation compared to transfected cells stimulated with hACTH(1-24). This indicates a likely two-step binding of ACTH to wsMC2R for functional activation. The results of this experiment will be discussed relative to the activation characteristics of the other cartilaginous fish studied in order to develop a full picture of the evolution of activation scheme among cartilaginous fish MC2R.

EVALUATING THE ROLE OF THE MELANOCORTIN-5 RECEPTOR IN THE HPA/HPI AXIS: A PHYLOGENETIC STUDY

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For several vertebrates (i.e., elephant shark, red stingray, rainbow trout, chicken) glucocorticoid cells not only express the canonical ACTH receptor, melanocortin-2 receptor (MC2R), but also express the melanocortin-5 receptor (MC5R). In these species the sensitivity of the MC2R ortholog to stimulation by ACTH is dramatically enhanced by co-expressed with the accessory protein, MRAP1. Since the *mc2r* gene the *mc5r* genes are the result of a local gene duplication, this study tested the hypothesis that the sensitivity of the MC5R orthologs in these species would be enhanced by co-expression with their respective MRAP1 ortholog.. Previous studies had shown that bony vertebrate MC2R orthologs require both the H(6)F(7) R(8)W(9) motif and K(15)K(16)R(17)R(18)P(19) motif in ACTH(1-24) for activation. In this study, MC5R orthologs were co-expressed with their respective MRAP1 in CHO cells, and stimulated with either ACTH(1-24) (positive control), a H/A F/A R/A W/A (A4) analog of ACTH(1-24), or a K/A K/A R/A R/A P/A (A5) analog of ACTH(1-24), and a cAMP reporter gene assay was used to measure activation of the MC5R ortholog. For all of the bony vertebrate MC5R orthologs, the A4 ortholog completely blocked activation, whereas, the A5 ortholog stimulated the receptor with comparable potency to ACTH(1-24). However, activation of the red stingray MC5R ortholog was completely blocked by both the A4 and A5 analogs of ACTH(1-24); whereas activation of the elephant shark MC5R ortholog was only blocked by the A4 analog. These results suggest that following the local gene duplication event that gave rise to MC2R and MC5R, a uniform ligand sensitivity pattern has evolved in the bony vertebrate lineage for MC5R, however among the cartilaginous fishes novel ligand sensitivity mechanisms have evolved. Possible mechanisms to explain the parallel evolution of the MC5R ortholog in cartilaginous fishes and bony vertebrates will be discussed.

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CORTICOTROPIN-RELEASING FACTOR EXERTS NEUROPROTECTIVE EFFECTS AGAINST AMMONIA NEUROTOXICITY IN ISOLATED LARVAL ZEBRAFISH BRAINS

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The neuropeptide corticotropin-releasing factor (CRF) is best known for its key role in the regulation of the hypothalamic-pituitary-adrenal/-interrenal axis. However, components of the CRF system are widely expressed throughout the brain and periphery, suggesting the existence of a broader physiological role for CRF. Our recent observation that CRF-related peptides can suppress heat shock-induced apoptosis during zebrafish embryogenesis as well as hypoxia/ reoxygenation-induced apoptosis in adult zebrafish heart, suggest that CRF may have cytoprotective effects in various tissues. Given the known neurotoxic effects of ammonia in fish, this study explored whether CRF confers neuroprotection during exposure to high environmental ammonia. *In vitro*, relative to control conditions, while exposure of cultured larval brains to ammonia (750 μM NH_4Cl) for 16 h reduced the gene expression of *neurod1* (a marker of neuronal differentiation), the addition of CRF prevented the ammonia-induced decrease in *neurod1* mRNA levels. In the presence of antalarmin (a CRF receptor type 1 antagonist), ammonia exposure reduced *neurod1* expression but CRF no longer had an effect. Ammonia exposure also elicited an increase in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL; a marker of cell death) in cultured larval brains and this increase was abolished by the addition of CRF. In the presence of antalarmin, ammonia exposure increased the percentage of TUNEL-positive cells independent of whether or not CRF was present. Together, these results demonstrate that CRF has cytoprotective effects in isolated larval zebrafish brains via type 1 CRF receptors and that CRF may confer neuroprotection against ammonia toxicity. The fact that CRF can provide cytoprotection against various environmental stressors in multiple tissues and life stages suggests that the CRF system, in addition to its canonical function in the endocrine stress response, has an important role in promoting cellular survival.

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GLUCOCORTICOID MODULATION OF THE IMMUNE RESPONSE IN ZEBRAFISH

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Glucocorticoids are steroid hormones secreted upon stress. In humans and in fish, the main endogenous glucocorticoid hormone is cortisol. Because of their immune-suppressive effects glucocorticoids are widely used as anti-inflammatory drugs. The effects of glucocorticoids are mediated by the glucocorticoid receptor and previously we have characterized this receptor in zebrafish. The anti-inflammatory action of glucocorticoids can be studied in zebrafish in the tail fin amputation model. Using this model we have demonstrated that glucocorticoids inhibit almost the entire transcriptional response to amputation. The migration of neutrophils towards the wounded site was also attenuated by glucocorticoids, but the migration of macrophages appeared to be unaffected, which could be explained by analysis of the expression of chemoattractants. Transcriptome analysis of FACS-sorted macrophages showed that amputation-induced transcriptional changes that are pro-inflammatory are generally blocked by glucocorticoid treatment, whereas pro-resolving changes are unaffected. Furthermore, we have investigated how glucocorticoid treatment affects the immune response to an infection with Mycobacterium marinum in zebrafish, which is a model to study tuberculosis. Glucocorticoid treatment increases the bacterial burden, but surprisingly, increase the expression of pro-inflammatory genes like *il1b*. We conclude that the modulation of the expression of immune genes by the glucocorticoid receptor is dependent on the signal that activates these genes during the immune response.

PROTEOGENOMICS OF FATHEAD MINNOW (PIMEPHALES PROMELAS) AS A FIRST STEP TO IDENTIFY TRANSCRIPT VARIANTS OF IMPORTANCE TO NEUROENDOCRINOLOGY

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The fathead minnow is the model of choice for ecotoxicology in North America as there are many studies relating toxicant exposures to changes in endocrine related endpoints in these fish. We developed a robust transcriptome with over 30,000 reads for several organs including brain, gut, liver, gonad, heart, gill, head kidney, and trunk kidney for the fathead minnow using long reads generated by the PacBio instrument. We had 17,382 transcripts that were ≥1,000 nts and 182 that were ≥5,000 nts. The transcriptome was assembled, and it was used as a scaffold for interpreting RNA-seq and proteomics data to determine tissue-specific transcripts for the hypothalamus, telencephalon, liver and gut. Overall, 28,616 transcripts met the requirements for statistical testing. Of those, 12,610 transcripts were not changed in any of the tissues. These are likely important housekeeping genes that are essential for all tissues. The number of significantly different transcripts varied by tissue. In general, the telencephalon and hypothalamus shared the most expressed genes while the gut and liver were the most distinct. Expression differences were confirmed by qPCR for a select set of genes including ERs and the brain aromatase isoform. Of note, there was overlap in enriched cell processes between transcriptomic and proteomic datasets from each respective tissue. We are currently examining the datasets for alternatively spliced variants that may be of interest to neuroendocrinology.

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PHYSIOLOGICAL EFFECTS OF STRUCTURAL ANALOGS OF KININS AND CAPA IN *RHODNIUS PROLIXUS*

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The kinin and CAPA family of neuropeptides are responsible for a variety of physiological processes in insects. In *Rhodnius prolixus*, Rhopr-kinins have been shown to stimulate hindgut contractions, and RhoprCAPA-2 is an anti-diuretic hormone. The effects of these neuropeptides are mediated by G protein-coupled receptors, with intracellular calcium and cGMP possibly acting as secondary messengers. In *R. prolixus*, the CAPA transcript encodes for 3 peptides: RhoprCAPA-1, RhoprCAPA-2, RhoprCAPA-pk1. A CAPA receptor that binds RhoprCAPA-2 and, in a minor way, RhoprCAPA-pk1, and a pyrokinin receptor that binds RhoprCAPA-pk1, are expressed on the hindgut of *R. prolixus.* Despite the presence of these receptors, there is no stimulation of hindgut contraction upon application of RhoprCAPA-1, RhoprCAPA-2 or RhoprCAPA-pk1. Application of a mixture of Rhopr-kinin 2 and RhoprCAPA-2 on the hindgut, however, results in a stronger contraction than that produced by Rhopr-kinin 2 alone. Neuropeptide analogs for Rhopr-kinin 2 and RhoprCAPA-2 have been synthesized with changes in their amino acid sequences, yet still possess the ability to bind to their specified GPCRs in other insects. The Rhopr-kinin 2 analog elicits stronger changes in hindgut contractions than that of Rhopr-kinin 2, and is more potent since it produces contractions at lower doses. The RhoprCAPA-2 analog elicits hindgut contractions, an effect which is not exhibited with RhoprCAPA-2. Using RNA interference to silence the kinin receptor, the effects of Rhopr-kinin 2 and its analog on the hindgut were reduced.

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INVOLVEMENT OF MULTIPLE PROGESTERONE RECEPTORS IN OVARY MAINTENANCE IN ZEBRAFISH

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Estrogen and androgen are well known steroid hormones that alter internal and external sex characteristics in vertebrates. Progesterone is well known for reproductive processes such as oocyte maturation and ovulation in vertebrates. Progesterone also plays an antiapoptotic role in ovarian cells. Progesterone receptor membrane components (Pgrmcs including Pgrmc1 and Pgrmc2) and membrane progesterone receptors (mPRs including $\alpha, \beta, \gamma, \delta, \varepsilon$) have all been suggested to play important roles in progesterone signaling and reproduction. However, determining physiological functions of these progesterone receptors *in vivo* is challenging due to gene duplication among progesterone receptor families and overlapping functions of these receptors. To overcome this obstacle, we have generated single as well as combined knockouts for Pgrmcs and mPRs. Surprisingly, no adult female could be found in a total knockout (*pgrmc1*$^{-/-}$/*pgrmc2*$^{-/-}$/*mprs*$^{-/-}$, N=127). In comparison, the sex ratios in other single or combined mutant lines (*pgrmc1*$^{-/-}$, *pgrmc2*$^{-/-}$, *pgrmc1/2*$^{-/-}$, *mprs*$^{-/-}$, and *pgrmc1*$^{+/-}$/*pgrmc2*$^{-/-}$/*mprs*$^{-/-}$) are similar as those found in wildtype. During early stage gonad development, 71% of total knockout (*pgrmc1*$^{-/-}$/*pgrmc2*$^{-/-}$/*mprs*$^{-/-}$, N=21) zebrafish had male biased gonads with early spermatocytes at 35dpf (day post fertilization), while only 31% wildtype contain early spermatocytes at this time point. At 45dpf, 86% of total knockout (*pgrmc1*$^{-/-}$/*pgrmc2*$^{-/-}$/*mprs*$^{-/-}$) fish (N=29) are male, and this ratio increased to 100% by 60dpf (N=92). In comparison, the ratios of male and female zebrafish in wildtype were normal (41% at 45dpf and 54% at 60dpf) when compared to total knockout (*pgrmc1*$^{-/-}$/*pgrmc2*$^{-/-}$/*mprs*$^{-/-}$). In conclusion, our novel results suggest that a redundant function exists between the Pgrmc family and mPRs family in ovarian maintenance in zebrafish.

DOES CHRONIC EXPOSURE TO AGRICULTURAL RETENTION POND WATER INDUCE ENDOCRINE DISRUPTION IN THE AMERICAN TOAD?

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Endocrine disrupting pesticides represent the largest group of endocrine disrupting compounds by number. Complex and temporally variable mixtures of these chemicals emanate from agricultural operations in runoff. Retention ponds are becoming more frequently implemented in the agroecosystem as a means to reduce the impacts of agricultural run-off directly entering surface waters. However, one of the consequences of using retention ponds is the attraction of wildlife to these constructed wetlands (e.g., as breeding grounds for amphibians). The objective of this research project is to study the effects of the retention pond water on the amphibian endocrine system. In the summer of 2018, pond water chemistry was assessed bi-weekly, revealing the presence of a suite of pesticides, including organophosphorus pesticides (e.g., atrazine, glyphosate) and emergent pesticides (e.g., neonicotinoids). In parallel, a chronic exposure to the pond water was conducted on American toads (*Anaxyrus americanus*) from early development stage until completion of metamorphosis. Throughout the exposure, survival, developmental, and morphological endpoints were assessed. Treated toads were significantly smaller in all morphometric indices (e.g., body mass, snout-vent-length, and hindlimb length) and completed metamorphosis on average 2 days earlier compared to control animals. To link these morphological biomarkers to molecular actions, liver and gonad-mesonephros complex tissues were sampled, and transcript level was measured for a suite of genes related to thyroid hormone signalling, energy metabolism, and sex-steroid hormone signalling. This work provides ecologically relevant information on the potential effects of agricultural activities on amphibian endocrine function.

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CAGE ENRICHMENTS NEGATIVELY IMPACT THE REPRODUCTIVE BRAIN IN MALE MICE

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The vertebrate reproductive brain comprises neurons that secrete gonadotropin-releasing hormone (GnRH) as well as their afferents/efferents. Increasing evidence suggests that the reproductive brain, like the cognitive brain, can respond to various forms of enrichment cues to alter its functions. Since enrichment cues provide sensory cues that may be processed differently by different neurocircuits, we hypothesize that the same enrichment may have highly divergent effects on different brain functions. The objective of this study was to examine if cage enrichments known to benefit the cognitive brain were also beneficial to the reproductive brain and downstream gonadal function in male mice. To test this, male mice were treated at the time of weaning on postnatal day (PN) 20 with either (1) no cage enrichments or (2) combined cage enrichments of nestlets, egg cartons, and igloos. Animals were sacrificed on PN35 and PN50 and assessed for reproductive hormone levels and gene expression as well as testicular histology. Cage enrichments did not significantly affect the expression of *GnRH* and *KiSS1,* an upstream stimulator of GnRH neurons, on PN35, but significantly decreased the expression of both genes on PN50. The expression of fibroblast growth factor 2, a factor known to enhance neural plasticity, in the preoptic area was also significantly decreased by enrichments. However, hypothalamic GnRH and pituitary luteinizing hormone (LH) stores were not significantly altered. Serum LH content as well as testicular morphology and spermatogenic function were also not altered by cage enrichments. These results suggest that environmental enrichments may not consistently have positive effects on all brain functions. Further, the negative impact of cage enrichments on the reproductive brain was manifested only in older animals with longer enrichment exposure, suggesting this is a slow process that may need more time to secondarily inhibit pituitary and testicular functions. Overall, the same enrichment cues may exert neurocircuit-specific effects, leading to highly variable outcomes depending on the brain region examined.

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CAPA NEUROPEPTIDES: ANTI-DIURETIC HORMONE ACTIVITY AND SIGNALING CASCADE IN THE DISEASE-VECTOR MOSQUITO, AEDES AEGYPTI

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Female *Aedes aegypti* mosquitoes face the challenge of excess water and ion intake after a blood meal. To cope with this, blood-feeding insects have a highly active excretory system that includes the Malpighian tubules (MTs), which are under rigorous control by neuroendocrine factors to regulate transepithelial movement of ions and osmotically-obliged water. While CAPA exhibits variable response in insect species, the role and signaling pathway of these peptides remains unclear in adult *Aedes* mosquitoes. Given that CAPA receptor transcript was localized to the principal cells of the MTs, the objectives of this study were to examine the effects of a mosquito CAPA peptide family member, *Aedae*CAPA-1, on adult female MTs stimulated with various diuretic factors. *Aedae*CAPA-1 was found to inhibit secretion of MTs stimulated by select diuretic factors, 5-HT and DH_{31}, while having no anti-diuretic action on MTs stimulated by other tested diuretic factors, including kinin-related and corticotropin releasing factor-related peptides. Additionally, although *Aedae*CAPA-1 elicits anti-diuretic activity, it does not influence the relative proportions of cations transported by adult MTs, thus maintaining the kaliuretic activity of 5-HT and the natriuretic activity of DH_{31}. Effects of the second messenger cGMP were tested on adult MTs which revealed that both 5-HT and DH_{31}–stimulated secretion is strongly inhibited by cGMP, similar to effects seen with *Aedae*CAPA-1. Furthermore, pharmacological inhibition of PKG/NOS signaling abolishes the anti-diuretic activity of *Aedae*CAPA-1, which collectively confirms the role of cGMP/PKG/NOS in the CAPA signaling pathway. Notably, the inhibitory effect of CAPA was also abolished through knocking down the receptor, verifying its role in anti-diuresis. Further understanding of the role of each specific hormone family, including both diuretic and anti-diuretic factors, will help resolve this complex regulatory network. Given the central importance of the excretory system, understanding the regulatory mechanisms controlling hydromineral balance may aid in developing improved pest management strategies, reducing mosquito populations, and thus mitigating their burden as disease vectors.

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INFLUENCE OF TEMPERATURE REGIME AND EPIZOOTIC SHELL DISEASE ON ECDYSTERONE CONCENTRATIONS IN AMERICAN LOBSTERS, *HOMARUS AMERICANUS*

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American lobsters (*Homarus americanus*, H. Milne-Edwards) comprise the most economically important shellfish fishery in the world, with the state of Maine contributing the majority of US landings. While lobster populations in Maine are still high, populations in southern New England collapsed more than 15 years ago, with no sign of recovery. Although the cause of the collapse is not fully understood, it was coincident with an increase in ocean temperature and the incidence of epizootic shell disease. Molting lessens the severity or eliminates the disease, and lobsters in the wild with shell disease have been shown to have increased concentrations of hemolymph ecdysteroids, key steroid hormone regulators of molting. Incidence of shell disease is significantly increasing along the Maine coast, highlighting an urgent need to understand factors affecting its etiology. This study examines the effects of three temperature regimes mimicking annual temperature cycles from southern New England, southern Maine, and northern Maine, on hemolymph ecdysterone (20E) concentrations. At baseline sampling from lobsters collected in the field, it was determined that 20E was significantly elevated in lobsters with severe shell disease, but not in those with low to moderate levels of the disease. In the winter sampling period, 20E was highest in lobsters from the southern New England temperature regime, but a reversed trend was apparent in the summer sampling period, although not significantly so. Therefore, these data suggest that temperature regime does impact hemolymph 20E concentrations in both diseased and healthy lobsters, and supports the findings of others studies that elevated 20E may be a mechanism to mitigate the effects of shell disease.

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HOW TO GROUP PRIMIPAROUS DAIRY COWS: BEHAVIOR, CORTISOL IN SERUM AND HAIR AND PRODUCTION PERFORMANCE.

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The beginning of lactation is extremely stressful for primiparous dairy cows, for the first time they must adapt to new managements, produce milk and compete for resources with bigger and more experienced cows, that's why, it has been suggested to group primiparous cows with primiparous cows in their first lactation. The aim of this study was to compare behavior, hair and serum cortisol levels and productivity of primiparous dairy cows grouped with or without multiparous cows. 44 Holstein cows were assigned into two groups: PM, 22 primiparous cows, grouped whit 128 multiparous cows and PP, 22 primiparous cows grouped with 128 primiparous cows. Animals were housed with plenty of food and space ($45m^2$/cow) and avoiding stress as much as possible. Social and maintenance behavior (standing, walking, eating, ruminating, lying and drinking), were observed daily from first day (0) to 90^{th} of lactation. On days 0, 30, 60 and 90 of lactation body condition and somatic cell counts (SCC) were measured, and blood and hair samples were obtained to analyze cortisol content by RIA. At milking, body weight, milk yield, activity and milk conductibility were measured from day 0 to 180 days of lactation. PP presented higher frequencies of aggression (PP: 3.32, PM:1.73, P=0.01) and displacement (PP:4.05, PM:1.59, P= 0.0003) than PM group. Non-statistical differences were found between PP and PM groups in maintenance behavior, milk yield (PP: 31.8, PM: 32.1Kg/day), SSC In PP group serum cortisol and hair cortisol were higher at 60 and 90 day of lactation (PP: 3.34, 8.56ng/ml, PM:6.75, 5.57ng/ml $P<0.05$) and hair cortisol at 60 days (PP: 7.94ng/ml PM:7.2ng/ml $P<0.05$). PM group had better Body Condition at day 90 of lactation than PP group, PP:2.58, PM: 2.96. $P>0.05$). Thus, in these situations of resources, management and space allowance, grouping primiparous cows with multiparous cows is recommended.

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SEXUAL MATURATION OF AFRICAN ELEPHANTS RAISED IN CAPTIVITY

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African elephant (*Loxodonta Africana*) is an emblematic species catalogued as vulnerable in the IUCN Red List and in CITES appendix. Elephants are threatened by human conflict as furtive hunting and habitat fragmentation. These animals have the largest mammal estrous cycle (12-18 weeks) and gestation (22 months) delivering only one calf per breeding. Data on serum hormones as well as behavior and zoometric data in the period that conducts to the puberty of this specie is scarce. Thereafter, the aim of this study was to obtain information on the reproductive changes that conducts to puberty in African elephants. A group of 9 prepubertal African orphan elephants 3 males (10, 9 and 5 year-old), and 6 females (10, 8, two of 7, and two 5 year-old) were rescued and adopted by a wild fauna Conservation Park (Africam Safari) in Mexico. The animals were trained to give samples and allow transrectal ultrasonography. Serum concentration of gonadotropins, testosterone and progesterone were determined by RIA fortnightly for one year. The physical development of the animals was measured. The agonistic interactions and reproductive behavior were recorded fortnightly throughout one year. Results showed that male testosterone concentration increased with age but did not reach values of musth. However, copulation was observed in one male. The low progesterone values found in the females suggest a low ovarian activity. LH peaks were not found. A corpus luteum was detected by ultrasonography in the oldest female (10 year-old) suggesting that puberty can be reached at this age. The behavioral information indicates that hierarchy is established before puberty.

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SERUM GLUCOCORTICOID PROFILES IN THREE SPECIES OF MEXICAN PRIMATES: RESPONSE TO CAPTURE-RESTRAINT

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Glucocorticoids (GC) are steroid hormones with multiple physiological functions, including the stress response. In different vertebrate species, exposure to stressful events activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in an increased blood GC concentrations. In most mammals, cortisol is the main GC used as a biomarker of the stress response; however, limited information on serum corticosterone profiles and its relation with cortisol exist for many mammal species. Although much research and conservation efforts have been placed on the three species of endangered Mexican monkeys, little information has been produced on their serum GC profiles. The objectives of this study were to investigate concentrations of serum GC in three species of primates (*Ateles geoffroyi*, *Alouatta palliata* and *Alouatta pigra*) and to evaluate the adrenal response to potentially stressful procedures such as capture and restraint. Seven adult males in captivity (Catemaco, Veracruz), 52 wild *A. pigra* and 36 wild *A. palliata* (Campeche and Tabasco) were included in this study. Two or three consecutive blood samples were collected during the capture procedure. Samples were analyzed using radioimmunoassay. Results showed that cortisol was the main serum GC in the three species. Corticosterone was also present in sera, but in significantly lower concentrations. GC values significantly increased for the three species in the last sample, reflecting a similar response for cortisol and corticosterone during the capture process. The spider monkeys (*A.geoffroyi*) tripled the cortisol concentration between the initial and final sample. The magnitude of the stress response was significantly higher in *A. pigra* than in *A. palliata*. This study provides information on the adrenal function of New World primates and suggests that the response to stress is different even in closely related primate species.

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TIMING OF A GONADAL COMMITMENT TO THE TESTICULAR DIFFERENTIATION BEYOND ESTROGEN-SIGNAL PRODUCING OVARY IN THE TEMPERATURE-DEPENDENT SEX DETERMINATION OF THE AMERICAN ALLIGATOR

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Many reptiles including the American alligator, exhibit the temperature-dependent sex determination (TSD), whose thermo-sensitive period is between developmental stages 21 through 24 in the alligator. Estrogen signal plays a central role in TSD which can be overridden by an estrogen-exposure. It is important to identify a timing of gonadal commitment to either ovary or testis for a better understanding of TSD and estrogen-signals, although it has been assumed to be sensitive to estrogen during a thermo-sensitive period of TSD. Moreover, some environmental contaminants are estrogenic, and their effects on the sex ratio and reproductive health of TSD-species have been concerned.

Utilizing eggs of the American alligator, an estrogen sensitivity in TSD was tested at the three developmental stages, 22, 24 and 26. The eggs were exposed to 5 μg/g egg of 17β-estradiol (E2) or vehicle ethanol alone at a male-biased temperature which produced 81% testis. The E2-exposure at the stages 22 and 24 induced more ovary than the control group, whereas it was not identified in a group exposed to E2 at the stage 26. These results indicated that there is a critical commitment in the testicular development between the developmental stage 24 (100% ovary in E2 Exposure) and 26 (39% ovary with E2), when gonadal differentiation are initiated as enlarging medullary cord and cortical germ cells in testis and ovary, respectively. Some individuals at the stage 26 were still plastic enough to become ovary, when they were exposed to E2. A gonadal commitment to testicular development could be later than a known thermo-sensitive period during the stage 21-24. Results generates a hypothesis that testicular structure may be a default form which stays capable of becoming ovary induced by estrogen-signal until later stages beyond the temperature sensitivity in the TSD.

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MECHANISMS OF LIFE-HISTORY TRANSITIONS: INTERACTIONS AMONG GLUCOCORTICOIDS, NEUROPEPTIDES, AND METABOLIC FACTORS REGULATE THE SEASONAL SWITCH FROM REPRODUCTION TO FORAGING BEHAVIOR IN GARTER SNAKES

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Transitions between life-history stages are often characterized by dramatic switches from one behavior mode to another and include examples such as reproduction, migration, and foraging. Behavioral switching helps organisms respond to changing resource availability, a process that is crucial to both survival and fitness. Red-sided garter snakes (*Thamnophis sirtalis*) are an exceptional model for understanding the mechanisms mediating life-history transitions. We previously showed that the hypothalamus-pituitary-adrenal (HPA) axis plays a central role in the seasonal transition from reproduction to migration and foraging. For example, males have decreased sensitivity to capture stress and adrenocorticotropic hormone during the mating season. Further, plasma glucocorticoids are elevated during mating in both sexes and decline as snakes begin to migrate away from the breeding grounds in search of food. Experimentally decreasing glucocorticoids with a synthesis inhibitor or glucocorticoid receptor antagonist prematurely induces the behavioral switch to foraging, as males choose to pursue feeding cues over mating opportunities in two-choice Y-maze trials. However, increasing the duration of elevated glucocorticoids with hormone implants does not extend the duration of courtship behavior, nor can the switch to feeding be reversed once the seasonal transition has been made. These results indicate that once glucocorticoids decline to some threshold level, it alters the fundamental nature of how glucocorticoids interact with the brain to mediate behavioral switching. To better understand these mechanisms, we asked if arginine vasotocin (AVT) and neuropeptide Y (NPY) are involved in mediating the transition from reproduction to foraging. We found that seasonal changes in AVT and NPY in males are specifically associated with the transition from reproductive condition to foraging activity. Moreover, experimentally decreasing plasma glucocorticoids not only induces feeding behavior but also increases NPY-immunoreactivity in specific brain regions. Collectively, these data provide a framework for understanding the role of the HPA axis in seasonal life-history transitions and the mechanisms by which neuroendocrine signals reconfigure regulatory systems.

TOO STRESSED TO EAT OR GROW: THE METABOLIC COST OF CHRONIC SOCIAL STRESS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Juvenile rainbow trout (*Oncorhynchus mykiss*) confined in pairs form social hierarchies, with subordinate fish showing prolonged elevation of the stress hormone cortisol, indicative of chronic social stress. Thus, subordinate social status provides a useful system for studying the effects of chronic stress on metabolism and growth. Whereas dominant fish monopolize and defend food resources, the combination of low food intake and elevated cortisol levels induces a catabolic state in subordinate fish. Subordinate fish exhibit elevated leptin-a1 transcript abundance in the liver, which likely contributes to the suppression of appetite in these fish. Thus, subordinate fish must tap into energy reserves, reducing liver glycogen and enhancing hepatic gluconeogenesis through increases in transcript abundance and activity of phosphoenolpyruvate carboxykinase (*pck1*). Lipid reserves are accessed for ß-oxidation as evidenced by elevated circulating levels of free fatty acids and enhanced transcript abundance of hepatic carnitine palmitoyltransferase 1A (*cpt1a*). In white muscle, subordinate fish show elevated activities of the ubiquitin proteasome pathway and the autophagy lysosomal system together with reduced rates of protein synthesis. Collectively, these metabolic effects account for the low growth rates of subordinate fish, illustrating the metabolic cost of chronic stress.

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PHYSIOLOGICAL COSTS OF CHRONIC SEASONAL HYPOXIA IN OKAVANGO TILAPIA

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The Okavango Delta in Botswana is a World Heritage Site and considered an almost pristine wilderness. Each year, from November to April, the Delta floods with rainwater from the mountains of Angola. As flood waters spread over the parched landscape, microbes spring to life and deplete the aquatic dissolved oxygen over hundreds of square kilometers. Using oxygen dataloggers, we documented lengthy periods of hypoxia that commenced 5-7 weeks after flood arrival and persisted for 3.5 – 5 months in 2017 and 2018. During this hypoxic period, dissolved oxygen rarely exceeded 3 mg/L and persisted below 0.5 mg/L for as long as two weeks. Field sampling of three tilapia species, collected during and after the flood, indicates that fish were ubiquitously affected by severe hepatic and splenic inflammation associated with melano-macrophage aggregates and extensive ceroid and hemosiderin. The inflammation and cellular damage are likely caused by direct tissue hypoxia along with iron accumulation arising from intense, hypoxia-induced red blood cell cycling. We suggest that Okavango tilapia have adapted to long-term, natural hypoxia by increasing red blood cell production, but at significant cost to their health and longevity. Our findings highlight seasonal hypoxia as an important natural stressor in the Okavango that may limit fishery resilience in the face of rising anthropogenic impacts (over-fishing, agricultural pollution, wildfires, timber-harvesting, mining, sedimentation, and invasive species).

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OF MICE AND MEN: RELATIONSHIP AMONG STRESS, GLUCOCORTICOIDS, AND COGNITIVE FUNCTION

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Stress, and the hormones associated with the hypothalamic-pituitary-adrenal axis (i.e., glucocorticoids), can negatively impact cognitive function. This phenomenon has been termed the glucocorticoid cascade hypothesis and it is supported by several lines of data. Recently my laboratory has been testing aspects of this hypothesis using APPswe/PS1dE9 transgenic mice, a beta amyloidogenic mouse model of Alzheimer's disease, and aging humans. Alzheimer's disease (AD) is an incurable and progressive disorder that presents with memory loss, impaired cognitive function, and behavioral changes. The underlying cause of AD is not fully understood, but, in-line with the glucocorticoid cascade hypothesis, elevated levels of stress and glucocorticoid (GC) hormones are associated with AD severity and progression. Little is known, however, about GC dynamics in the commonly used APPswe/PS1 AD mouse model. My lab is using various behavioral and endocrine endpoints to determine how GCs, aging, and memory interact. Data collection is on-going, and analysis of additional behavioral tests is still underway. But, thus far, in mice, baseline GCs increase with age from 2 to 10 months, but this increase did not differ by genotype (transgenic vs. controls). By 10 months of age, however, transgenic mice had higher post-stress GCs than age-matched controls. But, in a separate cohort of mice aged 18 months, genotypes did not differ in baseline or post-stress GCs. Preliminary results from the novel object test (short-term memory paradigm) suggest that at 18 months of age genotypes do not differ in memory performance and GCs, baseline or post-stress, are not correlated with test performance. In our sample of non-demented, aged humans, change in baseline GCs over a 3-yr period did not impact cognitive change over time, but single nucleotide polymorphisms in stress-responsive genes did relate to cognitive change on their own (CRFR1) or interacted with GCs to impact cognitive scores (FKPB5). Thus far our data suggest marginal support for the GC cascade hypothesis and highlight the importance of variables such as age and genotype when interpreting results.

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A NOVEL ROLE FOR LEPTIN IN FISH: BRANCHIAL LEPTIN IS INVOLVED IN SHORT-TERM SEAWATER ACCLIMATION IN ATLANTIC SALMON (*Salmo salar*, L.)

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The gills are considered the primary osmoregulatory organ: they sense changes in osmolality and actively manage ion homeostasis. Ionocytes contain ion-transporting proteins that take up or secrete ions to secure hydromineral balance, energised by the basolateral Na^+/K^+-ATPase (NKA). Adjacent glycogen-rich cells function as local energy supply for the ionocytes under acute salinity challenges. A key regulator of energy homeostasis is leptin, and fundamental differences between mammalian and fish leptin exist. Leptin is involved in liver glucose mobilisation to meet energy demands of catabolic stressors, including osmotic challenges. In the present study we explore the involvement of leptin in short-term acclimation to seawater in Atlantic salmon smolts (the anadromous 'Vosso' and the landlocked 'Blege' strain). We localised *lepa* in glycogen-rich cells of the gills using immunocytochemistry. Next, we analysed physiological variables (osmolality, glucose, cortisol), as well as transcript abundance of genes coding for the leptin system and osmoregulatory proteins, at several time points following seawater transfer (fish transferred to fresh water served as controls). Transcript abundance of branchial *lepa1* mRNA and hepatic *lepa2* increased after seawater transfer. Our findings suggest a differential role for salmon leptins in glycogenolysis to supply energy for osmoregulation. Blege plasma osmolality showed a stronger increase after SW transfer, compared to anadromous salmon, and only hepatic leptin (upregulation of both *lepa1* as *lepa2* after SW transfer) seems involved in osmoregulation: the branchial *lepa1* gene of landlocked salmon did not respond to SW challenge, suggesting that its role in gill-dependent osmoregulation was lost in the absence of osmotic challenges. Landlocked fish respond to SW challenge with upregulation of *nka-α-1a* only, the anadromous fish upregulate *nka-α-1b.* The loss of branchial *nka-α-1b* and *lepa1* upregulation in the landlocked salmon may explain their poorer osmoregulatory capacity. A Principle Component Analysis (PCA) identified components of different make-up in the salmon strains. Together, these results add to our understanding of leptin's role in energy metabolism in early vertebrates, which includes short-term seawater acclimation and osmoregulation.

POTENTIAL NEW ROLES FOR CRF AND NPY IN MIDBRAIN DEFENSE

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The optic tectum (OT) and superior colliculus (SC) are not only critical for the visual recognition of food in all vertebrates, but these brain areas also rapidly inhibit food intake when a visual threat is present. Recently collected data from our laboratory using an amphibian model suggest that the stress-related neuropeptides CRF and NPY may play a role in this feeding/predator avoidance trade off. Exposure to a reactive stressor increased CRF peptide and transcript abundance in the OT while forced reductions in food intake decrease tectal CRF content. Direct administration of oCRF to the tecta reduces food intake and alters certain prey capture behaviors, and these effects appear to be mediated by tectal CRFR1 receptors. Blockade of tectal CRFR1 receptors with NBI-27914 marginally impacts predator-induced changes in prey capture and locomotor behavior. The downstream effects of CRFR1 activation do not appear to involve modulation of glutamate or GABA release. We have observed that the anuran OT is richly innervated by NPY and NPY2R receptors, and that direct administration of pNPY to the tecta does not alter total amount of food consumed but does decrease the time it takes to eat as frogs approached food more quickly and spent less time in contact with food; these effects were reversed by tectal NPY2R blockade. Interestingly use of the selective NPY2R antagonist BIIE-0246 was more effective at reversing predator-induced food intake and prey-capture behavior than the CRFR1 antagonist NBI-27914. We conclude that stress related neuropeptides may modulate the response to predators and non-specific threats via action in the optic tectum. Current work is focused on identifying the sensory pathways targeted by tectal CRFR1 receptors and the neurochemical mediators downstream of CRFR1 and NPY2R activation.

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ACUTE AND LONG-TERM METABOLIC CONSEQUENCES OF EMBRYONIC ZEBRAFISH EXPOSURE TO AQUATIC CONTAMINANTS

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Metabolic disruption is a phenomenon that is increasingly observed in humans and wildlife. Several hypotheses of metabolic disruption have been postulated, including the developmental origin of disease hypothesis, the environmental contaminant hypothesis, and the life style hypothesis. Using the zebrafish model, we investigated the integration of these hypotheses by metabolically phenotyping zebrafish during acute early developmental exposure (0-6 dpf) to a range of concentrations in the low ppb range of known and suspected metabolic disrupting chemicals. For specific chemicals, we subsequently quantified growth rates and metabolic phenotypes in adults at baseline or in response to a nutritional challenge. Our results indicate that F-53B, a PFOS related contaminant detected in the Chinese environment acutely suppressed larval feed intake at 1% and 10 % of the measured LC_{50} concentration, while enhancing metabolic rate compared to control larvae. Investigation of gene expression patterns of key metabolic genes revealed a significant reduction of glucokinase in all embryos exposed to F-53B, regardless of concentration. Acute exposures to BPA and DEHP did not significantly alter larval the larval metabolic phenotype, but in the case of BPA resulted in significant decreases on subsequent growth. As well, BPA, following an embryonic exposure concentration of 1 ppb significantly decreased growth in response to overfeeding initiated at 100 dpf, suggesting long-term effects of embryonic BPA exposure on growth and metabolism.

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EVOLUTIONARY AND ECOLOGICAL ENDOCRINOLOGY OF INVERTEBRATE CARBOHYDRATE METABOLISM

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Arthropods (insects and crustaceans) metabolize trehalose as the energy source in addition to other carbohydrates: glucose and glycogen. Trehalose is a naturally occurring non-reducing disaccharide that is enzymatically formed by a 1-1 covalent glycosidic bond between two α glucose molecules. As the primary hemolymph sugar, trehalose levels change under different physiological conditions including feeding and starvation by the release of a hypertrehalosemic hormone (HTH) produced in insect corpora cardiaca. In decapod crustaceans, most studies have been focused on glucose metabolism and crustacean hyperglycemic hormone (CHH), because glucose is the energy source for an alternative ATP production under stressful conditions. Recently, it is reported that trehalose as the major hemolymph sugar is metabolized by the ubiquitous presence of multiple isoforms of trehalose-6-phosphate synthase (TPS) and trehalase in the tissues of decapod crustaceans. Physiological and environmental conditions such as feeding, temperature and dissolved oxygen influence the trehalose levels of hemolymph and the other crustacean tissues. Why do insects and crustaceans then utilize trehalose as an additional reserve energy source? Decapod crustaceans regulate glucose levels somewhat differently, compared to vertebrates and humans. Glucose levels in human blood are tightly controlled ranging between 3.7- 7.1mM. In crustaceans, hemolymph glucose remains much lower than one mM as the basal levels and then swiftly increases to >10mM under stressful conditions via trehalase cleaving trehalose into two glucose units. The paper discusses the benefits of trehalose being as the major hemolymph sugar over glucose in light of carbohydrate metabolism in ecological and evolutionary endocrinology.

THYROTROPIN-RELEASING HORMONE-DEGRADING ECTOENZYME CONTROLS THYROTROPIN SECRETION AND BODY WEIGHT IN MALE RODENTS

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In mammals, thyrotropin-releasing hormone (TRH) controls thyrotropin (TSH) secretion from the pituitary; it has also a potent anorexic effect independent of its pituitary actions. TRH is short-lived, hydrolyzed by the TRH-degrading ectoenzyme (TRH-DE). *Trhde* is expressed by β2 tanycytes, whose end-feet form synaptoid contacts with TRH terminals in the external layer of the median eminence (ME). Tanycyte TRH-DE activity may contribute to adjust thyroid axis activity to changing energy needs. To test whether TRH-DE is relevant for energy balance, we used mice in which *Trhde* exon 2 was deleted backcrossed to the C57BL/6NJ background for 11 generations. TRH-DE activity was reduced in heterozygote (HT) and eliminated in homozygote (KO) compared to wild type (WT) animals. Male adult mice were switched from a standard diet to a high fat high fructose diet (HFFD) for 9 weeks. On HHFD, KO mice ingested less Kcal and gained less body weight (BW) than WT animals. Compared to WT or HT mice, KO mice had a lower fat weight and a higher glucose tolerance. To clarify the specific relevance of tanycyte TRH-DE, we used adeno-associated virus (AAV) vectors in male rats to either knock down or overexpress TRH-DE in ME. Serum TSH concentration increased when TRH-DE activity decreased, and the converse occurred when TRH-DE activity increased. Thus, β2-tanycyte TRH-DE activity controls the concentration of TSH in the circulation, probably because it regulates the turnover of TRH before entry into the hypothalamus-pituitary portal vessels. However, knock down or overexpression of TRH-DE in ME did not affect BW 2 or 3 weeks after AAV injection. To further test the role of peripheral TRH-DE activity, a phosphinic analogue of TRH (P-TRH) was administered to adult male HFFD mice for 28 days. P-TRH treatment reduced TRH-DE activity in serum, but not inside the blood brain barrier, compared with mice treated with vehicle; however, treatment with P-TRH did not change food consumption and BW, although it tended to decrease white fat weight. In conclusion, ablation of TRH-DE impedes diet-induced obesity in male mice, possibly through enhanced thyroid axis activity and amplification of the anorectic effect of TRH. Supported in part by grants from DGAPA-UNAM (PAPIIT IN206712, IN206416, IN212719), and CONACYT (CB154931, CB254960 and PN562).

EVIDENCE FOR A LEPTIN-INSULIN AXIS IN THE MOZAMBIQUE TILAPIA *(OREOCHROMIS MOSSAMBICUS)*

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Leptin is a pleiotropic hormone known to influence growth, energy expenditure, and appetite in vertebrates. It is primarily associated with lipolysis in mammals and expressed in adipocytes, however data suggest that in tilapia and other poikilotherms, leptin is produced predominately in the liver and may act to regulate carbohydrate metabolism. Prior work in our lab showed that leptin A (LepA) stimulates hyperglycaemia and glycogenolysis in tilapia (*Oreochromis mossambicus*), thus we extended these studies to determine whether there are interactions between leptin, glucose levels, and the hypoglycaemic hormone, insulin, in mediating carbohydrate metabolism. Using primary hepatocyte cultures and *in vivo* injection studies, we first examined the effects of elevated glucose and insulin on *lepa* mRNA levels in tilapia liver. Results indicate that high extracellular glucose reduces hepatic *lepa* levels *in vitro*, while a bolus glucose injection caused a paradoxical 14-fold increase in hepatic *lepa*. We postulated that this disparate result may be due to glucose stimulating the release of insulin *in vivo*. Indeed, we found that insulin increased hepatic *lepa* by 3- and 50-fold *in vitro* and *in vivo*, respectively. As leptin and insulin have opposing actions on carbohydrate metabolism in tilapia, we hypothesised that there a negative feedback loop may exist between the liver and Brockmann bodies. To test this, we treated Brockmann bodies with recombinant tilapia LepA (rtLepA) *in vitro* and measured changes in *insulin* mRNA. Results show a reduction in *insulin* levels by rtLepA, supporting 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.

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MICRORNAS AS KEY PLAYERS IN ENDOCRINOLOGY

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MicroRNAs (miRNAs) are small non-coding RNAs that play important roles in regulating gene expression. In animal cells, miRNAs regulate gene expression primarily by binding to miRNA response element, mostly found at the 3' UTR of target mRNA, to decrease mRNA stability and to induce translational repression. Through regulation of genes encoding hormones, growth factors, their receptors, and/or intracellular mediators, miRNAs can control the activity of hormones and growth factors. For example, we have shown that several miRNAs regulate placental development by modulating the signaling of the transforming growth factor-ß (TGF-ß) superfamily. Among them, miR-378a-5p targets Nodal to inhibit trophoblast fusion and to promote trophoblast invasion. miR-218-5p induces endovascular trophoblast differentiation by inhibiting TGF-ß2 signaling while miR-376c enhances trophoblast invasion by reducing the expression of receptors for Nodal and TGF-ß. In addition, we have also shown that miR-590-3p promotes ovarian cancer development and one of the mechanisms underlying the actions of miR-590-3p in ovarian cancer cells is to promote Wnt/ß-catenin signaling. These examples show that miRNAs are important players in endocrinology.

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THE ROLE OF MICRORNA IN THE REGULATION OF OOCYTE MATURATION

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Cumulus expansion in the cumulus-oocyte -complex (COC) is associated with oocyte maturation and competency. The expansion is dependent on the production and remodeling of the extracellular matrix and regulated by autocrine/endocrine factors. How these complex and cohesive processes are regulated to ensure oocyte maturation was unclear. Our early work showed that miR378 and miR574 expression decreased, while miR21 increased in cumulus cell during porcine oocyte maturation. Over expression of miR-378 in cumulus cells attenuated cumulus cell expansion and suppressed oocyte progression from the GV to MII stage (54±3% vs. 31±5%). Further studies showed miR-378 targets aromatase, resulting in a significant decrease in estradiol production. The addition of estradiol to in-vitro maturation media was able to reverse the effect of miR-378 on cumulus expansion and oocyte maturation. This suggests decreased estradiol production via suppression of aromatase may be one of the mechanisms by which miR-378 regulates functions in COCs. MiR-574 directly targets the transcript for hyaluron synthase 2 protein (HAS2), a key enzyme in the production of extracellular matrix by cumulus cells. Over expressed miR-574 using a lentivirus resulted in a 50% decrease in HAS2 expression and nearly 20% reduction in oocyte progression through meiosis. ADAMTS1, and its partner VERSICAN, are important extracellular components in COC expansion. Our study found that one of the tissue inhibitors of the metalloproteinase (TIMP) family member, TIMP3, decreased the levels of ADAMTS1 and VERSICAN. In silico prediction revealed that a miR-21 binding site at the 3-UTR of the *TIMP3* mRNA, which was further confirmed to be the target site of miR-21 by luciferase reporter assays. MiR-21 decreased *TIMP3*, increased ADAMTS1, VERSICAN and enhanced cumulus cell expansion. Taken together, our findings add insights into the post-transcriptional regulation of oocyte maturation by these three miRNAs in the mammalian ovary.

YAP IS ANTAGONIZED BY ITS CIRCULAR RNA VIA SUPPRESSING THE ASSEMBLY OF THE TRANSLATION INITIATION MACHINERY

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Yap is the key component of Hippo pathway which plays crucial roles in tumorigenesis. Inhibition of YAP activity could promote apoptosis, suppress proliferation, and restrain metastasis of cancer cells. However, how Yap is regulated is not fully understood. Here, we reported Yap being negatively regulated by its circular RNA (circYap) through the suppression of the assembly of Yap translation initiation machinery. Overexpression of circYap in cancer cells significantly decreased Yap protein but did not affect its mRNA levels. As a consequence, it remarkably suppressed proliferation, migration and colony formation of the cells. We found that circYap could bind with Yap mRNA and the translation initiation associated proteins, eIF4G and PABP. The complex containing overexpressed circYap abolished the interaction of PABP on the poly(A) tail with eIF4G on the 5'-cap of the Yap mRNA, which functionally led to the suppression of Yap translation initiation. Individually blocking the binding sites of circYap on Yap mRNA or respectively mutating the binding sites for PABP and eIF4G derepressed Yap translation. Significantly, breast cancer tissue from patients in the study manifested dysregulation of circYap expression. Collectively, our study uncovered a novel molecular mechanism in the regulation of Yap and implicated a new function of circular RNA, supporting the pursuit of circYap as a potential tool for future cancer intervention.

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MIR-218-5P MODULATES NEUROPEPTIDE Y SIGNALING IN TROPHOBLASTS

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression primarily at the post-transcriptional levels. Many studies have shown that miRNAs play important roles in regulating placental development. We have recently found that miR-218-5p promotes trophoblast migration, invasion and differentiation into the endovascular trophoblasts, and accelerates the remodeling of uterine spiral arteries. Through microarray analysis, we identified neuropeptide Y (NPY) as one of the most strongly down-regulated genes by miR-218-5p. Although NPY is known to be produced by human placenta, little is known about the function of NPY during pregnancy. To investigate the regulation of NPY by miR-218-5p and the function of NPY in human placental development, we used an immortalized trophoblast cell line, HTR8/SVneo, to examine cell migration and invasion after NPY overexpression or knockdown. We found that silencing of NPY using siRNA resulted in an increase in trophoblast migration and invasion. Conversely, overexpression of NPY or treatment with NPY peptide led to a decrease in cell motility. Additionally, the effect of NPY on cell migration was attenuated when cells were also treated with an NPY receptor 1 (NPY1R)-specific inhibitor. These findings suggest that the NPY-NPY1R signaling pathway regulates trophoblast migration and invasion. Bioinformatics analyses predicted miR-218-5p binding sites on the NPY coding region and NPY1R 3'UTR; the possibility that miR-218-5p regulates NPY signaling through these genes is currently being investigated.

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Sunday, May 26 - Omics: Analysis of Genomes, Proteomes, Transcriptomes, and Metabolomes in Comparative Endocrinology

THE RHYTHMS OF METABOLISM: TRANSLATIONAL CHRONOBIOLOGY DECOUPLES TRANSCRIPTION FROM METABOLISM

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Life has evolved in the context of rhythmic daily cycles rooted in the earth's rotation. Almost all life forms have thus adapted to circadian cycles of light/dark, temperature and nutrient availability (chronobiology). Recently, these rhythms have been demonstrated at the level of metabolism in humans and model systems. Characterization of these small molecule oscillations across multiplexed metabolite measurements is challenging due to the quantitative nature of data required, and the large number of timepoints needed to define diurnal rhythms. Here we demonstrate that these metabolic rhythms persist across a cell autonomous oscillating system (human U2OS cell lines), in mouse liver, and in mouse primary hepatocytes.

Targeted metabolomics was preformed using an ion-switching method using liquid-chromatography tandem mass spectrometry (LC-MS/MS) to analyze between 120-180 metabolites per specimen. Analysis of circadian oscillation was performed using JTK-CYCLE. Clock-dependence was assessed by si-RNA knockdown of clock components, BMAL1, CRY1, and CRY2.

In liver, ~50% of metabolites were circadian, with enrichment of nucleotide, amino acid, and methylation pathways. In U2 OS cells, 28% were circadian, including amino acids and NAD biosynthesis metabolites. Eighteen metabolites oscillated in both systems and a subset of these in primary hepatocytes. These 18 metabolites were enriched in methylation and amino acid pathways. BMAL1 knockdown diminished metabolite rhythms, while CRY1 or CRY2 perturbation generally shortened or lengthened rhythms, respectively. Surprisingly, CRY1 knockdown induced 8 hr rhythms in amino acid, methylation, and vitamin metabolites, decoupling metabolite from transcriptional rhythms, with potential impact on nutrient sensing in vivo.

These results provide the first comprehensive views of circadian liver and cell-autonomous metabolism across species and tissues. We are following up these results to understand potential interactions of the circadian clock with endocrine disrupting compounds at the level of metabolism.

TWENTY YEARS? OMICS, ESTROGENS, AND FISH.

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Comparative endocrinology and toxicology saw the emergence of omics at the turn of the century. Since these initial studies, significant advances have been made over the past 20 years; as a result, much has been learned about the complex nature of estrogen receptor signaling in fishes and exogenous estrogens in our environment. Vitellogenin, the egg yolk precursor protein, was identified as a central estrogen-responsive gene, establishing itself as the prime biomarker for estrogenic chemicals in our environment. Since then, many studies have identified additional estrogen responsive genes, developing a wealth of information on estrogen-mediated regulatory networks. There have been more than 50 studies that characterize molecular responses (i.e. omics) to estrogens in fish. Many of these studies investigate the endogenous ligand 17beta-estradiol, but also potent pharmaceuticals such as 17alpha-ethinylestradiol and estrogenic chemicals such as bisphenol A. Recently, an inter-genomics study from six different laboratories using fathead minnows identified common transcriptional networks associated with estrogens. Networks related to amino acid activation and protein folding were increased while networks related to blood clotting, complement activation, triglyceride storage, and xenobiotic metabolism were suppressed with estrogens, and these responses were also observed across multiple species of fish. Noteworthy was that more than ~85% of the gene networks were suppressed by estrogens. Based on these data, and those from the Comparative Toxicogenomics Database, an estrogen-responsive network was developed (including estrogen receptor alpha, transferrin, myeloid cell leukemia 1, insulin like growth factor 1, and methionine adenosyltransferase 2A among other genes). Thus, we continue to hone our knowledge of estrogen-responsive gene networks. What may be on the horizon for omics, estrogens, and fishes in the upcoming decade? These may include (1) Conceptual frameworks for incorporating estrogen-responsive networks into environmental monitoring programs; (2) In vitro assays (ToxCast) and computational data to predict estrogenic chemicals and vitellogenin in male fish; (3) Discovery of new estrogen receptor signalling pathways in fishes; and (4) Development of predictive models for adverse outcome pathways for estrogenic chemicals. As we look ahead, research into estrogens over the past two decades can serve as a template for the array of hormones and endocrine disruptors yet to be fully characterized or discovered.

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MINERALOCORTICOID RECEPTOR SIGNALLING IN ZEBRAFISH LARVAE

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The glucocorticoid receptor has often been considered the primary regulator of glucocorticoid driven metabolic changes during stress and early development in vertebrates. However, given that fish lack an endogenous mineralocorticoid, such as aldosterone, cortisol is also thought to be the primary ligand for the mineralocorticoid receptor (MR). Despite the persistence of MR in the vertebrate linage there is no known physiological role for MR in lower vertebrates. In order to elucidate the MR-specific molecular mechanisms, we generated ubiquitous MR knockout (MRKO) zebrafish (*Danio rerio*) using CRISPR/Cas9 mutagenesis. Using this knockout model, and utilizing a transcriptomics approach (RNA-Seq), we evaluated MR target gene transcription during early development. Wildtype and MRKO zebrafish larvae (3 days post fertilization) were treated with cortisol for 48 h and the MR-specific genes identified. Key metabolic genes were upregulated in response to cortisol signaling, which were abolished in the MRKO mutant, indicating these genes as molecular targets of this receptor. In particular genes related to lipid and cholesterol metabolism were enriched. For instance, the transcript abundance of *hmgcs1*, which encodes the first enzyme in the cholesterol biosynthesis pathway, was 3-fold higher in cortisol-treated WT larvae, and this was abolished in cortisol-treated MRKO larvae, suggesting a role for MR signaling in cholesterol biosynthesis. Overall, the larval transcriptome, and targeted gene studies, reveal for the first time a key role for MR signalling in the cortisol-driven metabolic changes during early development in zebrafish.

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METABOLIC PROFILING OF MALE GOLDFISH LIVER REVEALS PATTERNS OF ENERGY ALLOCATION IN SUPPORT OF GROWTH AND REPRODUCTION

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Reproduction and growth follow a clear seasonal pattern in many fish species involving changes in gonadal development, growth, and metabolism, regulated by hormones of brain-pituitary-peripheral axes. 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 energetic shift is particularly important in oviparous species, such as fish, because they require a significant metabolic energy investment for the gametogenic process both in male and female. Seasonal changes are the result of complex reciprocal control of growth, reproduction and metabolism, regulated by the hormones of brain-pituitary-peripheral axis.

The main objective of this study was to investigate changes in metabolic profile and energy allocation patterns at different stages of reproduction and growth. Emphasis is placed on changes in liver metabolic pathways resulting from treatments with key neurohormones, GnRH and GnIH, to energetically sustain the physiological processes related to growth and reproduction. Using a targeted metabolomics approach, we measured the concentrations of metabolites by LC-MS in liver samples of adult male goldfish. Acute treatments resulted in significant shift in the metabolic profile revealing distinct patterns of energy allocation in the reproductive and growth seasons. Moreover, during growth phase, both GnIH and GnRH were found to significantly stimulate proteins and nucleotides metabolism highlighting their importance in support of growth processes.

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.

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GENOMIC AND TRANSCRIPTOMIC CONSEQUENCES OF THYROID HORMONE SENSITIVITY EVOLUTION IN SALAMANDERS

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Thyroid hormone signaling is fundamental to vertebrate development and regulates major ontogenetic transitions. Even though patterns of thyroid hormone regulation and sensitivity vary extensively among species, few studies have tested how this relates to the evolution of developmental patterns across clades. Salamanders exhibit an assortment of life cycles that involve shifts in the timing of metamorphosis within and among species. The biphasic life cycles of salamanders include an aquatic larval stage that metamorphoses into a terrestrial form before adulthood. An alternative life cycle is larval form paedomorphosis, where salamanders forgo metamorphosis and retain their aquatic larval morphology and ecology into adulthood. Thyroid hormone can induce metamorphosis in some recently derived paedomorphic species, but lineages that transitioned to paedomorphosis tens of million of years ago are no longer responsive. This suggests that thyroid hormone has decoupled from metamorphosis in salamander lineages that continuously maintained a larval form paedomorphic life cycle. Our talk will report on comparative transcriptomic analyses of thyroid hormone sensitivity and molecular evolution across salamander species that exhibit paedomorphosis or metamorphosis. We will emphasize the consequences of thyroid hormone dysregulation on the evolution of genomic and transcriptomic patterns.

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FISHING FOR PHYSIOLOGY IN BIG DATA: A MACHINE LEARNING ROADMAP FROM TRANSCRIPTOME TO PHYSIOLOGY IN THE TILAPIA

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The field of endocrinology has recently undergone a transformation due to the emergence of big data modalities associated with "-omics" science. As these studies produce large amounts of robust data, traditional statistical methods of analysis (i.e. ANOVA, post-hoc corrections, and false discovery rate) may fall short of finding true biological meaning. Machine learning pattern recognition serves as an alternative approach for analyzing "-omics" data that can predict meaningful biological relationships from big datasets without being tethered to the limitations of traditional statistics. In the present study, we describe a method of coupling a transcriptome study and machine learning analysis to generate biological predictions of leptin actions on prolactin cell function in the Mozambique tilapia (*Oreochromis mossambicus*). Pituitary RNA sequence data was mapped to the tilapia genome to obtain total reads per gene using CLC Genomics then imported into the machine learning platform (WEKA) in order to reduce the dimensionality of the data. A two-step approach involving ranker and sequential minimal optimization algorithms was used to identify 972 genes predictive of leptin action on the tilapia pituitary. These genes were used for downstream pathway analysis (DAVID). Utilizing this approach, we identified a variety of leptin-responsive metabolic pathways. Leptin treated prolactin cells showed upregulated gene expression of the anaerobic glycolytic pathway and downregulation of the aerobic TCA cycle. The glycolytic activity of leptin has since been confirmed by orthogonal testing. These results suggest an important role for leptin in regulating cellular metabolism and oxygen consumption in fishes. These findings are relevant considering leptin is a well-established hypoxia responsive hormone in vertebrates; a role echoed in this study as gene ontology analysis revealed leptin affected hypoxia responsive pathways. Our work provides a roadmap from a transcriptome dataset to novel hypotheses and whole animal physiology, linkages established via machine learning. Importantly, the usefulness of this approach is not limited solely to this example of transcriptomic research, but may also have application with a variety of other big, "-omics" datasets, providing a powerful tool to aid discovery.

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INGESTIVE OR REPRODUCTIVE BEHAVIOR? HORMONE-NEUROPEPTIDE INTERACTIONS THAT ORCHESTRATE THE TRADEOFF

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During the process of natural selection, survival and reproductive success are the result of decisions about behavioral options, rather than the outcome of single behaviors that occur in isolation from other behaviors. Under some environmental conditions, ingestive behavior is preferred over reproductive behavior. For example, the decision to engage in reproductive behaviors can be adaptive, but reproduction is energetically costly, and therefore ingestive behaviors might be the best option when stored energy levels are low. Thus, *ad libitum*-fed female Syrian hamsters show ever-increasing levels of body weight and adiposity coupled with a strong preference for interacting with conspecific males on every day of the estrous cycle. After a period of mild food restriction, however, they switch their preference to food hoarding on three out of four days of the cycle and restrict their interest in males to the day of ovulation. In food-restricted females, the low preference for males is reversed by systemic treatment with the adipocyte hormone, leptin, and ovarian hormones, estradiol plus progesterone, and with intracerebral infusions of either RFamide-related peptide 3 (RFRP-3) or alpha-melanocyte-stimulating hormone (alpha-MSH). Cellular activation in RFRP-3 cells (but not in kisspeptin cells) is closely, positively associated with the duration of food restriction and levels of food hoarding and closely, negatively associated with the preference for spending time with males. These and other results from our laboratories elucidate a mechanism that fails to regulate body weight and food intake during *ad libitum* access to food, but rather, optimizes reproductive success in environments where energy availability fluctuates.

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NEUROENDOCRINE REGULATION OF FOOD HOARDING IN BIRDS

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Food hoarding behavior is the storage of food when availability is high for consuming later when resources are scarce. Evidence from mammals indicates a positive influence of ghrelin on its expression and a negative effect of leptin but little is known about mechanisms underlying hoarding in birds. We are testing the hypothesis that avian hoarding behavior evolved as a modification of the neuroendocrine systems that regulate energy balance in non-hoarding species. In the coal tit (*Periparus ater*), systemic injection of mouse leptin or chicken ghrelin significantly decreased hoarding activity and mass gain (a proxy of food intake) over a 2-hour post-injection period. This is consistent with the inhibitory effect of both hormones on food intake in non-hoarding avian species and suggests an extension of their regulatory effects to hoarding. To investigate the interaction of central and peripheral nutritional signaling mechanisms, we have used *in situ* hybridization to localize agouti-related protein, neuropeptide Y and pro-opiomelanocortin gene expression in the arcuate nucleus of the hypothalamus of the coal tit and a related, non-hoarding, species the great tit (*Parus major*). We are currently quantifying the effect of acute food deprivation on arcuate nucleus gene expression between these species. In addition, an experiment is underway to quantify and compare the effect of acute food deprivation on hypothalamic gene expression, and that of gut peptides, in the coal tit and another, more similarly-sized, non-hoarding species, the blue tit (*Cyanistes caeruleus*). This will help us to elucidate where differences in nutritional signaling mechanisms between hoarding and non-hoarding species may lie.

THE ENDOCRINE REGULATION OF FEEDING IN SELECTED FRESHWATER TELEOST FISH

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In fish, food intake is ultimately regulated by feeding centers of the brain, which receive and process information from endocrine signals from both brain and peripheral tissues such as the gastrointestinal tract. These endocrine signals interact with each other and induce (orexigenic) or inhibit (anorexigenic) food intake. Feeding is also largely influenced by feeding habits, intrinsic factors such as levels of energy stores and activity, as well as environmental cues such as temperature, pH and light. This review provides an overview of hormones known to regulate food intake in selected freshwater teleost fish, and how feeding habits, fasting, exercise and environmental changes affect these endocrine networks.

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EFFECTS OF CHROMATIC LIGHT ON SOMATIC GROWTH AND ENDOCRINE FUNCTIONS OF FLATFISHES

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In teleosts, skin color plays an important role in social events, including predator avoidance and prey capture. Melanin-concentrating hormone (MCH), a hypothalamic hormone, and melanophore-stimulating hormone (MSH), a pituitary hormone, are major factors regulating body color through opposing functions. Pigments such as melanosomes in chromatophores are aggregated by MCH and dispersed by MSH. In mammals, these peptides have been shown to regulate food intake by opposing functions—MCH and MSH enhance and suppress food intake, respectively. Between these hormones, we have shown that MCH is associated with increased food intake in the barfin flounder (Verasper moseri). The fact that the production of MCH is enhanced under a white background led us to propose the hypothesis that increased MCH under a white background also stimulates food intake. This phenomenon has been previously described in several studies that recorded improved growth under a white background than under a black background in indoor rearing systems. We further investigated the effects of chromatic light with peaks at different wavelengths because compared to mammals, fishes possess well-developed color vision. Among blue, green, and red LED lights, green light had the highest growth-promoting effects on barfin flounder. The growth-promoting effects of green light have also been demonstrated in other flatfishes such as spotted halibut (V. variegatus) and Japanese flounder (Paralichthys olivaceus). The studies on spotted halibut showed that among the several hypothalamic hormones, the expression of MCH genes was responsive to green light in an increasing manner. The expression of the somatolactin gene decreased under green light among pituitary hormones. The hormones encoded on these genes may function as interfaces linking photic conditions, such as green light, to physiological/morphological processes of the fishes.

BRAIN-DERIVED NEUROTROPHOC FACTOR (BDNF) IS A MEAL-RESPONSIVE OREXIGEN IN ZEBRAFISH

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Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors. While BDNF is mostly abundant in the brain and exerts its neurotrophic actions locally, several studies in mammals have described the peripheral presence of BDNF and its involvement in a wide variety of physiological functions, including food intake regulation, glucose metabolism, cardiovascular functions and reproduction, among others. In fish, BDNF has been widely described in the brain of several species, but very little is known about its peripheral distribution and functions. We hypothesized that BDNF is abundant in peripheral tissues and modulates feeding in fish. Our objectives were to determine the peripheral tissue distribution of BNDF in relation to nutritional/meal status, and assess whether it has any role in food intake regulation in zebrafish (*Danio rerio*). BDNF was found to be widely expressed within the zebrafish, with the highest levels of mRNA detected in the brain, eye, liver, gut and spleen. Western blot and/or immunohistochemistry techniques confirmed the presence of the protein in these tissues. BDNF receptors, tropomyosin receptor kinase B (TrkB) and p75 neurotrophin receptors A and B (p75NTRA and p75NTRB), were also observed to be widely distributed within zebrafish tissues. Intraperitoneal administration of BDNF increased food intake at 1, 2 and 6 h post-injection. It also caused an upregulation of *npy*, *agrp* and *orexin* mRNAs, and a reduction in *nucb2* and *cck* in the brain, and an induction of *preproghrelin* in the gut. A period of 7-day fasting resulted in an increase in *bdnf* and *p75ntrb* mRNAs in the gut, while a reduction in the expression of *bdnf*, *trkb*, *p75ntra* and *p75ntrb* mRNAs was observed in the brain and liver. Additionally, expression of *bdnf* and its receptors was found to rise preprandially and decrease after a meal in gut and liver, in accordance with the orexigenic role of the peptide. No major periprandial variations were detected in the brain. Together, these results describe for the first time a role for BDNF in feeding regulation in fish, and demonstrate that the nutritional status is an important regulator of the BDNF system. The wide presence of BDNF and its receptors within the zebrafish tissues points to a multifunctional nature of the peptide.

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GOLDFISH (CARASSIUS AURATUS) GUT MICROBIOTA COMPOSITION AND THE EXPRESSION OF GENES RELATED TO APPETITE AND DIGESTION

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The gut microbiota refers to the large and diverse microbial community in the gastrointestinal (GI) tract. The communication between gut and brain is made through the meal-induced secretion of gut peptides, such as cholecystokinin (CCK), which send signals to the brain through either neural or endocrine mechanisms. The gut microbiota can thus influence this gut–brain communication via alterations in the absorptive and secretory capacity of the intestinal epithelial cells. The objectives of this study were to (1) analyze the composition of the goldfish (Carassius auratus) microbiota in various regions of the gut including the foregut, midgut, and hindgut; (2) examine the effects of probiotic administration and fed/fasted conditions on microbiota and the expression of genes related to appetite/digestion [e.g. cholecystokinin (CCK), glucagon-like peptide (GLP), peptide YY (PYY)]. The 16S rRNA V6-V8 region was targeted for microbiota analysis and further analyzed in Geneious and Qiime1. Small variations in microbiota composition were found among gut regions. Gene expression studies were analyzed in CFX Maestro and Prism8. The results indicate alterations of the gut microbiota, through fasting or the administration of probiotics, might regulate the central and peripheral expression of genes related to appetite and digestion in goldfish.

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BRAIN-DERIVED NEUROTROPHOC FACTOR (BDNF) IS A MEAL-RESPONSIVE OREXIGEN IN ZEBRAFISH

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DISCOVERY AND FUNCTION OF THE TENEURINS AND THEIR INTERACTION WITH LATROPHILINS IN VERTEBRATES: A PHYLOGENETICALLY ANCIENT MECHANISM OF RECEPTOR-LIGAND INTERACTIONS IN THE CENTRAL NERVOUS SYSTEM.

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The teneurins were initially discovered in 1994 independently by the laboratories of Prof. Ruth Chiquet-Ehrismann in Switzerland and by Prof. Ron Wides in Israel. Complex type-II proteins, the teneurins were established to be the result of a lateral gene transfer from prokaryotes to a single-celled eukaryotic choanoflagellate ancestor of the metazoans. The teneurins, are present in most metazoans and interact with their putative cognate receptor, latrophilins. Highly expressed in the central nervous system (CNS), the teneurin-latrophilin system plays a significant role in intracellular adhesion, synaptic organization, axonal pathfinding and CNS functional maintenance. The teneurins possess a peptide-like sequence (teneurin C terminal associated peptide, TCAP) at their distal extracellular tip that has been implicated in the regulation of neuronal energy metabolism, stress and behaviour in mammals. Together, these studies indicate that the teneurin-latrophilin system is an ancient intracellular and trans-synaptic mechanism that regulates intercellular activity.

INCREASED METABOLIC RATE BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-3: A COMPARATIVE ANALYSIS ACROSS ZEBRAFISH LIFE STAGES.

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Exercise rewards its host with improved skeletal muscle health and increased metabolism, which improves overall energy regulation. Recent studies have shown teneurin C-terminal associated peptide (TCAP)-1, a bioactive peptide, increases glucose uptake in skeletal muscle both *in vitro* and *in vivo* in rodent models. Here we investigate the role of TCAP-3, a closely-related paralogue, as a novel activator of muscle metabolism in zebrafish. To assess metabolic rate, oxygen consumption was measured in larval and adult zebrafish using two metabolic assays: Loligo systems respirometry chambers and resazurin conversion. TCAP-3 treatment increased maximum oxygen consumption rates and cumulative $NADH_2$ production in both larvae and adult fishes. These data demonstrate the conserved function of TCAPs in activating oxygen consumption across all life stages in zebrafish. Thus, this project will provide insights into the role of TCAP-3 as a muscle metabolism regulator. Future work will evaluate the role TCAP may play in preventing metabolism-associated muscle diseases, observed in diabetes, cancer, and aging.

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CORTICOTROPIN-RELEASING FACTOR (CRF) SIGNALING IS ANTAGONIZED BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP-1): INSIGHTS INTO THE INTERACTION OF TCAP-1 AND CRF IN NEURONS

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Teneurin C-terminal associated peptide (TCAP)-1 is a member of an evolutionarily ancient family of neuropeptides that regulates cellular energy production and protects against organismal stress. TCAP-1 acts antagonistically to corticotrophin releasing factor (CRF) in both invertebrates and mammals; however, the intracellular signaling pathways and downstream targets of this interaction are unresolved. An essential component of neuronal communication involves calcium signaling. Calcium cascades have important downstream effects on both neurotransmission and cellular energy metabolism. This suggests that the TCAP-mediated suppression of CRF actions in neurons may result from modulation of CRF-induced calcium signaling. Therefore, the aim of this study was to further investigate the physiological role of TCAP-1 during CRF-associated stress in neurons to assess if the anxiolytic effects of TCAP-1 are the result of the suppression of CRF-activated calcium signaling. We show that TCAP-1 prevents CRF-induced changes in intracellular calcium and that this is associated with modulation of mitochondrial energetics Together, these data indicate that the anxiolytic effects of TCAP-1 result from inhibition of CRF-induced calcium increases and may also result from stimulation of mitochondrial energy metabolism.

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USING COMPARATIVE MODELS OF MUSCULAR DYSTROPHY TO ASSESS TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-1 AS A NOVEL THERAPEUTIC APPROACH.

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Teneurin C-terminal associated peptide (TCAP)-1 (also called TENM1) is a bioactive peptide that has been shown to enhance overall muscle function and metabolism in wild-type Sprague-Dawley rats. TCAP-1 treatment increases glucose uptake into muscle myofibers with a concomitant decrease in serum glucose. In addition, tibialis anterior muscle function assessed via *in vivo* electrical stimulation revealed that TCAP-1-treated animals had higher peak twitch force production and more efficient calcium handling. Collectively, this data indicates that TCAP-1 is a potent regulator of muscle function and metabolism.

As TCAP-1 has demonstrated its capacity to modulate some of these critical pathways in muscle, we aim to test the therapeutic efficacy of TCAP-1 in Duchenne muscular dystrophy (DMD). DMD is an X-linked genetic disorder that results in the progressive degeneration of muscle due to lack of functional dystrophin. Moreover, the loss of muscle integrity results in impaired glucose metabolism, aberrant calcium regulation and increased fibrosis and inflammation. We hypothesized that the observed muscle functions of TCAP-1 in a dystrophic model may be beneficial towards ameliorating the muscle symptoms of the disease.

In order to best assess our aims, we utilized comparative models of the muscular dystrophy, specifically the DBA2J-*mdx* (D2-*mdx*) mouse model and the *sapje dmd*^{ta222a} zebrafish model. Both of these animal models have a mutation in the dystrophin gene that recapitulates the human muscle disease pathology progression. Our studies will determine whether or not this bioactive peptide will ameliorate dystrophic symptoms with the potential to be advanced towards eventual clinical trials for DMD.

DISTRIBUTION OF THE NOVEL REPRODUCTIVE PEPTIDE SECRETONEURIN IN THE BRAIN AND PITUITARY OF THE ZEBRAFISH

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Secretoneurin (SN) is a 31-34 amino acid neuropeptide, derived from the proteolytic processing of the precursor protein secretogranin-II (Scg2). Teleosts have two Scg2 proteins named Scg2a and Scg2b that are processed into SNa and SNb, respectively. We have previously demonstrated that exogenous SNa stimulates the release of luteinizing hormone (LH) *in vivo* and *in vitro* in goldfish, while mutation of *scg2a* and *scg2b* genes leads to decreased *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. SNa-immunorectivity (ir) was observed in neuronal cell bodies in the ventral telencephalon, preoptic area (POA) and hypothalamus in female brain. Neuronal fibers staining for SNa projecting from the magnocellular POA passed through the pituitary stalk and terminated largely in the neurointermediate lobe (NIL), The SNa-ir fibers were less abundant but clearly present in the pars distalis. Moreover, SNa colocalized with isotocin in cell bodies in the POA and fibers in the NIL. Using the *lhb*-RFP transgenic zebrafish line, we observed SNa-ir near LH-secreting cells but not in them. Counterstaining nuclei with DAPI revealed that some endocrine cells within the NIL and rostral pars distalis also expressed SNa-ir, but their identity is currently under investigation. *In situ* hybridization confirmed that magnocellular neurons and cells in the NIL are sites for Scg2a/SNa production. These data indicate that SNa or related peptides produced from Scg2a may affect gonadotrophs through neuroendocrine and paracrine pathways. These observations support our hypothesis that Scg2a/SNa are involved in vertebrate reproductive processes.

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FUNCTIONAL ANALYSIS OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN THE ZEBRAFISH – A GENETIC APPROACH WITH GENOME EDITING TECHNOLOGY

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As in other vertebrates, the reproductive system of teleosts is also governed by the hypothalamic-pituitary-gonadal (HPG) axis, which involves gonadotropin-releasing hormone (GnRH) from the hypothalamus, gonadotropins (FSH and LH) from the pituitary and steroidal and non-steroidal hormones from the gonads. In addition, various locally-produced factors work at different levels of the HPG axis via autocrine and paracrine manners, in particular those in the gonads. In the past several decades, the reproductive axis has been extensively studied in different fish species, and most key molecules that work in the axis have been identified and characterized. However, compared with our knowledge in mammalian models including humans, our understanding of the functional importance of these molecules in fish reproduction is rather limited, partly because of the lack of genetic approaches. This situation has changed in recent years with the availability of the new generation of gene editing technologies in fish species, in particular TALEN and CRISPR/Cas9. This review will discuss some key discoveries with these technologies concerning the HPG axis in the zebrafish model, with particular emphasis on pituitary gonadotropins and local ovarian growth factors.

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STUDIES ON MOLECULAR ADAPTION OF HORMONAL PEPTIDES FROM ANTARCTIC FISHES USING THE CRISPR-CAS9 TECHNOLOGY IN MODEL FISHES

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Many fishes are endemic and adapted to cold and freezing polar oceans. In recent years, whole genome sequencing has completed in several species inhabiting the polar oceans, including the Antarctic toothfish, *Dissostichus mwasoni*, an icefish *Chaenocephalus aceratus*, and the black rock cod, *Notothenia coriiceps*. The whole genome sequencing of these fishes yielded arrays of gene duplication and diversification events which may related to environmental adaptation. From the Antarctic toothfish genome, we identified multiple hepcidin peptides that are highly divergent from the original 8-cysteine containing peptides to only 4 cysteine residues. Being a sole hormone in iron regulation, how the duplicated and diversified hepcidin isoforms in the Antarctic fishes related to physiological adaption to the polar ocean is an interesting question to address. Besides the hepcidins, we also found a specific form of leptin gene from the Antarctic toothfish – which we hypothesized to involve in the specific strong lipid accumulation in this species, given the function of this peptide in lipid metabolism in mammals. We thus generated hepcidin/leptin mutants and transgenic zebrafish and medaka using CRSIPR-cas9 in which their native hepcidin and leptin genes are mutated or replaced by the ones from the Antarctic fishes. These mutant and gene-replaced fishes provided good models enabled us to study adaptive features of these hormonal peptides in coping with the extreme environmental stresses.

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THYROID HORMONE SIGNALING IS NOT NECESSARY NOR SUFFICIENT FOR FROG METAMORPHOSIS

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Gene disruption technology has strikingly revised our understanding of the roles of the two main endocrine players in the hormonal control of development in frogs. Whereas thyroid hormone (TH) is required for metamorphosis, TH receptors (TRs) do not appear to be required. Null mutations in TRα or TRβ allow metamorphosis to proceed to completion, albeit with discernable changes in timing, and result in fertile adults. Methimazole treatment to block TH synthesis in TRα knockout animals still allowed full growth and development of limbs, even though the limbs likely received little to no TH signaling. Nevertheless, because compensation by one TR for loss of the other may explain successful metamorphosis, double TRα/TRβ mutant animals will be required to comprehensively address the role of TH signaling in metamorphosis. The other main endocrine player regulating metamorphosis is corticosteroid hormones (CSs), which synergize with TH to accelerate metamorphic progression but have no known developmental role independent of TH. Mutations in adrenocorticotropic hormone (required to stimulate CS synthesis) or glucocorticoid receptor (one of two CS receptor) result in death during the climax of metamorphosis. The cause of death may be related to impaired lung function and likely reflects a TH-independent action of CSs. The dual results that TR knockout animals complete metamorphosis and that GR knockout animals die during metamorphosis depart from expectations based on previous endocrine experiments but are consistent with analogous studies on the developmental role of hormone receptors in mutant mice.

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ADVANCEMENT IN GENE EDITING AND THEIR APPLICATION IN TILAPIA SEX DETERMINATION

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Loss-of-function technologies, such as ZFN-and TALEN- and CRISPR/Cas9-mediated gene editing, are widely used to investigate gene function and its physiological significance in comparative endocrinology. In vertebrates, sex is determined either genetically (genetic sex determination) or environmentally (environmental sex determination), or a combination of both. In non-eutherian vertebrates including fish, environmental factors, especially estrogens, play a critical role in sex determination. Estrogen is produced via the conversion of androgen by aromatase, which is encoded by *cyp19a1a/b* in fish. The availability of monosex populations and genome editing technique in Nile tilapia, one of the most important species in global aquaculture, made it an excellent model for investigation of sex determination and sexual plasticity in teleosts. In tilapia, vast transcriptome analysis of XX and XY gonads at early developmental stages enabled us to identify sex-specific and sex-biased genes. Here, we report that disruption of a selected gene or two genes simultaneously in tilapia through TALEN and CRISPR/Cas9 system was achieved with high efficiency. Non-coding sequences, such as microRNA, lincRNA and gene promoter, were also successfully deleted by dual gRNAs system. Mutations induced by TALEN and CRISPR/Cas9 were efficiently transmitted through the germline which enabled us to establish mutant lines. In addition, obvious phenotypes, partial or even complete sex reversal, were observed in G0, F1 and F2 generations after mutation of genes related to sex determination and differentiation. Homozygous mutation of *cyp19a1a* in XX fish resulted in female to male sex reversal, which could be rescued by exogenous E2 treatment. In contrast, homozygous mutation of *cyp19a1b* in XX fish resulted in no defects in ovarian development, indicating its redundancy for ovarian differentiation. In addition, knockout of male pathway genes *amhy*, *dmrt1* and *gsdf* or overexpression of female pathway gene*foxl2* could up-regulate *cyp19a1a* expression, which in turn, increases estrogen level and finally leads to ovarian differentiation. Conversely, knockout of female pathway gene*foxl2*or overexpression of male pathway genes *amhy*, *dmrt1* and *gsdf* could down-regulate *cyp19a1a* expression and decrease estrogen level so as to promote testicular differentiation. Loss of function analysis of estrogen receptors in XX fish revealed that *esr2a* and *esr2b*, not *esr1*, are critical for mediating E2 signal in sex determination and sex differentiation. Taken together, these results strongly emphasize the critical roles of gene editing in investigation of tilapia sex determination and differentiation.

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OVARIAN DEVELOPMENT IN ZEBRAFISH REQUIRES ADAMTS9

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Adamts9 (A disintegrin and metalloprotease with thrombospondin type-1 motif, member 9) is expressed during the pre-ovulatory period by somatic cells that surround oocytes as a response to LH and/or progesterone signaling in zebrafish, mice and humans. However, the function of Adamts9 during ovulation has not been determined due to embryonic lethality of mouse and *Drosophila* knockouts. To identify the role of Adamts9 during ovulation we generated knockout (adamts9$^{-/-}$) zebrafish using CRISPR/Cas9 and characterized the effects of the mutation. From 1047 fish generated by crossing adamts9$^{+/-}$ pairs, we found significantly fewer adamts9$^{-/-}$ fish (4%) than predicted by mendelian ratios (25%). In the adamts9$^{-/-}$ knockout there was a significant male bias (82%). Only 3 female adamts9$^{-/-}$ were identified (7%), and they had smaller ovaries with fewer maturing oocytes compared to wildtype (wt) fish of similar size and age. Astoundingly, the remaining mutants (11%) did not appear to have normal testis or ovaries. Instead there was a transparent, cyst-like organ that filled the abdominal cavity. Histological examination revealed seminiferous tubules and various spermatocytes, including mature spermatozoa, on the periphery of these transparent cysts. No female or female like knockouts were observed to release eggs, and no ovulated oocytes were found in histological sections. To our knowledge, this is the first report of an established Adamts9 knockout vertebrate line and the first description of how gonadal sex and somatic structures are affected- highlighting the importance of Adamts9 during gonadal development and the value of zebrafish as a model organism.

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DELINEATE THE REGULATORY NETWORK FOR BMP SIGNALING DURING EMBRYONIC DEVELOPMENT

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The BMPs are important cytokines belonging to the transforming growth factor (TGF-β) superfamily. It is essential for the specification of the primary body axes, as well as for the formation and further differentiation of ectoderm, mesoderm, and endoderm during early embryonic development. The BMP signaling can be activated by binding of BMP ligands to a co-receptor in complex with dimers of type I and type II receptors. The binding, in turn, induces the phosphorylation of Smad1/5/8. Activated Smad1/5/8 binds to Smad4 and translocates to the nucleus, where the complexes interact with other transcription factors including to activate the transcription of target genes. We found the *Ets1*, a proto-oncogene, can down-regulate of BMP signalling revealed by suppression of the BMP-responsive gene *id3*. Ets1 is expressed in the neural crest (NC) and is essential for NC migration. Conversely, overexpression of id3 can partially rescue the phenotype induced by ectopic Ets1. Mechanistically, we found that Ets1 binds to the *id3* promoter as well as Histone Deacetylase 1 (HDAC1), suggesting that Ets1 recruits HDAC1 to the promoter of id3, thereby inducing Histone deacetylation of the id3 promoter. Thus, our studies indicate that Ets1 regulates NC formation through attenuating BMP signaling epigenetically. We further generated *ets1-/- kno*ckout *X. tropicalis* line and identified ets1 target genes using RNA-seq. Functional analysis of these genes is on-going. Our studies shed light on a new epigenetic mechanism on regulating of outputs of BMP signalling.

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Monday, May 27 - Hormonal Control of Germinal Stem Cell Development and Gametogenesis

MODELS TO STUDY CELL-CELL INTERACTIONS IN THE MAMMALIAN TESTIS

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Germline stem cells (GSCs) in the testis are the foundation of spermatogenesis and male fertility. Stem cell function is tightly controlled by the interactions of stem cells with their surrounding microenvironment, the stem cell niche. While aspects of GSC-niche interactions have been described in mouse models, much less is known in higher mammalian species including humans. Studying germ cell-niche interactions in the testis requires accessible model systems. To address this need, we developed two complementary systems: grafting of testis tissue or cells ectopically into mouse hosts, and organotypic testicular organoids. Testis xenografts recapitulate complete spermatogenesis from a wide variety of mammalian donor species. Organoids bear resemblance to the primary tissue, reflect the complex multi-cellular interactions and signaling found *in vivo*, and can serve as an intermediary between animal models and conventional culture models. We employed a microwell centrifugal forced aggregation approach to establish multicellular testicular organoids from pre-pubertal porcine, mouse, macaque and human testis cells. The organoids consist of germ cells, Sertoli cells, Leydig cells, peritubular myoid cells and endothelial cells with cell associations similar to those *in vivo*, including a distinct seminiferous epithelium and interstitial compartment separated by a basement membrane. Using these models systems, we investigated the effects of exposure to phthalate esters on somatic cell and germ cell function, and elucidated the role of primary cilia on testicular somatic cells in tubular morphogenesis and niche formation. Testis xenografts and testicular organoids recapitulate the three-dimensional organization of the mammalian testis and provide *in vivo* and *in vitro* platforms for studying cellular interactions in testicular development and function, and for screening drug toxicity in a cellular context representative of the testis *in vivo*.

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CORTISOL AND THYROID HORMONES: “NEW” PLAYERS OF ZEBRAFISH SPERMATOGENESIS

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It has been increasingly clear that metabolic hormones such as cortisol and thyroid hormones (THs, T3 and T4) can also influence fish reproduction either directly by affecting hormones of the hipotalamic-pituitary-gonadal axis or indirectly by different mechanisms. Although the effects of cortisol in reproduction were extensively studied, directs effects at gonadal level has received less attention in this process. Regarding THs, it has been shown a positive correlation between THs and fish reproductive status, where THs are frequently associated with testicular development, growth and maturation. The aim of this work was to study the effects of cortisol and THs on zebrafish spermatogenesis using an ex vivo organ culture. We evaluated germ cell proliferation activity, quantification of germ cysts, androgen release by ELISA and the mRNA expression of testicular genes. Cortisol has a positive role in zebrafish spermatogenesis by stimulating germ cells differentiation and increase of free spermatozoa in the lumen, in an androgen independent manner. T3 affected germ cells related genes, while T4 did not alter these evaluated genes. Moreover, in vivo studies showed that methimazole increased the area occupied by the undifferentiated type A spermatogonia and type B spermatogonia, while spermatozoa and the lumen area were reduced significantly, which suggests that hypothyroidism-induced impairs meiosis and modulates the mRNA expression of Sertoli and Leydig cells genes. In conclusion, this work showed that metabolic hormones, such as cortisol and THs have a direct and positive effect in zebrafish spermatogenesis.

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PARACRINE CONTROL OF GERMINAL STEM CELL DEVELOPMENT AND SPERMATOGENESIS BY GNIH IN ZEBRAFISH (*DANIO RERIO*).

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Gonadotropin-inhibitory hormone (GnIH) is known to plays a role in the regulation of reproduction in vertebrates by influencing pituitary gonadotropin hormone release and synthesis. While the endocrine actions of GnIH have been identified in a number of species, its paracrine effects in the control of germinal stem cell development and spermatogenesis are less defined. We have used the *ex-vivo* culture of zebrafish testis to investigate the role of gonadal GnIH in the regulation of testicular development and spermatogenesis. Our results demonstrate direct action of GnIH on basal and gonadotropin (LH and FSH)-induced spermatogenesis, using FACScan cell cycle analysis and morphometric histological analysis. Our results demonstrate that GnIH hormone significantly alter the number of germinal stem cells and production of postmeiotic haploid cells after seven days' culture, *in vitro*. We have also extended our study to analyze further the changes in DNA synthesis and germinal stem cell development using BrdU (5-Bromo-2´-Deoxyuridine) labeling. The results demonstrate both stimulatory and inhibitory effects of GnIH on the proliferation of germinal stem cells and spermatogenesis in adult zebrafish, depending on the stage of development. These results in addition to our previous findings show that GnIH produced locally in the testis is a critical component of the complex multifactorial system that regulate testicular development and function in adult zebrafish.

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GONADAL TRANSCRIPTOME OF HYBRIDS DERIVED FROM CLOSELY RELATED SPECIES WITH THE SAME SEX DETERMINING GENE: O. *latipes* and O. *curvinotus.*

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In vertebrates, fish is the group with the highest diversity of sex determining genes. This variability is observed not only in species from distant branches of the phylogeny, but also within the same genus. The genus *Oryzias* is a well-studied paradigm for this phenomenon to understand how the sex determination genetic network evolved in closely related species. *O. latipes* and *O. curvinotus* share not only the same sex chromosome system, XX/XY, but also the same sex determining gene, *dmrt1bY*. However, crossings between both species causes XY female sex reversal (SR) and gonadal abnormalities. We analyzed the genome of the parental species and the transcriptome of the adult gonads of offspring from *O. latipes* and *O. curvinotus* crosses (H:Y*lat* when the Y comes from *O. latipes* and H:Y*cur* when the Y comes from *O. curvinotus*). Histological analysis of the gonads showed similar morphology between H:Y*lat* and H:Y*cur* XX females and XY females SR. All exhibited a significant decrease of pre-vitellogenic and vitellogenic oocytes compared to parental fish. Males of both hybrids were sterile showing empty seminiferous tubules. Transcriptome analysis of genes involved in gonadal sex differentiation and gametogenesis showed that XY female hybrids SR from both interspecific breedings (H:Y*lat* and H:Y*cur*) have a strikingly similar expression pattern as XX females. On the other hand, the expression pattern between H:Y*lat* and H:Y*cur* sterile males was different. Genes involved in germ cell specification (*vasa*), germ cell differentiation (*daz*l) and meiosis (*sycp3*) were highly expressed in both hybrids when compared to expression in testis of the parentals.. Interestingly, normalized read counts for *dmrt1bY* showed higher values in H:Y*lat* males and even in XY females SR. One explanation for this higher expression of *dmrt1bY* in XY females SR might be attributed to the different regulatory composition of *dmrt1bY* promoter in both parental genomes. In conclusion, gonadal structure and transcriptomes of hybrids are different from the parentals. The expression of *dmrt1bY* of one species in the different background of another species interferes with the correct development of the gonad.

ROLE OF THYROID HORMONES IN ZEBRAFISH SPERMATOGENESIS

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Important physiological functions of vertebrates are controlled by thyroid hormones (THs) (T4/T3), such as metabolism and reproduction. In this work, we have evaluated the effects of 1mM methimazole-induced hypothyroidism on zebrafish spermatogenesis. We showed by morphometric analysis that hypothyroidism increased the proportion of type A undifferentiated spermatogonia (A_{und*}) and type B spermatogonia. However, the proportion of germ cells developing from type B into meiosis was affected. There was also a reduction of spermatozoa number. Interestingly, the amount of abnormal cells in the germ cell cysts, increased significantly when compared to control. On the other hand, the co-treatment with 100nM T4 partially reversed the effects of hypothyroidism, and the proportion of A_{und} and type B spermatogonia remain stable compared to the control. Moreover, the proportion of spermatocytes and spermatids increased when compared to the hypothyroid, which reflected the increased number of spermatozoa. Also, the number of abnormal cells decreased. These results show the effects of THs on different stages of spermatogenesis. Interestingly, the androgen (11-KT) plasma levels decreased in the methimazole group. In vitro testicular androgen capacity was affected in the hypothyroid-induced males. In the brain and pituitary, the *gnih* and *fshb* mRNA levels increased in the methimazole group. Zebrafish injected with 250ng of T3 showed reduced mRNA levels of *lhb*. Our results reveal that THs exert regulatory roles on spermatogenesis by acting locally in the testis and the different levels of hypothalamic-pituitary axis.

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ACCLIMATION OF FISH TO DYNAMICALLY CHANGING SALINITIES: INSIGHTS FROM THE EURYHALINE MOZAMBIQUE TILAPIA

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The Mozambique tilapia, *Oreochromis mossambicus*, is a teleost fish native to estuarine waters that vary in salinity between fresh water (FW) and seawater (SW). Nevertheless, most studies on the osmoregulatory capacity of euryhaline teleosts to date have focused on experimental paradigms consisting of steady-state salinity comparisons or one-way transfers, such as between FW and SW and vice-versa. Salinity acclimation involves the concerted regulation of pituitary hormones involved in osmoregulation and their targets, which include effectors of ion and water transport. Recently, we simulated a tidal regime (TR), where tank salinity is switched between FW and SW every 6 h, to experimentally test osmoregulatory responses of fish challenged with dynamically-changing salinities. While one-way salinity transfers of fish from SW to FW and FW to SW trigger the activation of hormones and genes that promote ion uptake and extrusion, respectively, the regulation of these parameters in fish reared in TR most closely resembles that of SW acclimated fish. Our results indicate that tilapia in TR maintain a narrow range of plasma osmolality, while dynamically up- and downregulating prolactin receptors (*prlr1* and *prlr2*) in gill and maintaining the pituitary expression of prolactin (*prl*), and branchial expression of Na^+/Cl^- and $Na^+/K^+/2Cl^-$ co-transporters (*ncc* and *nkcc*), Na^+/K^+-ATPase (*nka*α1a and *nka*α1b), the Cl^- channel, cystic fibrosis transmembrane conductance regulator (*cftr*), and the water channel aquaporin 3 (*aqp3*) relatively stable across FW and SW phases of the tidal cycle. Moreover, adult fish are able to acclimate from steady-state salinities to TR and the osmoregulatory profile of fish transferred to TR is similar to that of fish reared in TR from fry. Together these results demonstrate the remarkable capacity of Mozambique tilapia to acclimate to dynamically-changing salinities at various life stages.

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RELATIONSHIPS BETWEEN OSMOREGULATION AND IMMUNITY IN AMPHIBIANS

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Anthropogenic alterations in salinity of freshwater environments due to increasing drought, mining or fossil fuel extraction activities, road salt run-off, agriculture or sea-level rise, is affecting amphibian populations across the landscape, increasing the need to understand osmoregulation physiology and the physiological trade-offs exist that affect fitness. Our research focuses on the effects of elevated salinity in wood frogs, a species whose range extends from southeastern US to the Arctic Circle. In the Northeast US, wood frog larvae in ponds with salinity 2-4% of seawater are more likely to experience die-offs. Salinity within this range does not alter glucocorticoid production in larvae, although it slows growth or development rates. But when larvae developing in elevated salinity incur ranavirus infections, mount a magnified glucocorticoid response that is associated with increased viral replication and death at lower doses of ranavirus than those reared in freshwater. These results suggest a trade-off between osmoregulation and immunity and demonstrate synergistic effects of these stressors. Yet, in the Athabasca region, wood frog larvae experiencing similar elevations in salinity and increased primary productivity grow faster, show elevated glucocorticoids, and endure ranavirus infections at titers above those known to cause mortality. These data suggest that trade-offs between osmoregulation and immunity are condition dependent. Future research is needed to elucidate the pathways through which endocrine osmoregulators interact with energy stores and immune function across life history stages, species, and host-pathogen systems to understand the fitness consequences of the salinization of amphibian environments.

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EVIDENCE FOR A ROLE OF THYROID STIMULATING HORMONE, DEIODINASE AND THYROID HORMONE IN THE PHOTOPERIOD-DRIVEN SEASONAL CLOCK OF FISH

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Seasonal timing is important for many critical life history events of vertebrates, and photoperiod is often used as a reliable seasonal cue. In mammals and birds it has been established that a photoperiod driven seasonal clock resides in the brain and pituitary, and is driven by increased levels of thyroid stimulating hormone (TSH) and deiodinase (dio2) which leads to local increases in triiodothyronine. In order to determine if a similar mechanism occurs in fish, we conducted photoperiod manipulations in anadromous (migratory) Atlantic salmon that use photoperiod to time the development of salinity tolerance which accompanies downstream migration in spring. Exposure of juvenile Atlantic salmon to increased daylength (LD16:8) resulted in significant increases in *tsh* mRNA levels in the pituitary within 10 days that were sustained for at least 20 days. Increased daylength also resulted in significant increases in *dio2* mRNA levels after 20 days. Increased gill *nka*α1b mRNA levels that normally accompany increased salinity tolerance of salmon were also seen after 20 days of increased daylength. The results provide evidence for the presence of a photoperiod-driven seasonal clock in fish that involves TSH, dio2 and production of triiodothyroinine.

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DIVERGENT RECEPTORS FOR GROWTH HORMONE AND PROLACTIN DISCOVERED IN AGNATHANS: GENE SEQUENCES AND TISSUE EXPRESSION PATTERNS AT DIFFERENT LIFE STAGES OF SEA LAMPREY

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It was thought that Gnathostome receptors for growth hormone (GHR) and prolactin (PRLR) arose by local duplication of an Agnathan ancestor, GHR/PRLR. In this study, however, we identified distinct genes coding for GHR and PRLR from sea lamprey (*Petromyzon marinus*), one of the oldest extant lineages diverged from the ancestor of Gnathostomes. The two locate at different genomic scaffolds and have 25% sequence similarity. Lamprey GHR and PRLR (pmGHR and pmPRLR) share the highest amino acid identity with coelacanth PRLR, 26% and 36%, respectively. Both have the conserved ligand-binding domain, WSNWS motif, as well as intracellular box 1 and 2 and C-terminal tyrosine residues potentially for JAK-STAT activation. Phylogenetic analysis shows that pmGHR is at the base of the PRLR, GHR and CRFA4 clades, and that pmPRLR branch and Gnathostomata PRLRs are in a clade. Thus, pmGHR appears more ancient than pmPRLR. The highest mRNA levels of pmGHR in the liver, whereas the highest pmPRLR levels occur in the brain, suggesting their physiological divergence. Moreover, pmGHR and pmPRLR had different expression patterns in various tissues at different life stages. In the gill, juvenile had higher *ghr* and *prlr* mRNA levels than ammocoete (larvae). During metamorphosis, branchial *ghr* and *prlr* mRNA levels were both elevated at the final stage; however, *ghr* levels were further upregulated in post-metamorphic juvenile during downstream migration to ocean. This upregulation is likely associated to gill reorganization and chloride cell generation for commencing marine life. In juvenile, the *ghr* mRNA levels were elevated, whereas *prlr* levels did not change between individuals in fresh water to seawater. Conversely, hepatic *ghr* and *prlr* mRNA levels were both downregulated in juveniles, compared to ammocoetes; whereas *igf1* transcription had no significant change. Meanwhile, downregulation was seen in *prlr* mRNA levels in the anterior intestine (AI) of juveniles; this was not seen in *ghr* mRNA levels or in the posterior intestine. Taken together, the variation of *ghr* and *prlr* levels in the gill and AI may suggest a conserved role of pmGHR and pmPRLR in osmoregulation. Herein, we propose that duplication (1R, 2R) of the vertebrate GHR ancestor gave rise to GHR and PRLR, and both survived in the Agnathan lineage. (Supported by NSF grant IOS1558037.)

REVIEW OF WATER & ELECTROLYTE HOMEOSTASIS IN MARINE MAMMALS

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The diversity of habitats to which marine mammals have evolved present unique challenges to their abilities to maintain water and electrolyte balance. Marine mammals have evolved a number of unique anatomical structures and physiological mechanisms to facilitate their radiation into aquatic environments. The kidneys of pinnipeds and cetaceans are reniculate in structure, unlike terrestrial mammals (except bears); however, this difference does not confer any greater concentrating ability. Pinnipeds, cetaceans, manatees, and sea otters can concentrate their urine above that of sea water, with the urine concentrations of Na^+ and Cl^- of only pinnipeds and otters being similar to those in sea water. In theory, this ability would allow these groups of mammals to extract fresh water from purposefully or incidentally consumed sea water. However, with few exceptions, drinking sea water is not a common behavior in pinnipeds and cetaceans. Water balance is maintained in these animals via metabolic and dietary water, while incidental ingestion and dietary salt may help maintain electrolyte homeostasis. Unlike most other aquatic mammals, sea otters commonly drink sea water and manatees frequently drink fresh water. Among the various taxonomic groups of marine mammals, the sensitivity of the renin-angiotensin-aldosterone system appears to be influenced by the availability of Na^+. The antidiuretic role of vasopressin remains inconclusive in marine mammals, while the natriuretic function of atrial natriuretic peptide has yet to be examined. The application of molecular and cellular techniques has allowed researchers to further examine potential evolutionary adaptations to support osmoregulatory mechanisms in marine mammals.

DNA METHYLATION LANDSCAPE CHANGES DURING THYROID HORMONE AND GLUCOCORTICOID CROSSTALKS AT XENOPUS METAMORPHOSIS

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Anuran metamorphosis marks the end of embryonic development and the transition to juvenile animals. This profound remodeling is occurring at every scale; from molecular to cellular and physiological. Pre-metamorphosis is a period of integration of environmental and internal cues controlling the timing and pace of metamorphosis; a physiological process illustrating phenotypic plasticity. At the endocrine level, metamorphosis is controlled by thyroid hormone and glucocorticoid signaling. Their action mechanism is complex, but their mode of action is nonetheless well documented. In contrast, the molecular basis of their crosstalks is much less understood at the molecular level.

We set out to describe the molecular responses affected during TH and GC crosstalks of phenotypic plasticity during metamorphosis, by treating premetamorphic *Xenopus tropicalis* tadpole to TH and/or GC. Transcriptional responses, characterized by RNA-Seq, are limited to a relatively small number of response types. Surprisingly, and despite short treatment times, many genes involved in DNA modification are differentially regulated, suggesting that remodeling of the DNA methylation landscape takes place. meDNA dynamics, monitored by MethylCap-Seq, is very dynamic and shows a large range of response types. Interestingly, many dynamic regions respond only when animals are treated with both hormones and may therefore correspond to crosstalks-specific sites.

Overall, our results show that 1) both in term of transcription and meDNA, TH and GC crosstalks display a very specific component with novel responses, and 2) meDNA dynamic is large compared to the more restricted set of transcriptional responses.

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LOOKING FOR A SILVER LINING: THE IMPACT OF NANOSILVER ON THYROID HORMONE SIGNALING IN FROG TADPOLE METAMORPHOSIS

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Thyroid hormones (THs) are important regulators of metabolism, growth, behavior, and development in all vertebrates. THs function primarily to modulate gene expression by binding to nuclear TH receptor proteins (TRs) that regulate gene transcription and change cellular gene programs. Although a great deal is known about TH signaling, it is still unclear how THs can induce a wide range of cellular outcomes including apoptosis, proliferation, and reprogramming. One of the most striking examples of the diverse effects of THs the absolute requirement for these hormones for the metamorphosis of an aquatic, herbivorous tadpole into a juvenile, carnivorous frog. Virtually all tadpole tissues are sensitive to THs yet the ultimate outcome is tissue-dependent (e.g. the tail regresses, the skin thickens, the liver reprograms, and the olfactory system remodels). It is postulated that these differential responses may be dictated by TR-mediated modulation of epigenetic factors such as histone modifications and DNA methylation. Moreover, environmental contaminants that disrupt TH action may do so by affecting these epigenetic marks. As an example, I will present work on a commonly-used antibacterial agent and its impact on TH signaling in the North American bullfrog (*Lithobates [Rana] catesbeiana*). Nanosilver is a common additive in many consumer products and we exposed premetamorphic *Rana catesbeiana* (American bullfrog) tadpoles to 0.06 – 6.0 µg/L nanosilver for up to 28 days and found that nanosilver alters metamorphosis alone and in combination with exogenously administered TH. Analyses using a multi-omic approach reveal epigenetic changes in both scenarios. Our results reveal some surprising insights into the mode of action of this chemical. Moreover, the levels of silver that elicited an effect on this important environmental sentinel species were at or below North American water quality guideline levels for silver suggesting that a re-evaluation of these guidelines is warranted.

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EPIGENETIC CHANGES CAUSED BY FASTING AND LOW TEMPERATURE IN AMPHIBIANS

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Environmental stresses such as oxygen deprivation, food deprivation, drought and cold temperature can affect many biological processes. For example, bullfrog tadpoles can overwinter by arresting metamorphosis, decreasing metabolic rate, whereas adult bullfrogs overwinter through hibernation. *Xenopus laevis* can survive for long periods without food intake. Changes of energy metabolism may enable these amphibians to adapt to the environmental stresses. Recently, we clarified that the majority of the transcript amounts for energy metabolic genes were down-regulated under fasting condition in the liver and intestine of *X. laevis* [1, 2]. Additionally, it is known that epigenetic processes affect gene expression in response to extreme environmental stresses. These facts raised us the the question. How are the expressions of genes for energy metabolism controlled epigenetically? Recently, we have been examined the effects of fasting and cold temperature on global and gene-specific epigenetic states in amphibians. As a result, we found that the relationship between euchromatin-related epigenetic marks and transcript levels was different in the intestine or the liver of fed or fasted *X. laevis* [1, 2] when compared with higher vertebrates. We also found that there were at least two epigenetic controls in the liver of bullfrog tadpoles to acclimate or acclimatize to environmental temperature [3]. The role of the modification of histones and the specific composition of histone variants will be discussed.

PRECOCIOUS ESTROGEN SIGNALING DURING EMBRYONIC DEVELOPMENT UNDERLIES PERSISTENT ALTERATIONS OF OVARIAN TRANSCRIPTIONAL NETWORKS IN AN ENVIRONMENTAL MODEL OF ENDOCRINE DISRUPTION

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Environmental endocrine disrupting contaminants (EDCs) interfere with native functioning of the endocrine system and are linked to reproductive disorders in wildlife and humans. The American alligator population inhabiting Lake Apopka has historically served as a valuable “real world” model for understanding how exposure to EDCs negatively impacts reproductive development and subsequent function. When compared to a reference site, alligators from Lake Apopka are characterized by altered levels of circulating sex steroids, reduced sexually dimorphic gene expression, perturbed folliculogenesis within the ovary, and an abated ovarian response to FSH challenge. However, previous studies have relied on candidate gene approaches and are correlative in nature. Here, we combine transcriptome sequencing with the developmental tractability of the alligator to (1) identify novel genetic pathways associated with developmental EDC exposure under ecologically relevant conditions and (2) test if altered estrogen signaling during gonadal differentiation is functionally capable of recapitulating persistent reproductive alterations associated with Lake Apopka. Alligator eggs were collected from Lake Apopka and a nearby reference site (Lake Woodruff), and were treated with either vehicle alone or estradiol (E2) just prior to sex determination and gonadal differentiation. Neonatal alligators were lab-reared for five months and RNA-seq performed on resulting ovarian tissues. Profound divergence at the population level was observed, as a majority of genes identified in the analysis (76%, 13,911/18,435) were differentially expressed (FDR < 0.05) between Lake Apopka and Lake Woodruff. Interestingly, treating embryos from Lake Woodruff with a single dose of exogenous E2 during gonadal differentiation was sufficient to recapitulate approximately 77% of DEGs observed across populations. Polycomb Repressive Complex target genes were enriched in DEGs associated with both site and estrogen treatment, implicating a potential epigenetic mechanism linking developmental exposures to persistent alterations in ovarian transcriptional networks. Collectively, our findings inform our understanding of how EDCs interact with endocrine signaling to impact reproduction in natural systems.

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EPIGENETIC MODIFICATIONS IN THE DEVELOPMENT OF INTESTINAL STEM CELLS

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Adult organ-specific stem cells are essential for organ homeostasis and tissue repair and regeneration, but the underlying mechanism for their development is unclear. Intestinal remodeling during frog metamorphosis offers a unique opportunity to study the formation of such stem cells during vertebrate development. During the transition from an herbivorous tadpole to a carnivorous frog, the intestine is completely remodeled with the larval epithelial cells undergo apoptosis and are replaced by adult epithelial cells formed de novo. The entire metamorphic process is under the control of thyroid hormone (T3). We have shown that adult epithelial stem cells are induced by T3 through dedifferentiation of some larval epithelial cells. T3 exerts its metamorphic effects through T3 receptors (TRs). TRs recruit, in a T3-dependent manner, cofactor complexes for chromatin remodeling/histone modifications. We have demonstrated that the expression of two histone methyltransferases, Dot1L and PRMT1, are activated by T3 during intestinal remodeling. Our studies further suggest that both are recruited by TR during metamorphosis to function as TR coactivators to promote gene regulation and intestinal stem cell formation and/or proliferation through histone methylation.

DOES DNA METHYLATION PLAY A ROLE IN TEMPERATURE-DEPENDENT SEX DETERMINATION IN THE SNAPPING TURTLE?

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Sex determination in common snapping turtles, *Chelydra serpentina*, is regulated by temperature making it an ideal model for studying the role epigenetic mechanisms play in determining cell and gonad fate. DNA methylation has been studied for its role in temperature dependent sex determination (TSD), but the interplay between DNA methylation and TSD is largely unknown. We are investigating genome-wide DNA methylation patterns in snapping turtle embryonic gonads using whole-genome bisulfite sequencing (WGBS). We dissected gonads from embryos incubated at male- (MPT; 26.5°C) and female-producing temperatures (FPT; 31°C) on day 1 and day 5 of temperature sensitive period (TSP). Seven gonads from the same incubation temperature and time point were pooled together for each sample. DNA was extracted from the pooled gonads (total 12 samples = 2 temperatures x 2 time points x 3 replicates/temperature/time point). Bisulfite conversion was done using EZ DNA Methylation Gold Kit (Zymo Research). Bisulfite treated DNA samples were sequenced on Illumina Hi-seq platform in 150PE format to get at least 24x coverage. Quality filtered reads were mapped to the snapping turtle draft genome using Bismark tool and DNA methylation analysis was done using methylkit. We found significant differences in DNA methylation level between MPT and FPT at several loci in the genome on day 5 of the TSP. The results suggest that DNA methylation plays a role in mediating temperature effects in TSD species during sex determination and development. Further analysis is being done to correlate the WGBS data with RNA-Seq data to identify the expression changes linked with DNA methylation at specific loci.

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CHARACTERIZATION OF INSULIN PATHWAY MUTANTS IN DROSOPHILA

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We have characterized heteroallelic mutant combinations of three different genes in the insulin pathway. We have brought them to a common genetic background, a condition that enables comparisons between the different mutant hypomorphic states and a common wild type control. These three mutant combinations represent different models of diabetes type II, from the more general condition that includes all of the insulin signaling pathway (mutations in *Inr*, the sole insulin receptor in flies), some aspects of signaling: mutations in the *PI3K92E* gene, which are mutations in the catalytic subunit of the PI3K gene associated with the insulin pathway, and mutations in *S6k*, the S6 kinase homolog in flies, which is mainly involved in growth and anabolism. All three are different models of diabetes type II, and allow different aspects of the phenotype to be studied. We have studied weight, lipid and carbohydrate accumulation throughout adult life, as well as feeding behavior, activity and circadian rhythms. We have found that the flies are smaller than controls, and accumulate lipids and carbohydrates to different degrees. *Inr* mutants are more active, and present starvation resistance, whereas *S6k* mutants are less active and present only lipid accumulation. *Pi3k92E* mutants have more moderate defects. The phenotypes vary throughout adult life.

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INSULIN-LIKE GROWTH FACTOR 2 THROUGH THE AGES: LOCUS AND GENE CONSERVATION AND DIVERSIFICATION DURING VERTEBRATE SPECIATION

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The small, secreted protein, insulin-like growth factor 2 (IGF2), plays a central role in fetal and prenatal growth and development in humans and other mammals. Human *IGF2* and mouse *Igf2* genes are each located within a complex conserved linkage group and are regulated by parental imprinting, with *IGF2*/*Igf2* being expressed from the paternally derived chromosome, and *H19*, which encodes a long noncoding RNA, from the maternal chromosome. Analysis of data retrieved from genomic and gene expression repositories reveals a similar degree of complexity in 19 different mammalian species from 13 different orders spanning ~166 million years of evolutionary development. In most species, multiple potential *IGF2/Igf2* gene promoters were identified, each with individual 5' un-translated exons. DNA sequence conservation was high in *IGF2/Igf2* coding exons, but lower in non-coding exons and promoters, in a putative imprinting control region 5' to *H19*, and in potential enhancer elements 3' to *H19*. A similar analysis in 8 terrestrial vertebrates, 11 ray-finned fish, and 1 lobe-finned fish representing > 500 million years of evolutionary diversification demonstrates that vertebrate *Igf2* genes are simpler than their mammalian counterparts, having fewer exons and only a single gene promoter. *Igf2* genes are conserved among non-mammalian vertebrates too, especially in protein coding regions, and IGF2 proteins also are conserved, although less in fish than in terrestrial species. The *Igf2* locus in terrestrial vertebrates shares several additional genes with its mammalian counterparts, including tyrosine hydroxylase (*Th*), insulin (*Ins*), mitochondrial ribosomal protein L23 (*Mrpl23*), and troponin T3, fast skeletal type (*Tnnt3*), and both *Th* and *Mrpl23* are present in the *Igf2* locus in fish. Taken together, these observations support the idea that a recognizable *Igf2* was present in the earliest vertebrate ancestors, but that other features developed and diversified in the gene and locus with speciation, especially in mammals. These data also indicate that inaccuracies in data found in genome-level repositories could limit our ability to fully understand contributions of individual genes and multi-gene families toward vertebrate and mammalian physiology and evolution.

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SOMATOTROPE REGULATION: A NOVEL FUNCTION OF NUCLEOBINDIN ENCODED PEPTIDES?

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Growth is a multifactorial process influenced by diverse elements such as hormones, nutrition (e.g. metabolic status, meal plan, and diet composition), environmental factors (e.g. season), genetics or even age. Alteration of these factors results in growth disorders, being the regulation of the growth hormone (GH) the most critical point for proper growth. GH is produced and secreted in the pituitary and acts in an endocrine manner enhancing the secretion of insulin-like growth factors (IGFs) in the liver, and all together promoting somatic growth in different tissues. Recently, two peptides called nesfatin-1 and nesfatin-1-like peptide (NLP) (encoded in nucleobindins (NUCB), nucleobindin-2/NUCB2 and nucleobindin-1/NUCB1, respectively) have been identified. Nesfatin-1 and NLP are involved in different physiological functions including food intake and insulin secretion. We hypothesized that rodent somatotropes are a source and target of nesfatin-1 and NLP, and both peptides influence GH synthesis and secretion from somatotropes. The main objectives of the present study were to determine whether somatotropes are a source of NUCBs and encoded peptides, and analyze if they are involved in the production and secretion of GH in the mammalian pituitary. Using rat immortalized somatotropes (RC-4B/C and GH3), the expression of NUCB1 and NUCB2 was determined, as well as both dose- and time-dependent effects of nesfatin-1 and NLP on GH synthesis and release were evaluated. Somatotropes express both NUCB1 and NUCB2 mRNAs, and somatotropes were found to be immunoreactive for both NUCB1/NLP and NUCB2/nesfatin-1. Moreover, incubation of somatotropes in rodent 0.001 and 0.1 nM nesfatin-1 for 1 hour reduced GH mRNA expression. Additional signaling studies are underway with the goal of elucidating the mechanisms of action by which nesfatin-1 modulates GH in the somatotropes. Overall, these data indicate that rat somatotropes are a source of NUCB1/NLP and NUCB2/nesfatin-1, and that nesfatin-1 and NLP could act directly on these cells to regulate GH synthesis. Acknowledgments: Supported by SHRF (EJV), Ferring Innovation Grant (SU).

HOW OCEAN-WARMING COULD AFFECT GROWTH OF COLD-WATER MARINE TELEOSTS: GH-INDUCED STIMULATION OF ATLANTIC WOLFFISH GOWTH AT TEMPERATURES APPROACHING THE UPPER THERMAL TOLERANCE LIMITS

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The Atlantic wolffish (*Anarhichas lupus*) is a cold-water marine fish and is widely distributed in the North Atlantic Ocean. The species is demersal and sedentary, except during spawning and feeding migrations, and is found at temperatures ranging from -1.7 to 11°C. It is likely that spawning takes place at temperatures below 8°C. Following internal fertilization, the female lays a gluey clutch of a few thousand, relatively large eggs, which is subsequently guarded by one of the parents.

An Atlantic wolffish egg clutch was obtained by bottom trawling at 136 m depth off the North-West coast of Iceland. The mechanical disturbance induced hatching after which the larvae were transferred to an MFRI research facility at Grindavik, Iceland. The larvae were raised at 7°C through the yolk-sac stage and into the early first-feeding stage. Then, they were separated and transferred to four different rearing temperatures (3, 7, 11 and 15°C) where they were fed *ad lib* for nine weeks to determine optimal temperature for growth (T_{opt}). The species was found to be relatively stenothermic with fastest growth in the 11°C group and T_{opt} calculated to be 12.1°C. At 15°C, growth rate was severely inhibited, skeletal deformities developed and some mortality occurred, indicating this to be close to the upper thermal tolerance limits of the species.

The fish were then implanted with either a sham implant or a sustained-release bovine growth hormone (GH; Posilac®) implant, and growth monitored for further nine weeks. While growth rates differed significantly among the groups, increasing from 3 to 11°C, GH was found to induce a similar (30%) weight increase over controls. Two years later, the GH-treated fish were still 10-15% heavier than the corresponding sham-treated fish.

In sharp contrast, GH did not stimulate growth of fish at 15°C at all. This suggests that at the upper tolerance limit, the allostatic load is so great that that the available energy is shifted from growth towards the maintenance of vital cellular and organismal functions. The study offers new insights into physiological mechanisms that may define upper thermal limits of ectothermic vertebrates.

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NEUROREGENERATIVE EFFECT OF GROWTH HORMONE (GH) IN THE CHICKEN NEURAL RETINA

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Neuroregeneration is a process conserved in lower vertebrates but absent in mammals; in birds the retina can be partially regenerated for a short time after hatching. The avian retina is an interesting model to study molecular and physiological mechanisms underlying neuroregeneration. After excitotoxic damage, the avian retina is able to partially regenerate and to re-integrate neural cells originated from trans-differentiated Müller cells. Growth factors such as FGF2 and IGF-1 have a critical role during this process, however, GH actions during neuroregeneration are largely unknown. In this work, we propose that GH enhances retinal regeneration after an excitotoxic damage. The protocol to induce neuroregeneration included a single intravitreal KA (20 µg) injection at P1 followed by 10 post-injury GH (300 ng) injections from P2 to P16. Retinas were collected at P21 for histochemistry and mRNA quantification. We confirmed the regenerative effect of GH through retinal morphometry. We quantified relative changes of transdifferentiation markers such as FGF2, PCNA and Sox2 by qPCR. We also determined the participation of Notch signaling pathway on neuroregeneration through changes in Notch1, Hes5 and Ascl-1a mRNA expression. Finally, we determined the effect of GH on relative changes of glutamate receptors NMDA-R (NR-1) and KA-R (GRIK4) as markers for retinal healing. Changes in the expression of synaptogenesis markers (DLG1 and SNAP25) were also analyzed. Our results show that after the excitotoxic damage, GH is able to promote retinal tissue recovery and it increases the expression of FGF2 mRNA, although no effect for Ascl-1a was observed. In our neuroregeneration model, Notch signaling was activated by GH, promoting the expression of Notch1 and Hes5. In addition, GH increased levels of BDNF, Sox2, NR-1, GRIK4 and DLG1 mRNA, but not SNAP25. This work showed the activation of Notch pathway induced by GH and was associated with Müller glia transdifferentiation into neural progenitors. GH was able to induce the upregulation of transdifferentiation markers and to potentiate the expression of glutamate receptors and synaptogenic markers. In summary, we found that chronic treatment with GH potentiates the neuroregenerative process induced by excitotoxicity damage in the chicken neuroretina.

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MECHANISMS UNDERLYING TEMPERATURE-INDUCED REPRODUCTIVE BEHAVIOR: ARE OVERWINTERING ECTOTHERMS REALLY "DORMANT"?

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Many ectotherms rely on changes in temperature for timing seasonal reproductive activity. However, the mechanisms of temperature-induced reproduction are poorly understood, and we do not yet know if the effects of photoperiod and temperature are regulated by a conserved neuroendocrine pathway. We asked if low-temperature dormancy alters the synthesis of gonadotropin-releasing hormone (GnRH), the metabolism of thyroid hormones by deiodinase enzymes within the hypothalamus, and/or the synthesis of thyrotropin within the pituitary pars tuberalis. Studies in spring breeding birds and mammals have demonstrated that these neuroendocrine factors mediate the effects of long days on the reproductive axis. We used the exceptionally well-studied red-sided garter snake (*Thamnophis sirtalis*) to address these questions, as 4 weeks of low-temperature exposure is both necessary and sufficient to induce reproductive behavior in this species. Using a series of simulated hibernation experiments and immunohistochemistry, we found that increasing duration of low-temperature dormancy significantly increased GnRH-immunoreactive cell number and GnRH soma size (a proxy for relative cell activity) in males. Increases in GnRH mirrored temperature-induced changes in both male reproductive behavior and plasma androgens. Immunoreactive staining for deiodinase 2, which metabolizes thyroxine to triiodothyronine, within the hypothalamus of males also increased significantly during low-temperature dormancy, indicating that the metabolism of thyroid hormones within the hypothalamus is indeed temperature sensitive. Intriguingly, temperature-induced changes in GnRH and deiodinase 2 were sexually dimorphic, a finding that corroborates known sex differences in the timing of reproductive activity in this species. Finally, thyrotropin immunoreactivity within a pars tuberalis-like structure of the pituitary was not altered by low-temperature dormancy in either sex. These latter findings contrast with thyrotropin's known role in photoperiodic birds and mammals. Our results provide insight for understanding the mechanisms that mediate sex differences in reproductive timing, and suggest that changes in the neuroendocrine reproductive axis during winter "dormancy" play a role in mediating these differences.

A POSSIBLE ROLE FOR LEPTIN IN SEXUAL MATURATION AND REPRODUCTIVE FUNCTION OF THE MOZAMBIQUE TILAPIA OREOCHROMIS MOSSAMBICUS

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Leptin is a key regulator of energy homeostasis and a permissive signal for puberty and reproductive function in mammals. Thus far, the role for leptin in regulation of the reproductive axis and integration of the somatotropic and gonadotropic systems in ectotherms and teleost fishes, specifically, remains poorly understood. In the current study, we assess several aspects of leptin's potential role in regulating reproductive biology in the Mozambique tilapia (*Oreochromis mossambicus*). We evaluated leptin regulation during sexual maturation and by sex steroids, as well as its function in controlling key hypothalamic reproductive signals (kisspeptin, gonadotropin-releasing hormone). We also assessed leptin regulation of hepatic vitellogenins and insulin-like growth factor (*igf1*). Transcript levels of *lepa*, the dominant paralog of leptin in teleosts, in the liver, the primary source of circulating hormone, increased with gonadosomatic index and sexual maturation of male and female tilapia ($P < 0.01$, $r = 0.608$). Intraperitoneal injection and 6-hour treatment with recombinant tilapia leptin A (rtLepA) in adult male fish increased brain *kiss2* and *gnrh1* transcript levels versus saline control ($P < 0.01$). In primary hepatocyte culture, rtLepA suppressed *vtgb* and *vtgc* transcript levels ($P < 0.01$), while it stimulated *igf1* ($P < 0.0001$). Testosterone and estradiol reduced *lepa* levels ($P < 0.001$), but disparately regulated *igf1* whereby testosterone increased *igf1* and estradiol decreased *igf1* at 10 and 100 nM ($P < 0.001$). The non-aromatizable 11-ketotestosterone increased *lepa* and *igf1* transcript levels at 100 nM ($P < 0.05$). Our findings suggest that leptin expression rises with sexual maturation and the hormone exerts stimulatory effects on hypothalamic gonadotropic factors. By contrast, we show for the first time that leptin reduces estrogen-sensitive vitellogenesis while promoting elements of the somatotropic axis (e.g., *igf1*), suggesting the hormone may have dual roles in regulating reproductive and somatic growth processes. We speculate that this latter effect may be associated with limiting energy partitioning to reproduction in instances of negative energy balance when leptin has been shown to increase.

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LIGAND-BIAS IN GOLDFISH PITUITARY GNRH RECEPTOR ACTIVATION: INVOLVEMENT OF BETA-ARRESTINS

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In the goldfish pituitary, gonadotropin-releasing hormone (GnRH) signals *via* GnRH receptors (GnRHRs) to stimulate production/release of growth hormone (GH) and luteinizing hormone (LH). Previously, our lab has used this teleost neuroendocrine model system to highlight a ligand bias in GnRH signalling. Two endogenous GnRH isoforms (chicken GnRH-II, GnRH2, and salmon GnRH, GnRH3) differentially signal via a shared population of GnRHRs to bring about varying outcomes in a ligand-, cell type-, time-, and response-specific fashion. Through pharmacological interventions in perfusion experiments with primary pituitary cell cultures, differential recruitment of calcium stores, PI3K subunits and the Raf-MEK-ERK cassette has been shown to underlie some of the differences in GH vs LH synthesis and/or release. However, whether other mediators, such as B-arrestin, play a role in these differences is unknown. In general, ligand bias observed in other hormone-GPCR systems is largely attributed to the variable contributions of G protein- *versus* arrestin-dependent signalling. To begin to address this question, we employed a novel pharmacological inhibitor of arrestin interactions and downstream signalling (Barbadin) to examine whether arrestin contributes differentially to GnRH-induced GH and LH release in cell column perfusion experiments of primary cultures of dispersed goldfish pituitary cells. Barbadin had little effect on basal hormone secretion but reduced GnRH2- and GnRH3-stimulated LH release, as well as GnRH2-induced GH secretion; however, the GH response to GnRH3 was augmented. These results suggest that differential use of arrestin-dependent events is part of the biased GnRH signalling mechanisms in goldfish pituitary somatotrophs and gonadotrophs. How these differences in the use of arrestin-dependent mechanism(s) relates to the known selective use of Class 1 PI3K isoforms, MEK-ERK, arachidonic acid and pharmacologically distinct calcium pools by the two endogeneous GnRHs in this system would be important questions to pursue.

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CHALLENGING THE PARADIGM OF GNRH CONTROL OF REPRODUCTION: THE CASE OF GNRH3 IN ZEBRAFISH

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The recent developments in gene knockout (KO) technologies, especially in the intensively studied zebrafish (ZF) model, occasionally contradicted our knowledge and results from more traditional research methods. The hypophysiotropic gonadotropin-releasing hormone (Gnrh3) is one example of such a discrepancy. Inheritable gnrh3 gene KO has unexpectedly yielded fertile ZF, even though Gnrh3 neuronal ablation impedes oogenesis. Moreover, the double Gnrh KO line (gnrh3-/-;gnrh2-/-) also displayed normal reproduction, thus another Gnrh isoform is not simply replacing Gnrh3. These findings have sparked a debate over the relevance of Gnrh3 in reproduction. To re-establish the reproductive role of Gnrh3, gnrh3 was knocked down in adult brains, using HSV1 viruses expressing anti-sense gnrh3, and Gnrh receptors were blocked with LHRH receptor antagonists. Both Gnrh3 gene silencing and receptor blocking yielded impairment of reproductive capacity in females. To test the possibility of a compensation for the loss of Gnrh3, transcriptomes were compared between wild-type and gnrh3-/- adult brains and pituitaries. Several differentially expressed genes were identified. Peptides such as Gnrh inhibiting-hormone (Gnih) and vasoactive intestinal-peptide (Vip) were upregulated in the KO brain. Tyrosine hydroxylase that synthesizes dopamine was downregulated and monoamine oxidase that degrades dopamine was upregulated, possibly lowering its levels in KO fish. In the pituitary, dopamine receptor drd2 was downregulated. Vip was also tested for possible replacement of the hypophysiotropic function of Gnrh3 (via neuronal immuno-staining in the neurohypophysis, interactions with gonadotropes, and induction of gonadotropin secretion) and demonstrated a clear gonadotropin secretion ability. The results suggest that Gnrh3 is an important player in reproduction and that a multi-factorial compensatory mechanism is activated in its absence to guarantee proper reproduction.

EXAMINING VASOACTIVE INTESTINAL PEPTIDE AS A POTENTIAL REPRODUCTIVE COMPENSATOR FOR THE HYPOPHYSIOTROPIC GONADOTROPIN-RELEASING HORMONE LOSS-OF-FUNCTION IN ZEBRAFISH

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Gonadotropin-releasing hormone (Gnrh) is a major hypothalamic neuropeptide governing the hypothalamus-pituitary-gonad (HPG) axis that controls vertebrate reproduction by stimulating the synthesis and release of the pituitary gonadotropins, Follicle-stimulating hormone (Fsh) and Luteinizing hormone (Lh). During the past decade, our lab has applied various approaches, using zebrafish (*Danio rerio*) as a model, to better understand the exact roles and the mechanism of the hypophysiotropic Gnrh actions in teleost. Knockdown of the hypophysiotropic Gnrh form (*gnrh3*) using antisense morpholino oligomer in wild-type (WT) zebrafish results in abnormal Gnrh3 neuronal migration. In addition, laser ablation of Gnrh3 neurons during ontogeny, which eliminates all Gnrh3 neurons in the brain, causes infertility in female zebrafish. However, unlike the mammalian loss-of-Gnrh models that display hypogonadotropic hypogonadism and infertility, the *gnrh3* mutant (*gnrh3*$^{-/-}$), which lacks the Gnrh3 decapeptide, exhibits normal Gnrh3 neuronal migration and reproductive performance. These results suggest that a reproductive compensation mechanism is activated to mitigate the effects of the inherited loss of Gnrh3 functions in *gnrh3*$^{-/-}$ zebrafish. One of the most logical potential compensators is Gnrh2, the only other isoform of zebrafish Gnrh. However, a double-Gnrh loss-of-function mutant has recently been generated and displays normal fertility, suggesting that compensation by Gnrh2 is not occurring. These findings in *gnrh3*$^{-/-}$ zebrafish revived an old question as to how the hypothalamus communicates with the pituitary to drive reproduction. Thus, *gnrh3*$^{-/-}$ zebrafish has emerged as a unique model and may be the first demonstration of non-Gnrh-dependent reproduction in vertebrates. In order to identify potential reproductive compensators, WT and *gnrh3*$^{-/-}$ fish brain transcriptomes were compared. Analyses revealed that the expression of a 28 amino acid neuropeptide, Vasoactive intestinal peptide A (VipA), increases in *gnrh3*$^{-/-}$ fish. This finding is supported by an upregulation of VipA content in the *gnrh3*$^{-/-}$ pituitary, compared to the WT. Although VipA neurons play a key role in mammalian reproduction as a circadian pacemaker in the suprachiasmatic nucleus, the functions of VipA and its neurons in teleost reproduction are still unknown. Here, in order to examine VipA as a potential reproductive compensator for the loss of Gnrh3, we investigated its neuroanatomical distribution and its ability to induce the reproductive axis by immunohistochemistry, *in situ* hybridization and *in vitro* pituitary culture explants. VipA neurons were localized in the anterior part of the parvocellular preoptic nucleus and periventricular hypothalamus. Their axons innervated the proximal pars distalis (PPD), alongside Gnrh3 axons, making direct contact with gonadotropes. In a pituitary *in vitro* explant assay, a VipA peptide induced Fsh and Lh secretion, presumably through two VipA receptors (PAC1R, *adcyap1r1*; VPAC1R, *vipr1b*) expressed in the PPD. Taken altogether, for the first time in fish, a potential role for VipA as a regulator of reproduction has been demonstrated, whereby VipA acts directly on the pituitary gonadotropes. Moreover, the results suggest that VipA may be involved in overcoming the loss of Gnrh3 function as a reproductive compensator in *gnrh3*$^{-/-}$ zebrafish.

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SECRETONEURIN IS A PEPTIDE HORMONE THAT RESCUES IMPAIRED SPAWNING IN ZEBRAFISH LACKING THE PRECURSOR PROTEIN SECRETOGRANIN-II

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Secretogranin-II (Scg2) is a ~600 amino acid protein processed by prohormone convertases to produce an array of potentially bioactive peptides, one of which is secretoneurin (SN). In teleosts, a presumptive early duplication gave rise to the *scg2a* and *sg2b* genes, and the production of SNa (34 AA) and SNb (31 AA), respectively. The only peptide characterized thus far is SNa and it stimulates LH release from the goldfish pituitary *in vivo* and directly from dispersed cells *in vitro*. We have also shown previously that mouse SN stimulates LH release from the LbT2 cell line *in vitro*. However, critically missing is direct evidence for a role in vertebrate reproduction *in vivo*, so we generated Scg2-null mutant zebrafish using the TALEN approach. Rates of oviposition for *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ mutant females are 6% and 11% when crossed with *scg2a* $^{-/-}$*;scg2b*$^{-/-}$ and wild-type (WT) males, respectively, compared to 62% in WT pairings of sexually naïve fish. Histological examination of gonads of mutant fish indicates that both testes and ovaries are fully formed with a generally normal appearance. In contrast, the total duration of male courtship behaviour was significantly reduced in the *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ within-line crosses and when *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ mutant females were paired with WT males. Intraperitoneral (i.p.) injection of hCG in *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ fish significantly increased spawning success to 38% compared to control injected *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ fish. We synthesized zebrafish SNa and SNb using Fmoc chemistry followed by purification (~98%) and sequence confirmation by tandem mass spectrometry. A single i.p. injection of SNa rapidly increased spawning success in *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ fish ~3-fold to 30% compared to *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ fish injected with saline only. On the other hand, SNb did not rescue spawning success in *scg2a*$^{-/-}$*;scg2b*$^{-/-}$ fish. These data support our hypothesis that Scg2 is a precursor for the new stimulatory reproductive hormone SN.

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FEMINIZATION OF BEHAVIOR, PLASMA SEX HORMONE PROFILE, GONADAL HISTOLOGY AND BRAIN GENE EXPRESSION FROM ENDOCRINE DISRUPTION IN SEXUALLY LABILE ANEMONEFISH

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Endocrine disruptors, such as bisphenol A (BPA) and ethinylestradiol (EE2), are becoming increasingly concentrated in the marine environment from plastic waste and wastewater effluent. Despite significant data on the feminizing effects of these pollutants on the reproductive axis of freshwater fish and terrestrial vertebrates, little is known about effects on marine fish. Since sex determination in many marine fish is labile and subject to environmental cues, endocrine disruptors can be particularly damaging to normal development. The objective of this study was to determine the impact of 6 months exposure to environmentally relevant concentrations of BPA and EE2 on behavior, vocalizations, plasma sex hormone profile, gonadal histology and brain gene expression in *Amphiprion ocellaris*, the false anemonefish. *A. ocellaris* are sequential hermaphrodites and display post-maturational sex change from male to female in nature. Ambisexual, non-reproductive fish were paired together in 10 gallon aquariums and were fed twice a day with normal food (control), or food laced with either BPA (100 µg/kg) or EE2 (0.2 µg/kg) (n=4 tanks per treatment group). Behavioral and vocal responses to a conspecific intruder were measured at 1, 3, and 6 months. Blood plasma was collected at 3 and 6 months to measure estradiol (E2), 11-ketotestosterone (11-KT; main bioactive androgen in fish), and vitellogenin. At the end of the study, fish were euthanized and fixed for gonadal histology, and the brains frozen for aromatase gene expression measurements. The behavioral significance of vocalizations was established in a separate study, which measured behaviors directed towards a mirror while vocalizations were played back to focal fish through an underwater speaker. Hearing vocalizations reduced aggressive displays in focal fish, establishing their significance, but were infrequently displayed in the main study. By 3 months, BPA-treated fish were more aggressive towards intruders, and individuals displayed higher plasma levels of vitellogenin, E2, and lower 11-KT relative to control. Preliminary results suggests BPA significantly feminized the gonads. Quantification of brain gene expression is currently underway. Initial results suggest BPA exposure induces substantial feminizing effects in a sexually labile marine fish.

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DEVELOPMENTAL EXPOSURE OF CALIFORNIA MICE (PEROMYSCUS CALIFORNICUS) TO BISPHENOL A OR GENISTEIN AND EFFECTS ON THE GUT MICROBIOME, AND METABOLOME AND SOCIO-COMMUNICATIVE BEHAVIORS

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During the perinatal period, human offspring can encounter high amounts of phytoestrogens such as genistein (GEN) through the maternal diet and soy-based formulas. Such chemicals can exert estrogenic activity and thereby disrupt neurobehavioral programming. Besides inducing direct host effects, GEN may cause gut dysbiosis and alter gut metabolites affecting host function. Previously, we have shown that another endocrine disrupting chemical (EDC), bisphenol A (BPA), can affect socio-communicative behaviors and the gut microbiome. To determine whether perinatal exposure to GEN affects the gut microbiota and host neurobehavioral responses, California mice (*Peromyscus californicus*) dams were placed on a diet supplemented with GEN (250 mg/kg feed weight) or phytoestrogen-free control diet (AIN) two weeks prior to breeding and throughout gestation and lactation. At weaning, offspring socio-communicative behaviors were examined, and gut microbiota and metabolite profiles were assayed. Developmental exposure of offspring to GEN decreased the relative amounts of select bacteria but increased the amounts of others, e.g., *Lactobacillus spp.*, *Ruminococcus flavefaciens*, Bacteroidales, and Clostridiales were reduced in GEN-exposed female offspring, whereas Rikenaceae, Ruminococcaceae, Lachnospiraceae, and *Lactococcus spp.* were elevated in this group. In male offspring, GEN decreased the relative abundance of *Flexispira spp.*, Clostridiales, Bacteroidales, *Odoribacter spp.*, and Desulfovibrionaceae but increased the relative abundance of Lachnospiraceae and *Allobaculum spp*. Gut metabolite shifts were also evident in male and female offspring exposed to GEN. For socio-communicative behaviors, female offspring of dams exposed to GEN were less likely to investigate a novel mouse when tested in a three-chamber social test designed to measure interaction with novel mice. When placed in isolation, both GEN exposed males and females exhibited increased latency to elicit their first vocalization, which is suggestive of reduced motivation to communicate with other individuals. Correlation analyses revealed several interactions between GEN-induced microbiome, metabolome, and socio-communicative behaviors. Notably, data suggest early exposure to GEN disrupts normal socio-communicative behaviors in California mice, which are otherwise social rodents. Such effects may be due to early exposure to GEN disturbing normal neural programming. Results suggest that behavioral deficits might also be attributed to GEN-induced microbiota shifts and resultant changes in gut metabolites. Current findings indicate there is cause for concern in expectant mothers consuming soy products and providing soy formula to infants as early exposure to GEN might detrimentally effect the offspring microbiome-gut-brain axis.

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NONCLASSICAL ACTIONS OF STEROIDS IN THE MODULATION OF VOCAL AND AUDITORY CIRCUITS IN SONGBIRDS

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This talk will focus on the unconventional role of steroids as neuromodulators. Traditionally, steroids are viewed as hormones secreted by peripheral glands to impact brain function and behavior over long-term timescales (days-weeks). We now understand that steroids can be synthesized by neurons at synaptic junctions, and that they can have acute (secs-mins) actions on neural circuit function and behavior. Our work in this domain focuses on auditory processing circuits in the forebrain of songbirds. We have developed evidence that brain-derived steroids can fluctuate dynamically in auditory forebrain circuits, and that steroids can have minute-by-minute actions on auditory coding and communication behaviors. Evidence from in vivo electrophysiology, patch clamp electrophysiology, in vivo microdialysis, and behavioral experiments will be discussed. Together, the evidence shows that brain-derived steroids can influence neural circuit function and sensorimotor-dependent behaviors acutely, similar to traditional neuromodulators like serotonin and dopamine.

NAPHTHENIC ACIDS DISRUPT COURTSHIP BEHAVIOURS IN THE WESTERN CLAWED FROG *(SILURANA (XENOPUS) TROPICALIS)*

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Naphthenic acids (NAs) are a diverse array of carboxylic acids found in crude oil and are among the most toxic pollutants in tailings from the Alberta oil sands. This wastewater is held in tailings ponds under a zero-discharge policy, but NAs still contaminate surrounding ecosystems. Little is known about their effects on frogs, with studies focusing on tadpoles and almost no previous research with adults. In fish, NAs have shown potential endocrine disrupting effects at sublethal doses, especially as anti-androgens. Since reproduction is governed by hormones, we examined the effects of NAs on courtship behaviours in Western clawed frogs. Adults were found to tolerate exposure of up to 20 mg/L NA (a commercial mixture) without noticeable toxic effects. Individual males were exposed to control conditions or 20 mg/L NA (n=6) for 5 days, then injected with hCG to induce vocalizations that were recorded with underwater microphones. Total calling duration was lower in the NA treatment (p=0.008). The experiment was replicated (n=12) and calling was again inhibited by NAs (p=0.01). Gene expression in the testes of these hCG-injected males was measured with qPCR. Levels of *StAR* (R^2=0.81, p=0.0001), involved with cholesterol import, and *cyp17a1* (R^2=0.48, p=0.01), involved with testosterone production, were related to calling duration. Individual unexposed males were allowed to freely interact with two females, one control and one exposed to 20mg/L NA for 5 days. Males preferentially (p=0.009) amplexed control females (9/11) over exposed (2/11), suggesting that NAs reduce female receptivity. As hormone-dependent courtship behaviours are disrupted, sublethal NA exposure may impact reproductive success.

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(NEURO)ENDOCRINE DISRUPTION OF AMPHIBIAN REPRODUCTIVE PHYSIOLOGY AND BEHAVIORS

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Anthropogenic chemicals, which can interfere with the endocrine system of vertebrates, are called endocrine disrupting chemicals (ECD) and are suspected of disturbing developmental programming prenatally and during early childhood leading to developmental anomalies later in life. EDC, however, can also act acutely, and thereby affect physiology and behavior of adult vertebrates as well. A main sink of EDC are surface waters. Hence, fishes and amphibians are specific target species being affected by the adverse effects of those substances. We recently examined whether exposure to EDC at environmentally relevant levels can affect the reproductive physiology of male African clawed frogs (*Xenopus laevis*). We specifically investigated the effects of exposure to EDC on various reproductive behaviors including male courtship calling and clasping behavior, and examined females' responses to the altered male mating behavior as well. It turned out that the male mate calling behavior is modulated distinctively depending on the specific mode of action of the chemical the animal is exposed to. This specificity might even allow for potential predictions to be made on whether and by which mode of action a specific substance can disrupt the (neuro)endocrine system of male *X. laevis*. Co-exposure of male frogs to two EDC with different modes of action resulted in various effects, depending on the mode of action of the substances tested as well as on the concentration ratios used. Effects included antagonistic, independent, and synergistic impacts on mate calling behavior, vitellogenin induction, and heme metabolism, respectively. Furthermore, obvious alterations in females' responses to the courtship behavior of EDC exposed male frogs were detected, which resulted in decreased reproductive success of EDC exposed males.

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INTRA-GENERATIONAL EFFECTS OF EARLY LIFE-STAGE EXPOSURE TO TEBUCONAZOLE ON REPRODUCTIVE CAPACITY OF ZEBRAFISH (DANIO RERIO)

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Pulse exposures occur when rain follows application of a pesticide, causing it to mobilize and result in elevated concentrations for short duration in nearby waterbodies. In early life-stages of fish, epigenetic processes, such as clearing and re-establishment of parental methylomes, are vulnerable to chemical stressors, including endocrine disrupting chemcials. In this study, zebrafish (Danio rerio) embryos were exposed from prior to 1-hour post-fertilization until 24 hours post-hatch to either a control (0 µg/L), low concentration (10 µg/L), or high concentration (1000 µg/L) of tebuconazole (TEB), a herbicide that causes endocrine disruption via inhibition of aromatase (CYP19) activity. Upon termination of exposure, embryos were transferred to water without TEB and reared until sexual maturity to determine their reproductive capacity. There were no significant effects of TEB on mRNA abundance of genes along the hypothalamus-pituitary-gonad liver axis that regulates reproduction, nor were there effects on reproductive capacity. However, there were significant effects on expression of the biochemcial machinery that regulates the DNA methylome. mRNA abundance of ten-eleven translocase 3 (tet3) was greater in embryos exposed to 10 µg/L of TEB and mRNA abundacne of DNA methyltransferase (dnmt) 31, tet1, tet2, and tet3 was greater in embryos exposed to 1000 µg/L of TEB. mRNA abundance of tet 1 was lesser in testes from males reared from embryos exposed to 1000 µg/L. In brain from males reared from embryos exposed to 10 µg/L of TEB there was lesser mRNA abundance of dnmt2 and dnmt3b4 whereas in males reared from embryos exposed to 1000 µg/L of TEB mRNA abundance of dnmt2, dnmt 3b4, tet1, and tet2 were greater. In brain from females reared from embryos exposed to 10 µg/L of TEB there was a greater mRNA abundance of dnmt3a2 and dnmt3b1 whereas in brain from females reared from embryos exposed to 1000 µg/L of TEB there was lesser mRNA abundance of dnmt3a1, dnmt3b2, dnmt3b4, tet1, and tet2. Analysis of loci-specific DNA methylation via reduced-representation bisulfite sequencing is ongoing. To date, 16 differentially methylation regions have been identified in ovaries from females exposed as embryos to 10 µg/L of TEB. Results suggest that reproduction is not impacted by exposure to TEB during ealry life-stages, but the DNA methylome is a sensitive target of TEB.

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DEVELOPMENTAL EXPOSURE TO FLUOXETINE REDUCES OFFSPRING BASAL CORTISOL CONCENTRATION VIA LIFE STAGE-DEPENDENT MATERNAL TRANSMISSION IN ZEBRAFISH

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Fluoxetine (FLX) is a pharmaceutical used as antidepressant that reaches low µg/L concentrations in the environment, affecting wildlife. In humans and fish, acute FLX treatment/exposure is linked to endocrine disruption, including effects on the reproductive and stress axes. Basing on the recent finding that developmental FLX exposure can reduce cortisol production across generations, our study seeks possible parental and/or life-stage contributions to this transgenerational phenotype transmission. Using zebrafish as model organism, we measured basal cortisol levels in 12 days post-fertilization (dpf) larvae (F_1) that descended from control and FLX-exposed individuals (F_0), mated at 5 and 9 months old. To investigate potential molecular contributions, we profiled maternally deposited transcripts involved in stress regulation, epigenetic (de novo DNA methyltransferases) and post-transcriptional regulation of gene expression (miRNA pathway components and specific miRNAs) in unfertilized eggs. We found lower basal cortisol levels in the F_1 descended from FLX exposed F_0 females bred at 5 ($p<0.001$), but not at 9 months ($p=0.696$), revealing a maternal, life-stage dependent effect. No paternal effect was found in any case. Furthermore, maternal FLX exposure decreased transcript abundance of glucocorticoid receptor (*gr*), de novo DNA methyltransferases (*dnmt3-4* and *dnmt7-8*) and miRNA pathway components (argonaute *ago2* and ribonucleases *dicer* and *drosha*) in eggs collected at 5 months. Specific miRNAs predicted to target stress axis transcripts decreased (*miR-740*) or increased (miRNAs *-26*, *-30d*, *-92a* and *-103*) in eggs collected from FLX females at 5 months. Increased abundance of *miRNA-30d* and *miRNA-92a* even persisted at 9 months. In conclusion, our results shows that the reduced basal cortisol phenotype is maternally inherited and life-stage depending and it suggests that the phenotype transmission to the offspring can be driven via miRNA signaling (gamete miRNA abundance).

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MOVING THE LAST DECADES OF ENDOCRINE DISRUPTION WORK INTO A PROVINCIAL ROUTINE SCREENING PROGRAM FOR COMPLEX EFFLUENTS

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Endocrine disrupting chemicals (EDCs) are mostly xenobiotics, which have the ability to mimic or inhibit natural hormones. Their presence in aquatic ecosystems can affect the development and reproduction of aquatic wildlife. However, EDCs are not yet regulated in municipal and industrial wastewater, and this for any countries, including in Canada. The current EDC frameworks of the United States' Environmental Protection Agency, Japan's Ministry of Environment, China's Ministry of Environment, the European Union, and the Organisation for Economic Co-operation and Development currently aim to identifying EDCs using a single compound testing approach. However, the reality of cities (including hospitals and industry) is to deal with complex mixture effluents. These effluents could yield an overall endocrine disrupting (ED) activity different than the sum of individual ED activity of each compound. Therefore, we are proposing a two-Tier approach for testing complex wastewater mixture focused on reproductive bioassays. The first Tier consists in performing three *in vitro* bioassays: the transactivation assay of the human estrogen and androgen receptors, and the assay of steroidogenesis in H295R cells, while the Tier 2 entails conducting the fish short term reproduction assay to validate any positive scores obtained in Tier 1. The optimization and validation of the bioassays using estrogen and dihydrotestosterone is ongoing. When fully working, these bioassays will assist municipal, provincial, and federal government in a first phase of testing EDCs in municipal and industrial effluents. Altogether, this long-term research program aims to better manage the water quality being released into the Canadian ecosystems.

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EPIGENETIC REPROGRAMMING AND TRANSGENERATIONAL INHERITANCE OF EPIMUTATIONS IN MEDAKA

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Postfertilization epigenome reprogramming erases epigenetic marks transmitted through gametes and establishes new marks during mid-blastula stages. A mouse embryo undergoes dynamic DNA methylation reprogramming after fertilization, while in zebrafish the paternal DNA methylation pattern is maintained throughout early embryogenesis and the maternal genome is reprogrammed in a pattern similar to that of sperm during the mid-blastula transition. Here we show DNA methylation dynamics in medaka embryos, a biomedical model fish, during epigenetic reprogramming of embryonic genome. The sperm genome is hypermethylated and the oocyte genome hypomethylated prior to fertilization. After fertilization, the methylation marks of sperm genome are erased within the first cell cycle and embryonic genome remains hypomethylated from zygote until 16-cell stage. The DNA methylation (5-mC) level gradually increased from 16-cell stage through the gastrulation stage. The 5-hydroxymethylation (5-hmC) levels showed an opposite pattern to DNA methylation. The pattern of genome methylation in medaka was thus very similar to mammalian genome methylation but not to zebrafish. We also screened for environmental estrogen-induced epimutations that survived epigenetic reprogramming in primordial germ cells (PGCs) and represented in sperm. The present study suggests that a medaka embryo resets it's DNA methylation pattern by active demethylation and gradual remethylation similar to mice. Environmental estrogen-induced epimuations can survive reprogramming, and additional unique epimutations are established *de novo* during gametogenesis. Our results provide new information regarding endocrine disruption and environmental epigenetics research.

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GUT MICROBIOTA AND PHYTOESTROGEN-ASSOCIATED INFERTILITY IN SOUTHERN WHITE RHINOCEROS

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With recent poaching of southern white rhinoceros (*Ceratotherium simum simum*; SWR) reaching unprecedented levels, a robust *ex situ* population is likely necessary to ensure the survival of this species. Although SWR have reproduced well in managed settings historically, the global captive SWR population is not currently self-sustaining due to the reproductive failure of captive-born females. Dietary phytoestrogens have been established as a likely cause of this phenomenon as recent work has demonstrated a negative relationship between diet estrogenicity and fertility of captive-born female SWR. To further examine this relationship, we compared gut microbial communities, fecal phytoestrogens and their metabolites, and fertility of SWR to the greater one-horned rhinoceros (*Rhinoceros unicornis*; GOHR). Both species typically consume similar high phytoestrogen diets in managed settings, but GOHR exhibit much higher levels of fertility than SWR. Using 16S rRNA amplicon sequencing we identified distinct microbial communities in each of the two rhinoceros species. However, analysis of phytoestrogen and metabolite profiles by mass spectrometry failed to demonstrate any species-specific differences. Employing a hierarchical clustering approach, we identified three dominant fecal phytoestrogen profiles common to both species and then evaluated their estrogenicity using SWR and GOHR estrogen receptor activation assays. These profiles exhibited different levels of estrogenicity when tested *in vitro* with profiles dominated by the microbial metabolite, equol, stimulating the highest levels of receptor activation. Finally, we found that fertility of individual SWR varies with respect to phytoestrogen profile produced, and to the abundance of several bacterial taxa and microbially-derived phytoestrogen metabolites. Taken together, these data suggest that in conjunction with species differences in estrogen receptor sensitivity to phytoestrogens, fertility may be affected by the transformation of dietary phytoestrogens by gut microbiota in captive SWR females.

TRANSGENERATIONAL REPRODUCTIVE EFFECTS OF TWO SEROTONIN REUPTAKE INHIBITORS AFTER ACUTE EXPOSURE IN *DAPHNIA MAGNA* EMBRYOS

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Pharmaceutical release into the aquatic environment has been a major concern for some time but in many cases we lack a fundamental understanding of the underlying mechanisms through which these compounds can modulate the physiology and development of target organisms. Specifically, the long term transgenerational effects of acute exposures during embryogenesis remain largely unknown. The purpose of the present study was to determine the transgenerational effects of commonly used serotonin re-uptake inhibitors (SSRIs - fluoxetine and sertraline) on the life history of *Daphnia magna*, after acute exposure during embryongenesis. These SSRIs are commonly found in freshwater ecosystems for decades. We found that a 72h exposure of *D. magna* embryos had long lasting consequences for their life history into the 4th generation post exposure. Moreover, we identified direct effects on heart rate and swimming behavior in the first generation that carried over from embryonic exposure. Because of the short reproductive period of daphnia and their integral role in aquatic food webs, our results suggest that even low amounts of SSRIs in the environment can have population-level implications.

ACTIVE FEMINIZATION OF THE PREOPTIC AREA OCCURS INDEPENDENTLY OF THE GONADS IN *AMPHIPRION OCELLARIS*

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Sex differences in the anatomy and physiology of the vertebrate preoptic area (POA) arise during development, and influence sex-specific reproductive functions later in life. Relative to masculinization, mechanisms of feminization of the POA are not well understood. The purpose of this study was to induce sex change from male to female in the anemonefish *Amphiprion ocellaris*, and track the timing of changes in POA cytoarchitecture, composition of the gonads and circulating sex steroid levels. Reproductive males were paired together and then sampled after 3 weeks, 6 months, 1 year and 3 years. Results show that as males change sex into females, number of medium cells in the anterior POA (parvocellular region) approximately double in females over the course of 1 year. Feminization of gonads, and plasma sex steroids occur independently, on a variable timescale, up to years after the POA sex change has completed. Findings suggest the process of POA feminization is orchestrated by factors originating from within the brain as opposed to being cued from the gonads, consistent with the dominant hypothesis in mammals. Anemonefish provide an opportunity to explore active mechanisms responsible for female brain development in an individual with male gonads and circulating steroid levels.

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EVIDENCE FOR ROLES OF ANGIOGENESIS IN FOLLICULOGENESIS OF ZEBRAFISH

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Angiogenesis is the process of new blood vessel formation and maturation from pre-existing vessels, and vascular endothelial growth factor (VEGF) is a key regulator of physiological angiogenesis during embryogenesis, skeletal growth and reproductive functions. Our recent data using transgenic zebrafish *Tg(flila:EGFP)* showed extensive angiogenesis during follicle growth in the zebrafish ovary, especially at follicle activation when the primary growth (PG) follicles were recruited to enter the pre-vitellogenic (PV) stage. The PG-PV transition was characterized by extensive blood vessel formation in the follicle layer surrounding the oocyte. This observation is further supported by our transcriptome analysis during PG-PV transition. A large number of GO terms and pathways involved in blood vessel formation and angiogenesis were demonstrated to be up-regulated from PG to PV, including angiogenesis, sprouting angiogenesis, vasculogenesis, blood vessel development, blood vessel morphogenesis, lymph vessel formation, response to hypoxia, and regulation of VEGF receptor signalling pathway. To further understand how the machinery of angiogenesis works during follicle development, especially the VEGF signalling system, we are now carrying out experiments on spatial distribution of key molecules of the VEGF signalling and related processes in the two compartments of the follicle (oocyte vs. follicle cells). Our hypothesis is that as the major component that grows the most during follicle development, the oocyte may likely be the controlling center that releases angiogenic molecules to the surrounding somatic follicle layer where the blood vessels are located and develop. Gene targeting techniques are now being used to disrupt some key angiogenic genes to assess the functional importance of angiogenesis in folliculogenesis. Our study will provide critical information about a new regulatory mechanism underlying folliculogenesis of fish and vertebrates in general.

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INVOLVEMENT AND EXPRESSION OF GH/IGF SYSTEMS GENE IN THE OVARIAN DEVELOPMENT OF TURBOT

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Accumulating evidence suggests that the growth hormone (GH)/insulin-like growth factor (IGF) system participates in fish reproduction. To understand the physiological functions of the GH/IGF system, the mRNA expression profiles of all known members of the GH/IGF system, including hepatic and ovarian *gh*, GH receptor (*ghr*), IGFs (*igf-i*, *igf-ii*), IGF-I receptor (*igf-ir*) and IGF binding protein (*igfbp1*, *igfbp2*), pituitary *gh*, and hepatic vitellogenin (*vtg*) were investigated during ovarian development in turbot *Scophthalmus maximus*. Results showed that *ghr*, *igf-i*, *igf-ii*, *igf-ir*, and *igfbp2* were expressed in the liver and ovary, whereas *igfbp1* and *gh* were undetected. The hepatosomatic index (HSI) and gonadosomatic index (GSI) gradually increased and peaked during the late vitellogenesis (Latvtg) and migratory nucleus (Mig-nucl) stages, respectively. The mRNA expression profiles of ovarian *ghr*, *igf-ii*, hepatic *igf-ir*, *vtg*, and pituitary *gh* were similar to the HSI; ovarian *igf-i* and igf-ir expression was close to the GSI. However, the hepatic mRNA levels of *ghr*, *igf-i*, and *igf-ii* peaked at the early vitellogenesis (Evtg) stage, and then drastically declined during ovarian development. The mRNA expression of hepatic igfbp2 decreased and reached the lowest at the atresia (Atre) stage, whereas that of ovarian *igfbp2* increased and peaked at Latvtg stage. Furthermore, significant correlations between pituitary *gh*, ovarian *ghr*, *igf-i*, and *igf-ii*, and hepatic *ghr*, *igf-i*, *igf-ir*, and *igf-ii* were observed, respectively. These results suggest that GH/IGF members appear to play distinct roles in the regulation of ovarian development in turbot and will be valuable for fish reproduction and broodstock management of aquacultured fish species.

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G-PROTEIN COUPLED ESTROGEN RECEPTOR (GPER) IN FEMALE ZEBRAFISH REPRODUCTION

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G-protein coupled estrogen receptor (GPER) which is an orphan receptor belonging to the G-protein family has previously been shown to inhibit oocyte maturation in vitro In teleosts. This inhibition or meiotic arrest is vital to the maintenance of proper reproductive timing. However, studies in murine models indicate that GPER may be dispensable in the mediation estradiol's effects in reproduction. To address this question, we generated two GPER mutant lines using CRISPR/Cas9 resulting in a -8 and a -10 deletion both in the 2nd exon of GPER. In these mutants reduced fertility was observed, this phenomenon is in direct contrast to the lack of reproductive defects found in mice GPER mutants. Additionally, GPER mutant females have more stage III and stage IVa oocytes compared to the wildtype, possibly indicating dysfunction during these stages. We also found that stage IVa full grown immature oocytes from GPER mutant females showed no significant changes in oocyte maturation compared to wildtype in an in vitro GVBD assay. Interestingly we observed the gene expression changes in the hypothalamic–pituitary–gonadal axis (HPG axis). In the brain, estrogen receptor α and β are upregulated in GPER mutants. In the ovary, enzymes involved in steroid hormone synthesis are downregulated, including hsd3b1, which is essential for progesterone synthesis. Our results suggest an important role of GPER in female reproduction. Further experimentation is in progress to elucidate the role of GPER in mediating estradiol's effects in oocyte maturation arrest in the early stage oocytes.

STEROID-5ALPHA-REDUCTASE TYPE 2 KNOCK-OUT IN *SILURANA TROPICALIS*

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Steroid-5alpha-reductase (srd5a) is a class of enzymes capable of reducing the C4-C5 double bond of steroids. Throughout the body, the isoforms of srd5as are implicated in various biological functions. Allopregnanolone, a neurosteroid, is synthesized by srd5a1 and is able to decrease anxiety and seizure risk, among other functions. In contrast, srd5a2 is primarily known for the synthesis of 5a-dihydrotestosterone (5a-DHT), an androgen more potent than testosterone, mainly involved in the development of male secondary sexual characteristics. Finally, srd5a3, the most recently discovered isoform, is known so far to be involved in N-glycosylation. Based on an extensive review of inhibition and knock-out studies of srd5as across vertebrates, we found that srd5as could have an array of other functions, including roles in neurodegenerative disease prevention, clearance of glucocorticoids, and the development of bones. In addition, recent publications have identified novel regulation routes for srd5as through hormonal crosstalks and epigenetic pathways. This review also highlighted the critical lack of information in non-mammalian species. Previous studies have shown that inhibiting *Silurana tropicalis*' srd5a2 using finasteride lead to intersex froglet individuals (presence of testicular oocytes) and a female biased sex ratio. These data suggest that 5a-DHT plays a role into gonadal determination, which contrasts with mammal studies, and also highlights another molecular pathway by which endocrine disrupting chemicals (EDCs) could interfere with amphibian physiology. In order to understand the biological function of srd5a2 and to pinpoint the exact molecular mechanism leading to the intersex condition in frogs, a knock-out (KO) line of *srd5a2* in *S. tropicalis* was designed. Using CRISPR, a deletion of 4 bp was obtained which affects the reading frame of the protein. The heterozygous F0 and F1 generations have now reached the adulthood, while the homozygous F2 is still developing. This literature review will help to better understand the role of srd5as in vertebrates, while this KO study will complement our current understanding on srd5a2 regulation and function in amphibians. Ongoing analysis will be presented.

Acknowledgements: This work was done in collaboration with the National Xenopus Resource (Marine Biological Laboratory, MA, USA) and supported by a discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a Canada Research Chair (CRC) in Ecotoxicogenomics and Endocrine Disruption to VSL.

Tuesday, May 28 - GnRH-related Peptides in Metazoa: Recent Progress and Discoveries

CORAZONIN NEUROENDOCRINE PATHWAY ORCHESTRATES STRESS-ASSOCIATED PHYSIOLOGY IN *DROSOPHILA*

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Environmental factors challenge the physiological homeostasis in animals, thereby evoking stress responses. Various mechanisms have evolved to counter stress at the organism level, including regulatory neuropeptides and hormones. Corazonin (Crz) is a neuropeptide known to regulate stress-associated physiology in *Drosophila*. However, the neural circuits and hormonal pathways underlying this regulation are poorly known. To unveil targets of Crz we mapped the *Crz receptor* (*CrzR*) expression in the *Drosophila* central nervous system (CNS). The *CrzR* is expressed in peptidergic neurons in the adult CNS, including the median neurosecretory cells and certain clock neurons in the brain, Hugin neurons in the suboesophageal zone (SEZ) and *CAPA*-expressing neurosecretory cells in the SEZ and abdominal neuromeres (Va neurons). We focused on the Va neurons since they produce osmoregulatory peptides (CAPA-1 and CAPA-2) which mediate recovery from desiccation and cold stress. *Trans*-Tango labeling to determine synaptic partners failed to reveal connections between Crz and Va neurons, suggesting hormonal interactions. To validate the signaling between Crz and Va neurons, we show that knockdown of *Crz* in Crz-producing neurons and *CrzR* in Va neurons increases survival under desiccation and delays chill coma recovery. Moreover, immunolabelling data suggests that Crz is released under nutritional and osmotic stress, and *in vivo* Crz peptide injections influence responses to desiccation and chill coma. Thus, Crz modulates Va neurons to maintain osmotic/ionic homeostasis, which in turn influences stress tolerance. Taken together with previous data, our findings suggest that Crz acts via both the fat body and CNS peptidergic neurons to regulate stress-associated physiology.

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FROM ECHINODERMS TO HUMANS – EXPLORING THE EVOLUTION OF METAL-BINDING TO GNRH PEPTIDES.

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In vertebrates gonadotropin-releasing hormone I (GnRH-I) is a key regulator of reproductive development and function. The receptor-binding activity of human GnRH-I can be modified by the presence of divalent copper – metal induced structural change in GnRH-I is thought to influence receptor interactions and subsequent intracellular signalling cascades. Until recently it was not known if copper-binding was a general feature of GnRH-type peptides in all taxa or if it was restricted to vertebrate GnRH. We have characterised copper binding to a newly identified GnRH-type peptide from the starfish *Asterias rubens* (ArGnRH). Using a range of spectroscopic and biophysical techniques we showed that this peptide can specifically bind copper(II) and nickel(II). Copper(II) is bound in a square-planar, high-affinity (K_d ~10^{-12} M) site incorporating four nitrogen donor atoms from a histidine imidazole group, two amides and the N-terminal amine group. The ArGnRH copper affinity and geometry are quite different to GnRH-I which we suggest means the copper sites have evolved to suit the environment the peptides are exposed to. By comparing the copper binding sites in ArGnRH and human GnRH peptides and conducting a phylogenetic analysis of GnRH-type peptide sequences from a range of species, we predict that copper-binding is an evolutionarily ancient feature of GnRH-type peptides that has been retained, modified or lost in different lineages

ADIPOKINETIC HORMONE IN A GASTROPOD: INSIGHT FROM LOCALIZATION AND FUNCTIONAL STUDIES

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Adipokinetic hormone (AKH) is a member of the gonadotropin-releasing hormone superfamily. In arthropods, AKH is primarily associated with the mobilization of energy reserves during periods of heightened physical activity. However, its function is less understood in other protostome lineages. In 2014, the first lophotrochozoan AKH (ac-AKH) was discovered in a gastropod, *Aplysia californica,* and has since been a peptide in search of a function. The present study summarizes several known features of ac-AKH with the overarching goal of elucidating its key functions. Initial *in situ* hybridization studies revealed that *ac-AKH* was expressed only in a few neurons of three central ganglia: the cerebral, abdominal, and pleural ganglia. However, immunohistochemistry revealed a much wider central distribution of ac-AKH-positive fibers, suggesting *ac-AKH* is an inter-ganglionic neuromodulator transported throughout the entire central nervous system. This notion was further supported by a specific ac-AKH radioimmunoassay (RIA) showing high levels of ac-AKH peptide in not only the source ganglia, but also other central ganglia lacking ac-AKH-producing neurons. However, ac-AKH was undetectable in any the peripheral tissues examined, including the gills, ovotestis, accessory genital mass, and large hermaphroditic duct. Functional studies suggested ac-AKH had diverse effects on metabolic, reproductive, digestive and osmoregulatory systems. In sum, our data show that ac-AKH is produced exclusively by a few neurons in select central ganglia but is transported widely to the entire central nervous system as an inter-ganglionic communicator. These findings are consistent with the ability of ac-AKH to elicit diverse effects on most major physiological systems.

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INSIGHT INTO GNRH-RELATED NEUROPEPTIDE RECEPTOR SPECIFICITY REVEALED THROUGH ANALYSIS OF NATURALLY OCCURRING AND SYNTHETIC ANALOGS OF THIS NEUROPEPTIDE FAMILY

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Adipokinetic hormone (AKH), corzazonin (CRZ) and the AKH/CRZ-related peptide (ACP) are peptides considered homologous to the vertebrate gonadotropin-releasing hormone (GnRH). We recently characterized and functionally deorphanized all of the *Aedes aegypti* GnRH-related neuropeptide receptors, which individually exhibit high specificity for their native ligands. While homologous receptors in other insects exhibit some degree of promiscuousness showing activation by closely related GnRH-related ligands, the strong ligand selectivity observed in *A. aegypti* prompted us to investigate the contribution of ligand structures in conferring receptor specificity. We designed a series of analogs based on the native ACP sequence in *A. aegypti* and screened them against the ACP receptor using an *in vitro* heterologous system that revealed critical residues of ACP required for activation of its receptor. Specifically, free acid and analogs replacing aromatic residues abolished all activity, whereas replacement of charged residues did not have detrimental effects. Similarly, we also utilized this high-throughput approach against an *A. aegypti* AKH receptor testing a number of naturally occurring AKH analogs from other arthropods to determine how substitution to amino acids in the AKH ligand influences receptor activation. AKH analogs having single substitutions compared to the endogenous *A. aegypti* AKH revealed position 7 (naturally serine) had only minor consequences to AKH receptor activation. However, substitution to position 6 (naturally proline) had pronounced effects, with receptor activity comprised nearly ten-fold. Lastly, substitution at position 3 (naturally threonine) was quite detrimental to analog activity on the *A. aegypti* AKH receptor with a significant decrease in analog activity. Analogs with combinations of these substitutions demonstrated even further reductions to receptor activation. Collectively, these results will advance our understanding of how the three GnRH-related systems in *A. aegypti* sustain independence of function and signalling despite their relatively high degree of ligand and receptor homology.

Acknowledgements: Supported through an NSERC Discovery Grant (JPP), Petro Canada Young Innovator Award (JPP) and an Early Research Award from the Ontario Ministry of Research & Innovation (JPP).

P1 LIGANDED THYROID HORMONE RECEPTOR ACTIVATES METHYL-CPG BINDING DOMAIN PROTEIN 3 (MBD3) THROUGH BINDING TO AN INTRONIC TRE DURING XENOPUS METAMORPHOSIS

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Thyroid hormone (T3) is important for adult organ function and vertebrate development, particularly during the postembryonic period when many organs develop/mature into their adult forms. Amphibian metamorphosis is totally dependent on T3 and can be easily manipulated, thus offering a unique opportunity for studying how T3 controls postembryonic development in vertebrates. Numerous early studies have demonstrated that T3 controls frog metamorphosis through T3 receptor (TR)-mediated regulation on T3 response genes, where TR forms heterodimer with RXR (9-cis retinoic acid receptor) and binds to TREs in T3-response genes to regulate their expression. The unliganded TR recruits corepressor complexes to repress gene expression, while the liganded TR recruits coactivator complexes to activate gene expression. To identify direct T3 response genes during metamorphosis, we previously carried out a ChIP (chromatin immunoprecipitation)-on-Chip analysis with a polyclonal anti-TR antibody on the intestine from premetamorphic tadpoles treated with or without T3 and identified a number of putative TR target genes. Among them is the methyl-CpG binding domain protein 3 (MBD3) gene, which has been implicated to play a role in epigenetic regulation of cellular processes as a subunit of the Mi-2/NuRD (Nucleosome Remodeling Deacetylase) complex. We show here that MBD3 is upregulated in the intestine and tail of premetamorphic tadpoles upon T3 treatment and its expression peaks at stage 62, the climax of metamorphosis during natural development. We further show that a putative TRE within the first intron of the MBD3 gene binds to TR/RXR *in vitro* and *in vivo*, and mediates T3 regulation of the MBD3 promoter *in vivo*. The data demonstrate that MBD3 is activated by T3 through TR binding to the intronic TRE during *Xenopus tropicalis* metamorphosis, implying a role of MBD3-dependent epigenetic regulation in the T3-induced gene regulation cascade underlying metamorphosis.

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P2COLD HARD FACTS" ABOUT THE THYROID HORMONE-INDUCED MOLECULAR MEMORY OF RANA CATESBEIANA AT LOW ENVIRONMENTAL TEMPERATURES

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Thyroid hormone (TH) is an essential signaling molecule in amphibian metamorphosis. Alongside TH, temperature also plays a crucial role in controlling this complex developmental program. At warm temperatures (24°C) TH elicits induction of the metamorphic program, however at cold temperatures (5°C) this precocious response is eliminated. Interestingly, when tadpoles exposed to TH at 5°C are moved to 24°C they undergo accelerated metamorphosis, regardless of further TH presence. This indicates a TH-conferred memory is established at 5°C. As TH functions as a signaling molecule in metamorphosis through modulation of gene expression programs, it is proposed that this molecular memory is established and held on the molecular level. Previous targeted molecular analyses have identified that the transcription factor, TH induced b/Zip (thibz) may play an important role, however it is unknown what other transcriptomic factors are involved. Herein we use a cultured tail fin (C-fin) assay of the North American bullfrog Rana catesbeiana at both 5°C and 24°C to discover novel transcripts involved in the molecular memory and elucidate the dynamics of their regulation. Time course experiments determined thibz responds within 18 hours of exposure to TH at 5°C, demonstrating the earliest transcript response within the TH-conferred memory. When moved into 24°C there is an accelerated response of both thibz and other TH-response genes including TH receptor thrb. This occurs even when there is no additional TH added, illustrating that a priming event may occur in TH exposure at 5°C allowing an accelerated metamorphosis when more permissive temperatures occur. RNA-seq analyses identified one downregulated and seven upregulated contigs which also respond to TH at 5°C. Although the majority of the identified contigs are novel and as of yet do not have an annotation, those where an annotation is available are all involved in regulation of cellular processes. These results form the foundation of understanding early signaling events required in the establishment of TH-dependent gene expression programs and has implications for how environmental contaminants may influence hormone dependent developmental processes under changing environmental temperatures.

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P3 LOCALIZATION OF THE SODIUM-IODIDE SYMPORTER (NIS) IN THE BRAINS OF TELEOST FISH, AND ITS PROPOSED FUNCTION IN MAINTENANCE AND DEVELOPMENT

Holloway, Nick

Iodine is an essential element vital to bodily functions including metabolism, reproduction, and development in vertebrate organisms. In the body it is concentrated by the thyroid gland, which utilizes a protein called the sodium iodide symporter (NIS) to transport iodide from the blood into thyroid epithelial cells, where it is then organified to make thyroid hormones. Because iodine is an element that can only be obtained by dietary means, it is not surprising that aside from its location in the thyroid gland, NIS is also in the gastrointestinal tract of mammals where it transports iodine from ingested food into the circulation. Both the GI tract and the thyroid are intuitive locations for NIS, however others and we have found it in more physiologically cryptic locations, such as the brain. Its presence in the nervous system is surprisingly an atypical and still speculative finding, even given the brain's well-established role as a thyroid hormone target tissue during development and nervous system maturation. It has been shown that in early human brain development an absence of thyroid hormone leads to a lack of myelination in neurons and decreased cell migration and proliferation, along with increased cell death. So while there have been multiple sources indicating need for thyroid hormone transporters (OATP14 and MCT8) in CNS development, no such clear functions have been established for NIS in mammalian or non-mammalian species. Because thyroid hormone is activated and deactivated by removal of constituent iodine atoms, it has been our interest to elucidate where and if NIS is functioning in the developing nervous system. We have recently found NIS presence in 5 species of adult teleost fish (red drum, *Sciaenops ocellatus*; channel catfish, *Ictalarus punctatus*; hybrid striped bass, *Morone saxatilis*; tilapia, *Oreochromus niloticus* ; and zebrafish, *Danio rerio*). A tractable genetic model system (*Danio rerio*) was then used to perform *in situ* hybridization to localize the presence of NIS in developing embryos, as well as adult brain tissue. Embryos and tissues were then cryo-sectioned and imaged. It is our finding that NIS is not only in a broad distribution of adult teleost fish species, spanning 3 order, but also localized to areas of major endocrine feedback and still even more cryptic locations.

P4 RECEPTOR INDENTIFICATION OF THE CRF-RELATED PEPTIDE, TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP); RECEPTOR KNOCKDOWN BY siRNA AND CRISPR/Cas9 METHODS

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Teneurin C-terminal associated peptide (TCAP)peptide with structural similarity to antagonistic to CRF *in vivo* and *in vitro* Endogenously, the TCAPs represent the C-terminal 40-41 amino acids of the teneurin genes. The teneurins are a family of proteins with a unique architecture that resembles bacterial Tc toxins, where TCAP is part of the exposed 'toxin-like' domain. The putative receptor for the TCAPs are the latrophilins, which are a family of adhesion G-protein coupled receptors that bind to the teneurins trans-synaptically in both vertebrates and invertebrates. Recent evidence suggests that TCAP binds to latrophilin's hormone binding domain (which shares homology with the CRF receptor binding domain) and evokes an intracellular signalling cascade through $Ga_{q/11}$. To examine the role of latrophilin in TCAP's cellular actions, we knocked down latrophilin-1 and 3 in the murine skeletal muscle C2C12 cell line by siRNA and CRISPR/Cas9. There was a significant reduction in TCAP-1-mediated intracellular calcium release in the knockdown cells compared to wildtype cells. These studies provide a novel understanding of how TCAP modulates cellular activity in vertebrates and offer new insights into CRF regulation.

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P5 TRIIODOTHYRONINE AND 3,5-DIIODOTHYRONINE EXPOSURE SELECTIVELY AFFECT AXOLOTL METAMORPHOSIS PROCESS AND SPEED

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Axolotl (Ambystoma mexicanum) is a neotenic salamander, which under natural conditions does not undergo metamorphosis. Under laboratory conditions, several groups report that immersion or intraperitoneal administration of the thyroid hormone (TH) prohormone T4 or the bioactive TH T3 are able to artificially induce metamorphosis; however, these protocols differ in the way of administration, age of animals and frequency of administration. In another hand, 3,5-T2, is a TH which has been shown to exert thyromimetic effects in fish and mammals but its effects on amphibian metamorphosis has not been tested. To compare the effect of the different THs upon metamorphosis, groups of axolotls of the same age (18 months) we treated by immersion with 500 nM of T4, T3 and 3,5-T2 every 3 days for 9 days, but we monitored the experiment for a total of 30 days. Control animals were immersed in vehicle (0.001 M NaOH). The onset of metamorphosis was determined when external gills and dorsal fin began to reabsorb. T4 and T3 were the only hormones capable of inducing axolot metamorphosis, even when the treatment was suspended at 9 days. Gill reabsorption was induced earlier in T3-treated axolotls as compared to T4-treated animals. 3,5-T2 showed early reabsorption of external gills but metamorphosis was not completed when treatment was suspended. Interesting, only for 3,5-T2 treated axolotls, this reabsorption was reversed by day 40 after the treatment was suspended. Because 3,5-T2 has been shown thyiromimetic effects at higher concentrations in mammals, we modified the protocol and decided to change the frequency of administration and the 3,5-T2 concentrations to every 2 days and 2 uM respectively. As controls, we treated axolotls in the same manner with rT3 (a poor affinity ligand) to rule out pharmacological effects. Interesting, 3-5 T2 treatment promotes gill reabsorption at about day 18, whereas rT3 did not. After 40 days of 3,5-T2 treatment, axolotls morph into salamanders, whereas animals treated with rT3 and control animals remain in the larval stage. In conclusion, exogenous THs affect metamorphosis of axolotl in different way and speed, showing that 3,5-T2 is also able to induce metamorphosis in this neotenic specie.

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P6 EFFECT OF HYPOTHALAMIC HORMONES UPON THE EXPRESSION AND RELEASE OF GROWTH HORMONE IN B LYMPHOCYTES FROM THE CHICKEN BURSA OF FABRICIUS

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It is known that growth hormone (GH) is expressed in immune cells, where it exerts immunomodulatory effects. However, the mechanisms of expression and release of GH in the immune system remain unclear. Since classical hypothalamic regulatory hormones (such as growth hormone releasing hormone [GHRH], thyrotropin releasing hormone [TRH], ghrelin, somatostatin [SST]) and their corresponding receptors are also expressed in B cells, we analyzed the effect of these hypothalamic hormones (HH) in B-bursal cells (BBCs). We evaluated the expression of HH receptors (by RT-PCR and IHC), quantified the GH mRNA expression (by qPCR) and the intracellular content and the GH released to the culture medium (ELISA) as well as CREB phosphorylation (by SDS-PAGE-WB), and calcium mobilization (by colorimetric assay) after the secretagogue treatments. The expression of TRH-R, GHS-$R1_a$ and SST-$R_{(1-5)}$ were confirmed; however, GHRH-R was absent in BBCs. TRH treatment increased GH mRNA expression (control: 100% vs 10 nM: 152.5%) and CREB phosphorylation (control: 100% vs 10 nM:180%). SST decreased mRNA expression of GH (control: 100% vs 1 nM: 41.55%; 10nM: 28.31%) and increased both, mobilization of calcium (control: 100% vs 100 nM: 184.4%) and intracellular content of GH (control: 100% vs 100nM: 168.02%). Finally, GHRH and ghrelin showed no effects related to the expression and release of GH. Our findings suggest a differential effect upon regulation of immune GH expression and release in comparison with the pituitary GH. In BBCs apparently TRH and SST might activate intracellular mechanisms that contribute to the transcription and release of the GH, while GHRH and ghrelin do not show any effect.

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P7 EVOLUTION OF TRANSTHYRETIN FROM 5-HYDROXYISOURATE HYDROLASE AFTER GENE DUPLICATION: WHAT HAPPENED TO AN ANCESTOR OF TRANSTHYRETIN?

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Vertebrate genomes are considered to have experienced whole-genome duplication at least two times. Gene duplication is a major driving force for evolution of novel functional genes; however, the underlying evolutionary mechanisms remain to be elucidated.Transthyretin (TTR), a plasma thyroid hormone distributor protein, is an example of neo-functionalization. TTR is hypothesized to have diverged from a functionally unrelated protein, 5-hydroxyisourate hydrolase (HIUHase), at some early stage of chordate evolution. Although the primary sequences of the two families share approximately 35% identities, these families have common exon/ intron structures and 3D structures of the tetramers. Evolution from an ancestor of HIUHase toward functional TTR may have occurred through several critical processes: (1) a change in cellular localization from intracellular to extracellular compartments, (2) amino acid substitutions at critical sites to become suitable for binding of TH, (3) other amino acid substitutions that are involved in the formation of high-affinity binding sites, the binding specificity for THs, and the protein stability in extracellular fluids. Our recent studies have revealed several unique properties of TTRs of primitive vertebrates which include high contents of Zn^{2+} and His residues in the tetramer with the presence of His-rich segments. If these were ancient properties in TTR, it is likely that these properties have contributed to the acquisition of TH binding activity in an ancestral HIUHase. In order to test this hypothesis, we recombinantly constructed mutant HIUHases with two amino acid substitutions (R54E/Y119T) at the active sites and/or an N-terminal 3×His (3×H) tag and mutant TTRs with or without an N-terminal His-rich segment, and investigated HIU hydrolysis and TH binding activity of these proteins. Here, we propose an evolutionary history of TTR from HIUHase in which functional trade-offs between HIU hydrolysis activity and TH binding activity might have sequentially occurred before and after gene duplication.

P8 PEPTIDERGIC ACTIONS ON MITOCHONDRIAL ENERGETICS AND MOVEMENT: ROLE OF TENEURIN C-TERMINAL ASSOCIATED PEPTIDE-1

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Teneurin C-terminal associated peptides (TCAP) consist of the 40-41 amino acids present at the carboxy terminal end of all four vertebrate teneurin paralogues as well as most (if not all) teneurin orthologues present in most invertebrates. Teneurins are evolutionarily ancient, having been introduced into Metazoa via lateral gene transfer into a single-celled ancestor. Although these peptides show some primary sequence similarity to the Cortictropin-Releasing Factor (CRF) and Secretin family of peptides, the TCAP peptides predate these peptides by some 200 million years. Since then, TCAP has remained highly conserved throughout all Metazoans. As a result, TCAP has evolved to play a critical role in mammal neuronal function associated with both energy metabolism and cytoskeletal dynamics. However, TCAP also increases the metabolic rate of adult and larval zebrafish as well as increase the glucose uptake in mammal myogenic cell lines and in skeletal muscle tissue. These studies demonstrate the important role TCAP plays in energy metabolism, glucose uptake, and muscle function and may indicate that TCAP could play a role in mitochondrial metabolism. Although there is some evidence to suggest that TCAP can regulate mitochondrial activity, the mechanism by which this occurs is unclear. We are currently investigating the mechanism by which TCAP regulates mitochondrial energetics and movement within the central nervous system. Current studies indicate that TCAP-1 modulates neuronal motility in vitro, a mechanism that appears to be associated with its actions on cytoskeletal action related to mitochondrial activity within the cytosol.

P13 FUNCTIONAL CHARACTERIZATION AND TISSUE-SPECIFIC EXPRESSION PROFILING OF TWO PYROKININ RECEPTORS IN ADULT AEDES *AEGYPTI* MOSQUITO

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Aedes aegypti are anthropophilic mosquitoes that transmit a number of arboviruses causing yellow fever, dengue, Zika virus, and chikungunya. With the rise of these vector-borne illnesses, advancing our understanding of mosquito biology and related physiological processes is imperative in order to develop new methods for vector control. Pyrokinin neuropeptides, characterized by a conserved FxPRLamide C-terminal motif, have been identified as myotropic and pheromonotropic in some insects, but their functions remain unclear in blood-feeding arthropods. Herein, we have functionally deorphanized two *A. aegypti* pyrokinin receptors (PK1-R and PK2-R) by profiling their selective and dose-dependent activation in response to pyrokinins. We have also examined receptor transcript expression, which showed enrichment of PK1-R in the rectum, and PK2-R in the ileum and reproductive organs. Immunohistochemical mapping in female adult mosquitoes revealed pyrokinin-like immunostaining in nerve projections innervating the rectum and terminating in close association to the rectal pads, which are structures proposed to be involved in transepithelial transport. Based on PK1-R enrichment at the transcript level, we further examined prospective physiological roles of its confirmed ligand (*Aedae*PK1) utilizing *in vitro* bioassays. Interestingly, *Aedae*PK1 did not influence myotropic or ionomodulatory (Na^+) activity in isolated recta. As a result, ongoing studies are examining the effects of receptor knockdown on diuresis, excretion, and reproductive biology, processes that heavily rely on organs where these receptors are enriched, in an attempt to better understand the functional role of pyrokinin signaling in mosquitoes.

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P14 INSULIN LIKE GROWTH FACTOR 3, A TARGET OF ANDROGEN, IS REQUIRED FOR SPERMATOGENESIS IN TILAPIA

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Fish spermatogenesis is tightly regulated by stage- and cell-specific interactions of various hormones, such as 11-ketotestosterone (11-KT), retinoic acid (RA) and 17a, 20b-dihydroxypregn-4-en-3-one (17α, 20β-DP, DHP). However, detailed information on the molecular mechanisms that regulate spermatogenesis by these hormones is not clear in fish. Here, we reported that fish specific *igf3* is required for spermatogenesis in a teleost fish, Nile tilapia (*Oreochromis niloticus*). *In vitro* gonadal culture with testosterone (T) and 11-KT treatment significantly increased *igf3* expression in a time- and dose- dependent manner. Consistently, knockout of androgen catalyzing enzyme gene *cyp11c1* led to down regulation of *igf3* expression. *In vivo* genetic studies strongly demonstrated that ablation of *igf3* severely inhibited spermatogonial differentiation and blocked the initiation of meiosis. Transcriptome analysis revealed that absence of Igf3 resulted in dysregulation of many genes that are critical for meiosis initiation and spermatogonial proliferation/differentiation. However, spermatogenesis resumed later and postmeiotic germ cells including spermatocytes, spermatids, and spermatozoa were observed in the mutants at 180 days after hatching. Adult *igf3*$^{-/-}$ male mutants were subfertile with drastically reduced sperm volume, which is similar to the phenotype of *cyp11b2* knockout males. In contrast, overexpression of Igf3 in XY fish resulted in precocious initiation of spermatogenesis by stimulating spermatogonia proliferation and differentiation. Taken together, our data from the present study have, for the first time, provided genetic evidences for *igf3* in regulation of spermatogenesis and Igf3 is identified as a downstream mediator of androgen in teleost species.

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P15 DO CATEGORICALLY DISTINCT STRESSORS AFFECT VISUAL ATTENTION TO FOOD IN HUMANS?

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Neuroanatomical and physiological studies support the idea of two (or more) pathways relaying stressor information to CRF neurons in the paraventricular nucleus (PVN); anticipatory stressors that reach the PVN through limbic system pathways and so-called reactive stressors that ascend from the brainstem via ventral noradrenergic pathways. While both anticipatory and reactive stressors have been reported to modulate food intake, there has been little work comparing how anticipatory and reactive stressor influence behavior. We examined the influence of an anticipatory stressor (Trier-social stress test, TSST) and a reactive stressor (cold-pressor test, CPT) on visual attention to food images in human participants. Participants (n = 60) were divided equally between control, TSST, or CPT groups. We measured salivary cortisol before and after stressor exposure. Following stressor exposure participants performed an eye tracking test using a standardized picture database. We analyzed three metrics in balanced pairs of food and non-food images: saccade latency, gaze duration, and saccade bouts. Missing data were replaced using harmonic means. Salivary cortisol was elevated over baseline in both stressor groups. Linear mixed model with repeated measures revealed main effects of image type for all three eye tracking variables, with initial saccades of shorter latency to food images and longer gaze duration and more saccade bouts with food images. There were no main effects of stressor group on any eye tracking variable. There was a statistically significant interaction between image type and stressor group for gaze duration ($p = 0.03$), both stressors significantly reduced gaze duration on food images relative to controls. There was a trend ($p=0.051$) for an interaction of the two independent variables on saccade bouts, with CPT tending to reduce the number of gaze bouts on food images. We conclude that both anticipatory and reactive stressors decrease time spent looking at food, but not non-food, images. These data are partly consistent with the idea that stressors adaptively reduce attention to non-critical visual signals.

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P16 AMHY DETERMINES MALE SEX VIA SIMULTANEOUS ACTIVATION OF GSDF AND REPRESSION OF FOXL2/CYP19A1A EXPRESSION IN TILAPIA

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The sexual fate of the bipotential gonads in vertebrates is under the control of specific genes that initiate the developmental pathway. In our current study, we provide further strong genetic evidences that *amhy* was the sex determining gene in Nile tilapia. We propose that male sex determination is initiated by the expression of the Y linked gene *amhy*, which in turn activates the *dmrt1* and *gsdf* in Sertoli cell precursors. Subsequently, *dmrt1/gsdf* initiates Sertoli cell differentiation, the critical supporting cell of the testis. Disruption of female promoting pathway, such as Foxl2 or estrogen deficiency, leads to complete rescue of gonadal sex reversal in XY *amhy* mutants, suggesting that the primary role of Amhy signaling is the repression of female-promoting genes. The differences in sex reversal ratio of XY, YY $amhy^{+/+}$, YY $amhy^{+/-}$ fish with estrogen treatment further demonstrated that *amhy* could antagonize the effects of estrogen induced feminization. In wild type XX fish, Foxl2 directly repress male pathway gene *gsdf* expression to promote ovary development, which might be the top gene determining ovary fate. Although ablation of germ cells masculinizes the somatic cells, the number of germ cell cannot contribute to sex determination because its difference occurs later than the time of genes expression differences in gonads. Taken together, these studies firstly provided further strong genetic evidences to support *amhy* as the sex determining gene of Nile tilapia. Secondly, *amhy* determine male sex via simultaneously activation of male downstream *dmrt1*/*gsdf* and repression of female *foxl2*/*cyp19a1a* genes expression. This is the first report clarifying male and female molecular cascades of sex determination and their mutual antagonism in teleost fish using knockout models.

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P17 REGULATION OF LEPTIN BY GLUCOSE, CORTISOL, AND EPINEPHRINE IN TILAPIA *(OREOCHROMIS MOSSAMBICUS)*

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Leptin is a cytokine that enhances energy mobilization and suppresses appetite in vertebrates, two processes often involved in the stress response. The hormone is known to increase under various stressors such as food deprivation, hyperosmotic challenge, or hypoxia in teleosts including the Mozambique tilapia (*Oreochromis mossambicus*). Currently, the regulatory interactions between classic stress hormones (e.g. cortisol, epinephrine), metabolites (e.g. glucose), and leptin in fishes and ectotherms generally is poorly understood. Using hepatocyte incubations and hormone injections, we evaluated the actions of glucose, cortisol, and epinephrine in regulating leptin in the liver, the major site of hormone production in tilapia. We show that synthesis and secretion of leptin A (LepA), the dominant paralog of leptin in fishes, declined as ambient glucose levels increased *in vitro* (10-25 mM). By contrast, bolus glucose injection increased *lepa* mRNA levels 14-fold at 6 hours, suggesting systemic factors regulated by glucose may counteract the direct inhibitory effects of glucose on hepatic *lepa* observed *in vitro*. Cortisol at physiological concentrations (100 nM) stimulated LepA secretion from hepatocytes at all timepoints becoming significant by 6 hours. Interestingly, *lepa* levels were suppressed, showing discordant regulation between synthesis and secretion of leptin by cortisol *in vitro*. Lastly epinephrine stimulated LepA secretion from hepatocytes in a dose-dependent fashion within 15 min but had little effect on *lepa*. The response was accompanied by increases in glucose release likely indicating a classical glycogenolytic effect by epinephrine. An epinephrine injection into tilapia stimulated a rapid rise in blood glucose which was followed by a 4-fold increase in hepatic *lepa* at 2.5 and 6 hours. Plasma LepA was also elevated by 6 hours relative to controls. These data show that tilapia leptin is negatively regulated by rises in extracellular glucose at the level of the hepatocyte but stimulated by hyperglycemia *in vivo*. Cortisol and epinephrine stimulate leptin suggesting these classical stress hormones may modulate glucose in part through their regulation of leptin. Together these results demonstrate that leptin is integral to the endocrine stress response and maintenance of energy homeostasis.

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P18 EARLY LIFE EXPOSURE TO 17β-ESTRADIOL AND NONYLPHENOL AFFECTS THE GH/IGF SYSTEM AND EXPRESSION OF ESTROGEN RECEPTORS IN MOZAMBIQUE TILAPIA

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Particular compounds released into the environment from anthropogenic activities affect the endocrine systems of vertebrates, including fishes. These endocrine disrupting compounds (EDCs) include hormones, pesticides, plasticizers, and other compounds that have short-term physiological and/or long-term developmental effects. We exposed Mozambique tilapia (*Oreochromis mossambicus*) yolk-sac fry to 17β-estradiol (E2) and nonylphenol (NP) and investigated their effects on growth and reproductive parameters in adults. Fry were exposed for 21 days to waterborne E2 (0.1 and 1.0 μg/L) and NP (10 and 100 μg/L). After exposure to the chemicals, juveniles were maintained in FW for an additional 112 days until when males were sampled. Gonadosomatic index was increased following exposure to 0.1 μg/L E2, while hepatosomatic index decreased with exposure to NP at 100 μg/L. Hepatic *growth hormone receptor (ghr)* mRNA levels increased following exposure to 0.1 μg/L E2. The high concentration of E2 (1 μg/L), and both concentrations of NP, increased hepatic *insulin-like growth-factor 1* (*igf1*) expression, while no effects were found on hepatic *igf2* and pituitary *growth hormone* levels. Exposures to 1 μg/L of E2 and 10 μg/L of NP induced hepatic *igf binding protein 1b* (*igfbp1b*) while 100 μg/L of NP increased hepatic *igfbp2b*. By contrast, hepatic *igfbp6b* was reduced in fish exposed to 1 μg/L of E2. There were no effects of E2 or NP on hepatic *igfbp4* and *igfbp5a* levels. Although the expression of three *vitellogenin* transcripts was not affected, E2 and NP stimulated hepatic *estrogen receptor (*α and β*)* levels. Our results indicate that tilapia exposed to E2 and NP during early life stages exhibit long-term changes in the endocrine systems controlling growth and reproduction at later life stages.

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P19 CORTICOSTERONE LEVELS AND IMMUNOLOGICAL STRATEGIES IN JUVENILE OF Caiman latirostris EXPOSED TO EXTREMES ENVIRONMENTAL CONDITIONS

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Evidence suggests that chronic stress may have a profound negative effect on the immune activity of all vertebrates. Apparently, the increments in plasma corticosteroids reduce immune activity because the resources are redirected towards activities that are more immediately valuable to survival. However, the effect of stress in wild vertebrates has been little investigated, especially in crocodilians. Crocodilians have to face to numerous stressors such as climatic factors, toxicant exposure, and microorganism in the habitats where they live. Exposure to stressors may affect the physiological processes of crocodilians, with consequences on their fitness and survival. Under controlled conditions, it was investigated the effect of environmental challenges (food restriction, water restriction and high temperature) during 4-week on growth, immunological investment and corticosterone levels of juvenile *Caiman latirostris*. White blood cells counts, natural antibody (NAb) levels and complement system activity were evaluated to characterize the influence of those treatments on the immune system. It was found that deprivation of food resulted in reduction in growth and body condition but high NAb levels, whereas high temperature had a beneficial effect on the variables measured. Individuals exposed to heat treatment (pulses of 37±1°C) grew more, and obtained better body condition, higher NAb levels, and significantly lower corticosterone levels relative to other groups. Our findings suggest that *C. latirostris* juveniles are able to tolerate environmental stressors and they are even favored by peaks of high temperatures. In spite of that, they activated immunological strategies to face the conditions evaluated in this study.

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P20 GENDER AND DOSE SPECIFIC IMPACTS ON LIVER METABOLOME OF C57BL/6J MICE AFTER DIRECT OR GESTATIONAL EXPOSURE TO BISPHENOL A

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Endocrine disrupting chemicals (EDCs) 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. BPA is persistent in the environment, and biomonitoring studies reveal pervasive human exposure. Rodent models demonstrate BPA-induced impacts on body weight, pancreatic function, glucose homeostasis and insulin signaling. However, 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. The potential for EDCs to disrupt circadian clock and circadian-driven cellular metabolism is not well characterized. Mass spectrometry-based metabolomics provides a platform to assess EDC-driven impacts on cell and organ-specific metabolite profiles. The purpose of the current study was to assess metabolomic changes in C57BL/6J adult mouse liver after direct or gestational BPA exposure. Mass-spectrometry based metabolomics was conducted to profile 340 aqueous phase liver metabolites harvested from adult mice after direct or gestational exposure to lower dose BPA (10 µg/kg/day), upper dose BPA (10 mg/kg/day) or to control (7% corn oil). Multivariate modeling using orthogonal partial least squares discriminant analysis (OPLS-DA) revealed significant metabolomic impacts following BPA exposure. In females directly exposed to BPA, metabolomic changes were observed as a result of exposure to upper but not lower dose BPA, with alterations seen in pyrimidine and cyanoamino acid metabolic pathways following upper dose BPA exposure. Metabolomic alterations were observed in adult male offspring as a result of gestational exposure to both lower and upper dose BPA. Pathways impacted by BPA exposure in male offspring include purine and pyrimidine metabolic pathways and aminoacyl-tRNA biosynthesis. The current analysis has revealed metabolic fingerprints of BPA exposure stratified by generation, dose and gender. Exposure induced impacts on amino acid as well as nucleotide metabolic pathways warrant further investigation as these pathways are important for nutrient cycling and energy homeostasis and have a strong aspect of circadian regulation.

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P21 ANDROGEN RECEPTOR IMMUNOREACTIVITY LOCALIZATION IN HATCHLING AMERICAN ALLIGATOR CLITERO-PENIS

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In many vertebrates, penis and clitoris differentiate during early development from a common bi-potential analgen. Much of male sexually dimorphic external genitalia growth during this period has been shown to be androgen-dependent and regulated through hormone binding of androgen receptors. The beginning of gross morphological size divergence of crocodylian phalli occurs around the time of hatching. While similar in size at hatch, male alligators double their penis length during the first three months post-hatch, as compared to a modest increase in clitoral lengths over the same time period. It stands to reason that sexually dimorphic circulating androgen concentrations contribute to this differential growth, but that external genitalia of both sexes have the capability of responding to the signal via androgen receptors. Here we localized androgen receptor immunoreactivity in histological sections of one-week-old alligator external genitalia from animals produced at male-producing (33°C) and female-producing (30°C) egg incubation temperatures. We characterize the overall histological architectures of penis and clitoris at this early life stage and present the AR-staining patterns observed in the various tissue types: epithelium, stromal tissues, and the semen-conducting sulcus spermaticus. By comparing and contrasting AR-immunoreactivity between genital tissue types and gonadal sex we begin to identify the androgen-responsive tissues that proliferate to masculinize crocodylian genitalia and those female tissues that potentially are responsive to exogenous androgenic signals.

P22 ENDOCRINE DISRUPTING EFFECTS OF ORGANIC ULTRAVIOLET-FILTERS ON MOLTING AND DEVELOPMENT IN *DAPHNIA MAGNA*

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Ultraviolet-filters (UV-Fs) are emerging contaminants of concern found ubiquitously in the aquatic environment. Many organic UV-Fs are endocrine disrupting compounds (EDCs) in vertebrates. However, few studies have assessed their effects on invertebrates. The process of molting, or the shedding of the exoskeleton, may be a target of these compounds. Molting is necessary for growth and development and is regulated by an arthropod specific endocrine system, the ecdysteroid pathway. Alterations of this process by EDCs can result in improper development, reduced growth, or death. These outcomes have potentially significant implications for organismal and population health. In this study, we investigate the effects of organic UV-Fs in a crustacean. *Daphnia manga* are chronically exposed to three common organic UV-Fs: 4-methylbenzylidene camphor (4MBC), octylmethoxycinnamate (OMC), and benzophenone-3 (BP3) and assessed for alterations in normal molting and development. We show that UV-Fs cause significantly more deformities in offspring Additionally, we evaluate the effects of acute exposure to 4MBC, OMC, and BP3 on the expression of several ecdysteroid-regulated genes (*EcRA, EcRB, HR3,* and *FTZ-F1*). This study is the first to assess the effects of organic UV-Fs on ecdysteroid regulated processes in a crustacean.

P23 ACTIVATION OF CALIFORNIA CONDOR ESTROGEN RECEPTOR VARIANTS BY XENOESTROGENS

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In 1987 the 27-remaining, free-flying California condors (*Gymnogyps californianus*) were brought into captive breeding programs. The population has since grown to over 450 individuals with over half of all condors living in small, reintroduced populations in Arizona, California and Baja California, Mexico. California condors are listed as critically endangered, and are under intensive human management. As a result, the pedigree for nearly every living condor is known. Whole genome sequencing of the 22 founder birds identified two genetically linked variants for condor estrogen receptor 1 (ESR1) (N161S, E162D) and one variant for ESR2 (T114M) present in the current population. These ESR variants were confirmed by direct PCR sequencing in 6 founder birds for ESR1 and 5 birds for ESR2. Given that coastal condors are exposed to high levels of xenoestrogens through scavenging of marine mammal carcasses, differences in vulnerability to xenoestrogens may exist depending on which ESR variant(s) an individual condor possesses. Site-directed mutagenesis was performed on wild-type receptors (ESR1 mutations were included in a single construct) to produce each of the full-length ESR variants and activation of variant and wild-type receptors by xenoestrogens was compared. The variant form of ESR1 was significantly more sensitive ($P<0.05$) to diethylstilbestrol (DES), ethinyl estradiol (EE2), bisphenol A (BPA) and *p,p'*- DDT, though there was no significant difference in activation by the endogenous estrogen (E_2), *p,p'*-DDE, *o,p'*-DDT or trans-nonachlor. Interestingly the condor ESR2 variant was only activated to a higher degree by one compound, *o,p'*-DDT. Although significant differences in activation of condor ESR variants by xenoestrogens occurred at high (micromolar) concentrations, they correspond to circulating concentrations previously reported in coastal birds. Release and relocation of California condors to the coast is a promising avenue for recovery, however, reproductive problems associated with xenoestrogen exposure pose a sub-lethal threat to long-term success. Based on above findings, future release decisions could be informed by individual ESR type to potentially reduce deleterious effects of xenoestrogen exposure and ultimately improve reproductive success in wild populations.

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P24 THE ENDOCRINE DISRUPTOR VINCLOOLIN INDUCES PENILE MALFORMATIONS BY MODULATING APOPTOSIS IN THE GENITAL TUBERCLE

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In recent decades, there has been a rise of endocrine-related diseases and disorders, including an increased incidence of genital anomalies, low semen quality, adverse pregnancy outcomes, neurobehavioral disruption, endocrine-related cancers, earlier onset of breast development, obesity, and type 2 diabetes (UNEP and WHO, 2013). An example of increased genital anomalies is seen with congenital penile anomaly (CPA) frequency, which has increased to a rate of 1 in 120, or 0.83%, of male newborns (Nelson et al., 2005). The most commonly reported CPA is hypospadias, which is characterized by an atypical urethral opening along the penile shaft, within the scrotum, or in the perineum. It was previously established that by specifying cell type and timing of deletion of the androgen receptor gene, a range of CPAs can be induced in the mouse that mimic the spectrum of human CPAs (Zheng et al., 2015). Male mouse embryos exposed to the endocrine disruptor vinclozolin develop external genital anomalies, and these mimic mouse androgen receptor-deletion defects and human CPAs. Quantification of sex steroid hormones and their respective receptors indicated that they are affected by vinclozolin treatment. Examination of cell death and proliferation demonstrated that these genital anomalies result from disruption of sexually dimorphic cellular processes that normally pattern the genital tubercle.

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P25 ENVIRONMENTAL ESTROGEN-INDUCED TRANSGENERATIONAL DIFFERENCES IN EXPRESSION OF OSMOREGULATORY GENES IN THE GILL OF MEDAKA FISH

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Embryonic bisphenol A (BPA) and 17α-ethinylestradiol (EE2) exposure can have far reaching health effects in fish, including adult onset transgenerational reproductive abnormalities, anxiety, cardiac disorders, etc. It is not known whether these two environmental estrogens can induce transgenerational osmoregulatory abnormalities in gills. The present study, therefore, examined transgenerational effects of BPA or EE2 exposure on genes that are critical for osmoregulation in medaka. Embryos were exposed to either BPA (100 µg/L) or EE2 (0.05 µg/L) for the first 7 days of embryonic development and never thereafter. Expression of osmoregulatory genes (NKAa1a, NKAa1b, NKAa1c, NKAa3a, NKAa3b, NKCC1a, and CFTR) was examined in the gills of the first-generation (F0) adults which were directly exposed as embryo and in the fourth-generation adults (F3), which were never exposed to these two environmental estrogens. Results showed significant alterations in expression of osmoregulatory genes in both F0 and F3 generations. At F0 generation, a sex-specific expression pattern was observed with a downregulation of genes in males and an upregulation of genes in females. At F3 generation, the pattern reversed as expression of majority of the genes were upregulated in males and downregulated in females, suggesting that an exposure to BPA and EE2 during embryonic development induces transgenerational impairment of molecular events associated with osmoregulatory functions. The transgenerational alterations in osmoregulatory gene network suggests possibility for osmoregulatory stress in fish inhabiting aquatic environments contaminated with estrogenic chemicals.

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P26 CHARACTERIZATION OF XENOPUS LAEVIS IODOTHYRONINE DEIODINASE (DIO3) ENZYME AND CHEMICAL INHIBITION COMPARISON WITH HUMAN DIO3

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Thyroid hormones are necessary for normal sequential development and metamorphosis of amphibian tissues and organs. These processes are regulated in part by activity of deiodinase (DIO) enzymes in peripheral tissues where deiodinases catalyze the removal of an iodine from thyroid hormones to either activate or inactivate the hormone. As part of an effort to evaluate chemicals for thyroid hormone disrupting potential, we previously conducted chemical screening using recombinant human DIO enzymes. To determine whether screening results from human proteins can be a surrogate for inhibition in other species, empirical cross-species comparisons are needed. Initial efforts to address this by expressing the *Xenopus laevis* DIO Type 3 in a human cell line (HEK293) produced low activity. Because deiodinases are selenoproteins containing the rare amino acid selenocysteine, they require not only the protein-coding region of the gene but also the selenocysteine insertion sequence (SECIS) within the 3'-untranslated region (UTR) to produce a functional protein. Replacing the *X. laevis* 3'-UTR with the 3'-UTR from the human DIO3 gene in the construct transfected into HEK293 cells resulted in doubling of *X. laevis* enzyme specific activity. Although the highest *X. laevis* DIO3 activity achieved was 1/20th that of human DIO3, the enzymes' affinities (measured Km) for the thyroid hormone substrate were similar. Amino acid identity is highly conserved between these species within the enzymes' catalytic sites, which can often be predictive of similar sensitivity to chemicals. Indeed, chemicals we tested for inhibition of DIO3 in concentration-response mode most often resulted in highly similar or identical curves between species. However, curves for some chemicals were significantly different. These results suggest that: (1) amino acid identity conservation within protein active sites generally informs cross-species predictions of chemical sensitivity, but more empirical data are needed to identify other contributing structure-activity factors, and (2) to accomplish high throughput chemical screening, species-specific cellular compatibility issues must be resolved to increase non-mammalian protein expression in mammalian cells.

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P27 DEFAULT SEX IN FLOG GONADS IN TREMS OF GENETICS, HORMONE, MORPHOGENESIS, AND GERM CELLS

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Default sex in gonads could be interpreted to be female or male from some points of view; genetic sex-determining systems supported by sex-determining genes, gonadal morphogenesis, germ stem cells, and gonadal hormones including sex steroids. Previously, we discovered a sex-determining gene *dm-W* in the African clawed frog *Xenopus laevis* holding a ZZ(male)/ZW(female)-type system, indicating male default. We also discovered a unique "mass-in-line" structure in both ZW and ZZ undifferentiated gonads of the species; the structure was formed with a number of cell masses aligned on the AP axis consisting of somatic cells having a potential to produce sex steroids. Importantly, the structure was deconstructed only in developing ZZ gonads, maybe by TGF-beta signaling, which could represent female default. As the third point, we observed ZW sex-reversed testes, when they had few germ stem cells during early gonadal development, which could propose "germ cell-supported female default". Then I will discuss different opinions for default sex in not only *X. laevis*, but also various vertebrate species.

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P28 ENDOCRINE DISRUPTION ON LARGEMOUTH BASS DUE TO GLYPHOSATE AND RODEO EXPOSURE

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Glyphosate is the herbicide most used worldwide, with no historic comparison. It is used for genetically modified crops, and particularly in Florida, it is used as a sugar cane ripener. It is also formulated to treat aquatic weeds in waterbodies as Rodeo®. Because of its extended used, it can run off or be sprayed directly into waterbodies and aquatic wildlife can be chronically exposed. Exposure in animal models has been associated with kidney and liver damage and it has been suggested as an endocrine disruptor. We exposed adult male largemouth bass for 21 days to two doses of glyphosate and Rodeo (chemically equivalent concentration of glyphosate) including 0.5 mg/L and 10 mg/L and clean water as a control (n=4/tank in quadruplicate). Concentrations during the experiment were corroborated with LC-MS/MS. Steroid hormones were measured in 50 µl plasma with LC-MS/MS. Based on generalized linear models, testosterone concentration was significantly increased (p-value < 0.05) in all treatments except for the lowest dose of glyphosate. Moreover, 11-ketotestosterone concentration was significantly reduced in all doses except the high dose of glyphosate (p-value < 0.05). Total RNA was isolated from the trunk kidney and RNAseq was performed for the high doses compared to controls (n=4/treatment). Transcripts were analyzed with Pathway studio and mapped to mammalian database. As expected, cellular processes associated with cellular stress, endocrine system and oxidative damage were significantly enriched for glyphosate and Rodeo. Glyphosate significantly enriched pathways of insulin response, hormone secretion and pancreas development while Rodeo significantly enriched hypothalamus function, endocrine pancreas development, beta-cell function and hormone secretion. Transcripts were also mapped to Zebrafish metabolic pathways using Paintomics and the most significantly enriched pathway was steroid hormone biosynthesis. Glyphosate and Rodeo produce endocrine disruption corroborated at the hormone and gene expression level and the probable mechanism of toxicity is through oxidative damage. Rodeo and glyphosate share gene expression pathways, however, Rodeo inerts can have their own toxicity.

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P30 THE OBESOGEN DIETHYLHEXYL PHTHALATE AND ITS EFFECTS ON THE GASTROINTESTINAL SYSTEM-MICROBIOME OF ZEBRAFISH (*DANIO RERIO*)

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Diethylhexyl phthalate (DEHP) is an endocrine disrupting chemical and a commonly used plasticizer, notably in polyvinyl chloride. Because of this, traces of the compound have been found virtually everywhere, including aquatic environments. It has also been proposed that DEHP acts as an obesogen, which is defined as any chemical that promotes adiposity. However, further studies are required to clarify the relationship between DEHP and the gut, and to determine the underlying mechanisms. This study aimed to better understand the impact of DEHP in zebrafish by (1) assessing histopathological changes in the gut and liver tissue; (2) assessing the effects of DEHP on the microbiome and host transcriptome; (3) measuring the expression levels of transcripts related to gastrointestinal hormone signaling as this was a dietary exposure. We hypothesized that DEHP would shift the microbiome towards dysbiosis and this would be associated with endocrine disruption and inflammation. A two-month dietary exposure was conducted in which male zebrafish were fed DEHP (3 ppm). Fish were collected at 0, 1, and 2 months for histopathology, microbiome analysis, and transcriptomics. There was no discernible pathology or inflammation in the gastrointestinal tract nor liver. However, both Fusobacteriaceae and Enterobacteriaceae, both disease causing bacterial genus, were increased in relative abundance while the abundance of Verrucomicrobiaceae was decreased with DEHP. In the host gastrointestinal system, a peroxisome proliferator-activated receptor gene network was affected in expression with DEHP. However, there were no changes in the expression of gastrointestinal hormones (*cck*, *ghrelin*, *sst1*, *sst2*), nor were there any changes in neuropeptides active in the brain-gut axis (*npy*, *cart1*, *grp*) with DEHP treatment. Our data suggest that a two month dietary exposure to DEHP does not significantly impact gut morphology or inflammation, but it can affect the host-gut microbiome communication. This may subsequently lead to pathophysiological changes in the gut over time.

P31 ELUCIDATION OF MOLECULAR MECHANISM UNDERLYING TEMPERATURE-SENSING DURING SEX DETERMINATION IN ALLIGATOR AND TURTLES

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Sex determination is a critical element in development that greatly influences the individual on multiple levels, including physiological, reproductive and behavioral phenotype. In contrast to sex determination based on intrinsic genotypic factors, as commonly seen in many vertebrates, certain reptiles, including the crocodilians and turtles, display temperature-dependent sex determination (TSD), in which the temperature of the surrounding environment during embryonic development determines the sexual fate of the individual. However, much of the details concerning its underlying molecular mechanism remain to be elucidated, such as how the developing embryo initially detects the external temperature signals and directs the gonadal fate accordingly. We have investigated several thermosensory factors, and particularly focused upon transient receptor potential (TRP) channels as main initiation candidate. Functional characterization of alligator TRPV4 channel reveals that it is activated in temperatures proximate to alligator TSD. We also found that selective inhibition and activation of TRPV4 channel induces both down and upregulation, respectively, of male gene expression cascade, and higher prominence of Müllerian duct in males by TRPV4 inhibition. In addition, we have investigated the effects of several TRP agonists and antagonists on the turtle gonadal differentiation. Our findings provide several insights to genetic framework underlining TSD, and our potential novel findings serve as a basis for further understanding gonadal fate pathway during vertebrate sex determination.

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P32 SCREENING OF CYP1A GENE EXPRESSION IN FATHEAD MINNOW JUVENILES TO ASSESS THE EFFECTIVENESS OF NOVEL SILICA-BASED NANOPARTICLES TO CAPTURE CRUDE OIL COMPONENTS

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Oil spills and exposure to toxic PAHs within complex crude oil mixtures are of major concern to the aquatic environment. To remediate this, different approaches are being developed in order to minimize adverse outcomes of this type of accident. Here, novel engineered nanoparticles (NP) are being developed and tested for their capability to uptake the toxic compounds that are in the water-soluble fraction of oil. These NPs have a silica-NP core; grafted with different amphiphilic non-toxic chemicals that will confer a hydrophobic environment around the core to capture the oil components while being soluble in water. This "unimolecular micelle" resembles the mode of action of traditional oil dispersant such a Corexit, but without the need of achieving a critical micellar concentration and they are less harmful to aquatic biota. In order to be released in the environment, first these new substances should undergo several toxicity tests. In this study, we exposed fathead minnow embryos and juveniles to five different engineered NPs. NPs were re-suspended (1 mg/mL) in Hanks solution at 20% strength and then added to the water-accommodated fraction (WAF) of the oil to make a final concentration of 20 mg/L of NP in WAF. The duration of the exposure was 96h with 50% water change every day. In total, 32 juveniles and embryos were tested per condition, divided into 4 separate replicate testing chambers. Endpoints measured were mortality and gene expression of CYP1A as a biomarker of exposure to oil components. There was no mortality observed in either life stage, meaning that NPs were not acutely toxic. Cyp1A gene expression evaluation was only performed using juveniles. Results indicate that WAF elicited the expression of CYP1A as expected; on the other hand, 2 out of 5 NPs lowered the expression but 3 out of 5 NPs induced a higher expression. In conclusion, two NPs were effective in capturing the oil component making it less bioavailable to fathead minnow juveniles.

P33 IDENTIFICATION OF CYTOKINES ASSOCIATED TO THE NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN THE RETINA

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Growth hormone (GH) neurotrophic actions in the CNS include neurogenesis, neuroprotection and neuroregeneration. In the neural retina, GH is able to promote axonal growth, synaptogenesis and cell survival. However, the molecular mechanisms involved in GH actions during neuroprotection and neuroregeneration are still largely unknown. In addition to its direct neurotrophic actions, we propose that GH could act modulating the neuroinflammatory response in order to decrease excitotoxic damage. In neonatal chicken neuroretinas (*in vivo*), we evaluated the anti-inflammatory effect of GH against LPS-induced neuroinflammation. To induce microglial mediated neuroinflammation, LPS (10 µg) was intravitreally injected to induce a local short-time response (6 h). We observed that LPS induced a strong increase on IL1B, IL2, IL6, IL8, LITAF and interferon-γ. Interestingly, GH injection without LPS similarly increased IL1B, IL2, IL6, IL8 and LITAF but not interferon-γ. GH was able to reduce the expression of interferon-γ in retinas co-treaded with LPS. Treatments with LPS+GH resulted in similar levels of IL1B, IL2, IL6, IL8 and LITAF in comparison to independent LPS or GH treatments. To determine if GH modulates the NF-kB signaling through changes on TLR4 expression, we administrated LPS resulting in a significant decrease after the microglial activation. However, GH did not change or restored TLR4 expression loss at short-time. Using immunoblot analysis, we observed that GH decreased TNF-R1 and increased TNF-R2 immunoreactivity after excitotoxic damage (96 h post-injury). Our data suggest that GH anti-inflammatory/neuroprotective actions in the retina involve IL1B, IL2, IL6, IL8, LITAF and TNF-R2 upregulation together with interferon-γ and TNF-R1 downregulation. We conclude that GH protective actions in the neural retina involve a complex communication network that includes pro- and anti-inflammatory cytokines.

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P35 MECHANO-GROWTH FACTOR: A HYPOTHESIS OF THE EVOLUTION AND ACTION OF THE EXERCISE-INDUCED IGF-1 VARIANT

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Insulin-like growth factor 1 (IGF-1) is a protein involved in growth and differentiation of cells. IGF-1 is expressed in response to growth hormone (GH) signaling and is predominantly produced in the liver as a variant known as IGF-1Ea. The IGF-1 gene undergoes alternative splicing resulting in differential splice variants that conserve the mature peptide, with variation primarily in the E-domain located on the carboxy terminal of the mature protein. A unique variant of IGF-1E responds to exercise induced muscle damage, instead of growth hormone signaling, and is therefore named mechano-growth factor (MGF). This variant has also been referred to as human IGF-1Ec or IGF-1Eb in rodents in the literature due to inconsistency in nomenclature assignment. The aim of this paper is to consolidate previous work into a unifying hypothesis of the mechanism of which MGF acts to induce increased cell growth and differentiation, as well as to hypothesize the evolutionary history of the MGF variant. Finally, this paper will propose consistency in nomenclature surrounding the mechano-growth factor variant of the IGF-1 gene across phyla.

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P36 PROXIMATE AND PERSISTENT EFFECTS OF ECOLOGICALLY-RELEVANT THERMAL FLUCTUATIONS DURING TEMPERATURE-DEPENDENT SEX DETERMINATION IN THE AMERICAN ALLIGATOR

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Many non-mammalian organisms lack sex chromosomes and sex is instead determined through genome-by-environment interactions experienced during discrete developmental periods. Temperature-dependent sex determination (TSD) provides a unique window to examine how external stimuli are integrated into physiological responses that shape phenotypic diversity in terms of both inter- and intra-sexual variation. Yet our understanding of these fundamental processes has historically been shaped by experimental studies employing constant incubation temperatures that do not accurately reflect the environment experienced by embryos in nature. In order to understand the scope of thermal variation experienced during development by the American alligator, a species with TSD, this project used field data from 86 alligator nests monitored over the course of 9 years at two geographically distinct sites. Interestingly, the majority of alligator nests experience both male- and female-promoting temperatures during the thermosensitive period in development, frequently within a daily cycle. Laboratory incubations based on empirically-derived nest thermal profiles were further implemented to examine how these opposing environmental cues are integrated into concerted developmental programs and their consequences for later reproductive function. Proximate effects of thermal fluctuations on transcription of genes involved in sex determination as well as persistent effects of fluctuating thermal regimes on hatchling morphology demonstrate the importance of studying temperature-dependent sex determination in the context of the dynamic environments in which it evolved.

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P37 SOMATOSTATIN DISTRIBUTION IN THE BRAIN OF CHIROSTOMA HUMBOLDTIANUM (ATHERINOPSIDAE: TELEOSTEI)

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Somatostatin (SS) is an important multifunctional and multimember of family of related peptide. Usually, SS is playing as an inhibitor in different systems. In the axe brain-pituitary in fish, the first evidence like growth hormone inhibitor was reported. Posteriorly, SS showed inhibited prolactine and recently somatolactin. There is no information available about the SS distribution in the brain for any member of Atherinopsidae family , then, the aim of this work is determinate the somatostatin brain distribution in *Chirostoma humboldtianum*.

For localization of somatostatin , brains of Ch. humboldtianum were cut in transversal sections and a specific antibody against mammalian somatostatin 14 was used . Somatostatin has a widespread distribution in the brain, through out the brain since telencephalon to hindbrain. The immunohistochemical showed somatostatin reactivity in areas such as dorso medial and ventro central in telencephalum, in the diencephalum nucleus: preglomerulosus, glomerulosous, anterior tuberalis, difussus of inferior lobe, periventricular, for midbrain and hidenbrain in: torus longitudinalis, optic tectum, valvula cerebelli, corpus cerebelli, between others. As other species, somatostin has an extensive distribution in the brain. Some reports showed one similar condition when the expression or the peptide have been localized for in situ hybridizaton or immunohistochemical approach.

P38 TESTOSTERONE AND VITELLOGENIN CONCENTRATIONS IN FREE-RANGING GREEN TURTLES (*Chelonia mydas*) SHOW POTENTIAL FOR SPECIES-SPECIFIC VARIATION AND SIZE-BASED TIMING OF GONADAL RECRUDESCENCE IN SEA TURTLES

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Sea turtle reproduction is energetically costly, entailing long-distance migrations, courtship and mating, selection of nest sites, and production of multiple clutches of eggs. In a changing world, where climatic phenomena alter nutritional pathways, understanding the regulation of sea turtle reproduction is crucial for conservation. In female sea turtles, the pituitary gland stimulates the ovaries to produce the steroid hormones estradiol (E2) and testosterone (T) that are linked to the initiation of the reproductive cycle and to follicle formation. In response to E2, the liver produces vitellogenin (VTG), a protein deposited in ovarian follicles during a process called vitellogenesis and that largely constitutes egg yolk. Reproductive success relies heavily on yolk deposition, and understanding the timing of completion of vitellogenesis is essential for identifying threats to sea turtle reproductive success. Aiming to fill the gap in the knowledge left by the paucity of endocrine studies focusing free-ranging sea turtle populations, we used Enzyme-linked Immunosorbent Assays (ELISA) to measure concentrations of T and VTG in female green turtles (*Chelonia mydas*) of the North Atlantic population throughout a nesting season at Tortuguero National Park, Costa Rica. Our results corroborate previous studies where VTG concentration ($\bar{x}$ = 27.9 ± 6.6 mg mL^{-1}) correlates positively to T ($\bar{x}$ = 1,100 ± 1,000 pg mL^{-1}). This relationship ($p < 0.01$, $R^2 = 0.318$) is not causal, and it seems to result from the synchronization of the enlargement of ovarian follicles with the increased production of T by the external layers of follicular cells during vitellogenesis. However, T and VTG concentrations in our study significantly increased throughout the nesting season ($p < 0.05$), which contrasts with previous findings for loggerhead turtles and suggest a species-specific timing of completion of vitellogenesis, likely associated with the intake of protein. Additionally, VTG levels were negatively correlated to the weight of the females ($p < 0.05$, R2 = 0.235), leading us to suggest that larger turtles start laying eggs earlier in the season than smaller turtles.

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P39 GH AND IGF-1 IN GILTHEAD SEA BREAM: ENDOCRINE AND NUTRITIONAL SECRETAGOGUES

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Despite it is well known that Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) are the main hormonal regulators of growth in gilthead sea bream, there is still an important lack of knowledge about how the secretion of these hormones is regulated by other endocrine and nutritional factors. In the present study, we set up two *in vitro* approaches in order to study the secretion of GH and IGF-1, which are the incubation of hemipituitary and cultured primary hepatocytes, respectively. The pituitary obtained from 600 g to 1 Kg animals were split along the sagittal axis, and after stablishing a baseline GH release, one of every two halves were incubated for 18 h with different concentrations of amino acids or hormones (remaining the other half as control). Moreover, primary hepatocytes were isolated, seeded and incubated for 24 h with similar treatments. In both cases, the incubation media was stored to perform the analysis of either GH or IGF-1 by radioimmunoassay and the tissue or cells were properly sampled for gene expression analysis by real-time quantitative PCR. The validation of the hemipituitary trial was performed using natural stimulators and inhibitors of GH release. The results showed that the GH levels were highly increased with ghrelin incubation and decreased with IGF-1, as it would be *in vivo* by negative feedback. Moreover, the potent stimulatory effect of ghrelin was reflected in the downregulation of *gh* gene expression. Regarding the amino acids, lysine resulted to be the most potent GH secretagogue by causing a high increase in both protein and gene expression levels. However, arginine also induced an upregulation of *gh* mRNA, not reflected in plasma levels. Regarding the primary hepatocytes, difficulties in the detection of IGF-1 due to low concentration are being solved. However, at gene expression level, arginine and specially methionine resulted to be strong stimulators of *igf-1* and thus, are potential IGF-1 secretagogues. Finally, the treatments assessed *in vitro* were tested *in vivo* by an intraperitoneal injection model, which confirmed that arginine, lysine and methionine are powerful IGF-1 secretagogues, whereas only arginine and lysine induced the secretion of GH, suggesting that methionine could stimulate IGF-1 secretion only through a GH-independent pathway that demands further research.

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P40 SEASONAL VARIATION IN ESTRADIOL TRANSFER AMONG MALE AND FEMALE BIG BROWN BATS

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A growing body of research suggests that unconjugated steroids are excreted in the urine of male mice and alter the reproductive physiology/behaviour of female conspecifics. These observations support the notion that steroids may act as pheromone in mammals. Using radioactive tracers, past research from our lab demonstrates that female big brown bats (*Eptesicus fuscus*) readily absorb both exogenous estradiol and progesterone via cutaneous and intranasal exposure, with radioactivity measured throughout their neural, peripheral, and reproductive tissues 1 hour after exposure. Additional experiments using radioactive steroids have shown the reliable transfer of estradiol from male to female conspecifics, as well as progesterone between female conspecifics, in *E. fuscus* during the reproductive mating season. The current project aims to explore seasonal variation in estradiol transfer between male and female big brown bats at three time points: Autumn (coinciding to mating season), Spring (coincides with female ovulation and implantation), and Summer (coinciding with maternal colony formation and care). Using radioactive tracers, we observe seasonal variation in male estradiol transfer to a variety of female tissues including the frontal cortex, heart, liver, uterus, and blood serum, with a number of other tissues approaching statistically significant differences among seasons. Additionally, we present preliminary data demonstrating the presence of unconjugated bioactive estradiol in male urine across the various time points, supporting our hypothesis that urinary steroids are involved in the observed steroid transfer. Thus, the seasonal variation in estradiol transfer may influence sexual behaviours and reproductive physiology of female bats during reproductively relevant timepoints, as the transferred steroids are found in both neural and reproductive tissues.

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P41 COMPARATIVE NEUROENDOCRINE REGULATION OF PITUITARY GROWTH HORMONE EXPRESSION AND RELEASE IN MAMMALS, BIRDS AND REPTILES

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The neuroendocrine regulation of growth hormone (GH) in most vertebrates is regulated by hypothalamic and systemic factors. It is known that GHRH, TRH, PACAP, ghrelin and GnRH increase GH synthesis and release, whereas somatostatin and IGF-I act as negative regulators. We compared the *in vitro* effect and potency of these secretagogues in mammalian (rat), avian (chicken) and reptilian (iguana) pituitaries to study how these mechanisms have evolved in vertebrates. We found a differential effect between the secretagogues in their potency and ability to stimulate the synthesis and secretion of pituitary GH. I) The results for GH mRNA expression indicated that: a) GHRH significantly stimulates it in rat and iguana with respect to their control, having a largest effect in iguana; b) TRH stimulated GH mRNA expression in chicken and iguana significantly, in comparison to their respective control and also showed higher effects in iguana; c) PACAP promoted the highest GH mRNA expression in iguana, and to a less extent in chicken but not significantly in the rat; d) ghrelin did not show significant changes on GH mRNA expression in any of the three species; e) GnRH showed an effect in the rat but not in the other two species; f) somatostatin showed no significant differences in the three species, although a stronger effect to inhibit GH mRNA expression was observed in iguana; g) lastly, IGF-I decreased GH mRNA expression with stronger potency in chicken. II) Regarding GH secretion, we found that: a) GHRH significantly stimulated GH release to the culture media in rat and iguana; b) TRH had no effect upon GH release in neither species; c) PACAP did not show significant differences in any of the cultures tested; d) ghrelin significantly stimulated GH secretion in chicken, but not in the other species; e) GnRH increased GH release significantly in chicken and iguana, but not in the rat, and was more potent in iguana; f) somatostatin inhibited GH release in the rat but no difference was observed in chicken and iguana when compared to their control; g) IGF-I showed a stronger tendency to inhibit GH secretion in iguana. Our data indicate that a complex interaction and a sophisticated network between secretagogues for pituitary GH expression and release has developed during vertebrate evolution.

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P42 INTEGRATED CONTROL OF REPRODUCTIVE AND GROWTH PHASE IN GOLDFISH, HORMONAL EFFECTS ON GENE EXPRESSION OF THE PITUITARY AND PERIPHERAL TISSUES.

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In fish and many other vertebrates, reproduction and growth follow a clear seasonal cycle. This cycle is important because significant energy investments are required to sustain reproduction and growth, so that the two functions can not occur simultaneously. This shift between reproduction and growth phase is associated with changes in hormone levels in the animal, at both the pituitary and peripheral tissues level.

Seasonal effects of GnIH (gonadotropin-inhibitory hormone) and GnRH (gonadotropin-releasing hormone) responses were investigated in the gonads and liver during different seasonal phases: mid recrudescence (October-January), late recrudescence (March-April), and growth phase (June-August). Using qPCR, we measured transcript levels of genes of interest, including hormones and hormone receptors in peripheral tissues. Growth hormone and luteinizing hormone levels in the serum were also analyzed using radioimmunoassays during these three seasons. From these transcript and hormonal analyses, there were significant sex dependent differences in gene expression patterns in the liver, and gonads, as well as seasonal changes in expression.

These results provide novel information on integrated reciprocal control of reproduction and growth which is applicable to all oviparous species and other vertebrates undergoing seasonal reproductive cycle.

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P43 VARIABLE ORGANISMAL GROWTH POTENTIAL CORRESPONDS TO DIFFERENTIAL GROWTH HORMONE SIGNALING MECHANISMS

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

P44 HEMOLYMPH PROTEIN AND METHYL FARNESOATE HEMOLYMPH TITERS IN JUVENILE PACIFIC WHITE SHRIMP IN RESPONSE TO EYESTALK ABLATION

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Methyl farnesoate (MF) fulfills many critical functions in crustacean development and reproduction and is, in some respects, analogous to JH in terrestrial insects. MF titers have been demonstrated in many crustacean species at various developmental stages in both intact and eyestalk ablated individuals. However, despite its favorability in aquaculture and commercial significance, MF titers have not been demonstrated in Pacific White Shrimp (PWS). Insight here would serve valuable given the need to monitor and regulate PWS reproduction and development for industry production. The objectives of this study were to determine the effect of eyestalk ablation on MF titers, total protein concentration and protein biomarkers within the hemolymph of juvenile PWS. Juvenile PWS were either eyestalk ablated or left intact and bled one week later. Samples were pooled from three to six individuals, combined with equal parts 0.9% saline and acetonitrile and extracted twice with hexane. The precipitant of each sample was further subjected to a complex protein digestion protocol. Hemolymph titers of MF in eyestalk ablated and intact juvenile male and female PWS were measured via gas chromatography tandem mass spectrometry (GC-MS-MS). Electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS) was used for hemolymph proteomic analysis. MF titers in juvenile PWS were significantly higher in ablated individuals. The data also revealed that MF titers were highest in males (both intact and ablated). Conversely, total protein was highest in intact individuals, while being only slightly higher in males. Furthermore, proteomic analysis revealed a higher abundance of immune related proteins in ablated individuals. Additional studies will investigate whether MF titers, total protein concentration and protein biomarkers within the hemolymph differ at different developmental stages.

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P45 PRODUCTION OF RECOMBINANT GREEN IGUANA (*Iguana iguana*) GROWTH HORMONE (GH).

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Growth hormone (GH) is a protein (~MW=22 kDa) synthetized and secreted in the pituitary gland, which promotes growth and development, among other roles, in all vertebrates. In reptiles, little is known about GH functions and thus it is necessary to develop specific experimental tools to study GH physiology in these organisms. The aim of this work was to develop an *in vitro* constitutive expression system of recombinant green iguana GH (rgiGH) and to evaluate bioactivity of the recombinant hormone. Green iguana GH (giGH) mRNA was amplified and subcloned in a constitutive expression vector (pCAG). A quail neuroretina derived (QNR/D) cell line was transfected with pCAG-giGH. Later, the culture medium was collected after 24 h and 48 h post-transfection and analyzed by SDS-PAGE/Western Blot using an antibody directed against chicken GH (cGH). An immunoreactive band of 26 kDa was observed under reducing conditions (similar to the positive control, rcGH), which indicated that rgiGH was effectively synthesized and secreted from QNR/D cells. To determine rgiGH bioactivity, a Nb2 rat lymphoma cell line was used as a cell proliferation assay, and a significant increase ($P<0.0001$) was found in the cell cultures when media containing rgiGH was added, in comparison with untreated Nb2 cells. Rat growth hormone (rGH) was used as a positive control. Also, pCAG-GFP and untransfected QNR/D cell media were evaluated and showed no effect upon cell proliferation. Thus, an efficient method to produce giGH has been developed, which will allow the possibility to obtain reasonable amounts of the reptilian hormone. Even though, media containing rgiGH increased Nb2 cell proliferation, we might see the effect in an indirect way probably mediated by the increase of growth factors that are produced in the QNR/D cells when they are expressing rgiGH. On the other hand, purification of rgiGH needs to be done for further bioassays and for a biotechnological application for livestock purposes, to control growth and development of the green iguana, which in Southern Mexico and Central America is used as a food source.

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P46 THE EFFECTS OF LIFE HISTORY TRANSITIONS ON MALE SALAMANDER REPRODUCTION

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Many temperate organisms exhibit seasonal reproductive cycles, and the stability and stochasticity of their environment can strongly influence these patterns. The reproductive cycle of temperate salamanders is seasonal and some species express multiple life histories that occupy varying habitats. In this study, we test how life history affects seasonal reproduction of male Oklahoma salamanders, *Eurycea tynerensis*. Metamorphic (biphasic) populations of *E. tynerensis* have an aquatic larval stage that undergoes metamorphosis to become a terrestrial adult, whereas paedomorphic populations retain aquatic larval characteristics into adulthood. We analyzed the seasonal cycle of spermatogenesis, reproductive gland development, and pheromone expression in these disparate life histories. The Oklahoma salamander develops courtship glands during the breeding season that help deliver pheromones to increase female receptivity. We integrated morphological analysis through gland histology and transcriptomic profiles to understand how life history impacts seasonal patterns of courtship that could result in reproductive isolation. Based on habitat stability, we predict that paedomorphic salamanders will have a longer reproductive window as well as start the cycle earlier. This study represents an integrative approach to understanding how habitat variability shapes reproductive development among alternative life cycle modes.

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P47 EFFECT OF BENZO[A]PYRENE ON THE EXPRESSION OF GENES INVOLVED IN THE GH/IGF-I AXIS OF MALE TILAPIA (OREOCHROMIS NILOTICUS)

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Benzo[a]pyrene (BaP) a polycyclic aromatic hydrocarbon (PAH) has been implicated in causing effects on the survival, development and reproduction in fish. Recently, it has been reported that endocrine disrupting chemicals (EDCs) such PAHs could interfere with the Growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis in teleost. However, the effects of BaP on the GH- IGF-I axis is unknown. In this study we investigate the effects of BaP on genes involved in the GH/GF-I axis (by quantitative RT-PCR) in the liver and testis of reproductive adults male tilapia (*O. niloticus*) after a single dose of BaP (40 mg/kg) at 24h, 48h, 72h and 120h. In the liver, GHR1 mRNA expression was diminished at all time points but significant only at 72 h, while in testis it was highly variable but elevated significantly at 120 h. GHR2 was also highly variable in both the liver and testis but not significant in comparison to the controls. No effects was observed on hepatic IGF-1 mRNA levels, but in testis, it was diminishes at all time points and significant only at 72 h. We also quantitated vitellogenin (vtg) and cytochrome P450 1A (cyp1A) which are key genes for estrogenic endocrine disruption and in biotransformation and bioactivation of xenobiotics in fish respectively. Vtg mRNA expression was very low and highly variable but significantly diminished at 48h and 72 h, while cyp1A mRNA levels were elevated at all time points but only significant at 48 and 72 h. Our results revealed that BaP elicited changes in GH/IGF-1 axis, mainly in GHR1, and in IGF-I mRNA levels without affecting GHR2 expression. Further studies are necessary to understand the effect of PAHs on the GH/IGF-I axis in teleost due to its widespread nutritional and economic impact.

P48 DEVELOPMENTAL EXPRESSION PATTERNS OF GONADOTROPHINS DURING TURBOT METAMORPHOSIS

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Gonadotropins (GtHs) play a pivotal role in regulating the reproductive axis and puberty. In this study, full-length sequences coding for common glycoprotein α subunit (CGα) and luteinizing hormone β (LHβ) were isolated from female turbot (*Scophthalmus maximus*) pituitary by homology cloning and a strategy based on rapid amplification of cDNA end-polymerase chain reaction. Results showed that the two cDNAs consisted of 669 and 660 nucleotides encoding 129 and 139 amino acids, respectively. CGα and LHβ manifested typical characteristics of glycoprotein hormones, high homologies with the corresponding sequences of available teleosts and significant homology with that of *Hippoglossus hippoglossus*. *cg*α, *fsh*β, *lh*β mRNAs were abundant in the pituitary, but less expressed in extra-pituitary tissues. The *cgα*, *fsh*β, and *lh*β were detected at 1-day post hatching (dph) and peaked simultaneously at early-metamorphosis (22 dph). *cgα* and *fsh*β mRNA levels were significantly increased at pre-metamorphosis, peaked in early-metamorphosis, and then gradually decreased until metamorphosis was completed. Conversely, *lh*β mRNA levels gradually decreased at pre-metamorphosis, dramatically peaked at early-metamorphosis, and then decreased during metamorphosis. In addition, the mRNA levels of cgα were significantly higher than those of *fsh*β and *lh*β during turbot larval metamorphic development, whereas no significant difference was found between *fsh*β and *lh*β. In addition, turbot *fshr* and *lhr* mRNA were detected after hatching and showed different expression patterns during turbot early larval development These results suggested i) an early activation of the GtHs system after hatching, which was the highest at early-metamorphosis, and ii) FSHβ and LHβ were together involved in the establishment of the reproductive axis during larval development in turbot. These findings contribute to fully understanding the potential roles of GtHs during fish larval development.

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P49 2B OR NOT 2B: MSTN IS THE QUESTION

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Myostatin (MSTN) is a member of the transforming growth factor ß family that functions to negatively regulate skeletal muscle growth. Mammals express one MSTN gene, predominately in skeletal muscle, while fishes express several paralogs due to whole genome duplications (WGD). Salmonids express 4 MSTN paralogs (MSTN-1a, -1b, -2a, and -2b), due to a salmonid-specific WGD, that exhibit differential spatial expression across multiple extra-muscular tissues. Recent studies in poultry predict that a truncated form of MSTN, caused by alternative splicing of the transcript, can bind the full length MSTN and sequester activity. The salmonid MSTN-2b paralog has a premature stop codon, which has led to the hypothesis that this paralog is non-functional in salmonids. We hypothesize that MSTN-2b is differentially regulated in rainbow trout, and likely plays a key role in self-regulating MSTN function. Trout spleen tissue expresses higher levels of MSTN-2b mRNA, therefore our study focuses on the regulation of MSTN-2b in isolated and cultured rainbow trout splenocytes.

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P50 STARFISH SPAWNING INDUCED BY NON-NEURONAL ACETYLCHOLINE WITHIN GONADS

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Starfish are suitable animals for the study of hormonal regulatory mechanism of oocyte maturation and ovulation. Although the pituitary gland is absent in starfish, a gonadotropic hormone is present in the radial nerve cords. Starfish gonadotropin is not a glycoprotein but a relaxin-like peptide so that is designated as relaxin-like gonad-stimulating peptide (RGP). RGP is the primary mediator of oocyte maturation and ovulation. However, the effect of RGP is indirect. RGP secreted from the radial nerve cords stimulates ovarian follicle cells to produce a second mediator, maturation-inducing hormone, identified as 1-methyladenine (1-MeAde) in starfish. When isolated ovaries are incubated in artificial seawater (ASW) in the presence of either RGP or 1-MeAde, spawning occurs within 30 min. Neither RGP nor 1-MeAde induces contraction of gonadal walls, although contraction of gonadal walls is essential in shedding of gametes. It is thus considered that a contraction-inducing substance exists within gonads. In this study, imaging mass-spectrometry observations showed that non-neuronal acetylcholine (ACh) was present in ovaries and testes in *Patiria pectinifera*. However, spawning could not occur upon ACh application, because oocytes are adherently surrounded by follicle cells. It is easy to separate oocytes from follicle cells by treatment of ovaries in Ca^{2+}-free seawater (CaFSW). When ovaries were incubated in CaFSW in the presence of 1-MeAde, germinal vesicle breakdown was observed within ovaries but no spawning occurred. In contrast, ACh could induce spawning in CaFSW. It is important that atropine inhibited 1-MeAde-induced spawning, although mecamylamine had no effect. These suggest that ACh acts on the muscarinic ACh receptor in ovarian walls to induce contraction. Verapamil also showed to inhibit 1-MeAde-induced spawning. Thus presumably, non-neuronal ACh brings about peristaltic motion caused by the Ca^{2+}-influx *via* calcium channel in gonads in order to shed gametes.

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P52 THE BRAIN TRANSCRIPTOME OF THE SEX CHANGING MARINE FISH HALICHOERES MELANURUS

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Teleost fishes have evolved impressive strategies to maximize reproductive fitness. One strategy is to change sex, which involves the "re-modeling" of sexual dimorphic brain regions that control sex-specific reproductive behaviors. The teleost brain shows tremendous plasticity and, unlike mammals, synthesizes high levels of aromatase to control this transformation. Halichoeres melanurus or tail-spot wrasse is a species of wrasse endemic to the western Pacific from Japan to Samoa and south to the Great Barrier Reef. Larval fish first develop into smaller females, and transition into larger males. Thus, this species is sexually dimorphic and phenotypic sex is discernable, although little is known about the transition state during female to male sex change. We employed SMRT sequencing (PacBio IsoSeq) to obtain a reference transcriptome for gene mapping and de novo sequence assembly for H. melanurus. We are optimizing a primary cell culture for radial glial cells in this species, with the intent to understand how sex steroids regulate this cell type in a marine fish. This is important because radial glial cells express aromatase (AroB), the enzyme that converts testosterone (T) into estradiol (E2) and one which is considered to be a master switch for sex change. We have cultured primary cell cultures of RGCs for freshwater species (zebrafish and goldfish), demonstrating that these cultures are more than 95% glia; we aim to characterize novel molecular pathways regulating aromatase expression and bioenergetics in male and female wrasse. These investigations are expected to shed novel insight into the plasticity of sex in teleost fishes.

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P53 EXTRACTION OF STEROID HORMONES FROM WHOLE TISSUE OF SMALL FISH AND QUANTIFICATION USING UHPLC-MS/MS

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Quantification of steroid hormones from small fish is always challenging, since, the blood volume is too low to allow measuring various hormones in plasma. On the other hand, measuring hormone concentration in small model fish is most interesting, especially in toxicology research. In this study, adult zebrafish (*Danio rerio*), both male and female, were used as a model for simultaneous detection of steroid hormones including, cortisol, 11-ketotestosterone, testosterone, progesterone, 17OH-progesterone, estrone and estradiol. Fish weight was measured and sex was confirmed by dissection of the body and visual inspection of gonad. Whole tissue was ground to powder in liquid nitrogen using a mortar and pestle and homogenized in BPS buffer and protease inhibitor cocktail. A mix of internal standards of steroid hormones was added to the homogenate and then hormones were extracted using methyl tert-butyl ether. A part of the homogenate was used to measure total protein content. To measure estradiol in the samples, dansyl chloride was used for derivatization. Extracted samples were analyzed using ultra-high performance liquid chromatography tandem mass spectrometry, quantified according to the calibration curves and expressed per mg protein in gram weight of tissue. According to the results, six hormones were simultaneously detected in 0.25~0.35 g weight fish and the derivatized-estradiol was quantified, separately. The lower limit of detection was calculated for each hormone using a calibration curve, and these are 0.02 ng/mg for cortisol; 0.05 ng/mg for 11-ketotestosterone, testosterone, progesterone and estrone; 0.1 ng/mg for 17OH-progesterone and 0.2 ng/mg for estradiol. This technique can be extended to quantify steroid hormone content in other small fish, which are being used in endocrinology studies.

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P54 INTERACTION OF NEUROGENETIC VARIATION AND STRESS ON ZEBRAFISH BEHAVIOR

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Zebrafish (*Danio rerio*) is a powerful well-established model for vertebrate developmental and neuroscience studies. More recently, due to new genetic tools, a well-characterized neural development and life history, it has also been recognized as a tractable species in which to dissect underlying mechanisms of complex behaviors and psychiatric disorders. Zebrafish also have great potential to identify the genetics of neurological disorders, as the nucleotide sequence of zebrafish genes are often highly similar to corresponding human genes (usually showing more than 70% homology), and their function is often similar. Normal aggression in zebrafish is controlled by a complex interaction of genetics and environmental stimuli. However, there is a limited understanding of the molecular basis of maladaptive behavioral effects in zebrafish, especially related to chemical and physical stress. Potential genes that promote maladaptive behavior can be identified in humans, where extreme violence and aggression has been linked to the monoamine oxidase A low-activity genotype (MAOA) contributing to low dopamine turnover rate. Zebrafish has a highly similar ortholog of *mao* indicating that it could have similar effects in zebrafish. An additional environmental stimulus of trauma also appears to be necessary to trigger extreme behavior in humans. Here we use zebrafish to examine how genetic and environmental factors interact to cause aberrant behavior. To do so, we analyzed the behavior of zebrafish mutants having a low expression of *mao* after exposure to severe environmental stress (physical and chemical). Our results show that transgenic fish show a different behavior from wild type, and stressors affect them differently.

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P55 A COMPREHENSIVE, GENOME-WIDE ANALYSIS OF BRAIN AND GONAD TRANSCRIPTS REVEALS EXPRESSION CHANGES OF KEY SEX REVERSAL-RELATED GENES IN THREE STAGES OF *MONOPTERUS ALBUS.*

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The mechanism of the sex reversal process occurring in bisexual animals is of great interest and not yet understood clearly. Here we provide a comprehensive study of transcriptomes of brain and gonad tissue in three sex stages from the rice field eel (*Monopterus albus*), a species which experiences natural sex reversal during its life cycle. Approximately 195 thousand unigenes were generated and over 44.4 thousand were functionally annotated. Comparative study between stages provided multiple differentially expression genes in brain and gonad tissue. Overall 4668 genes were found to be of unequal abundance between gonad tissues, far more than that of the brain tissues (59 genes). A number of 231 genes were found with different levels in gonad in each stage, with several reproduction-related genes included. More gonad development related genes were validated for their expression patterns among stages by RT-qPCR. The expression of *spef2*, *maats1*, *spag6* and *dmc1* were abundant in testis, but was barely detected in females, while the *17β-hsd*, *zpsbp3*, *gal3* and *foxn5* were only expressed in ovary. In situ hybridization analysis showed that the expression of *foxn5* was detected mainly in the egg envelope and the early phase of follicles, but not in the mature ones, suggesting the functional importance of *foxn5* in the early development of the follicles in the rice field eel. Our study provided crucial data for further analysis of sex transformation mechanisms.

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P56 EFFECT OF MICE *TAENIA CRASSICEPS* WFU CYSTICERCI INFECTION ON THE OVARIAN FOLLICULOGENESIS, ENZYME EXPRESSION AND SERUM ESTRADIOL

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The murine infection with *Taenia crassiceps* WFU (*T. crassiceps* WFU) cysticerci has been widely used as an experimental model to better understand human cysticercosis. Several reports has been that the host hormonal environment determines the susceptibility and severity of many parasite infections. Female mice are more susceptible to infection with *T. crassiceps* cysticerci suggesting that a rich estrogen environment facilitates their reproduction. The aim of this study was to determine the effect of chronic intraperitoneal infection of *T. crassiceps* WFU cysticerci on mice ovarian follicular development, ovulation, expression of ovarian 17α-hydroxilase/17, 20 lyase ($P450_{C17}$) and P450-aromatase, and serum 17β-estradiol. key enzymes of the ovarian steroidogenic pathway. Ovarian androgens and estrogens are synthesized by key enzymes as P-450 aromatase and $P450_{C17}$. To perform this study ovaries and serum were obtained at two, four and six months from *T. crassiceps* WFU cysticerci infected mice, and compared to those of healthy animals. The ovaries were fixed and processed for histology or lysed in RIPA buffer for Western blotting using specific antibodies for $P450_{C17}$ and P450-aromatase. 17β-estradiol serum concentration was measured by ELISA. The results showed that the infection with *T. crassiceps WFU* cysticerci significantly reduced the number of developing and mature follicles, and of corpus lutea, whereas atretic follicles increased. The expression of ovarian $P\text{-}450_{C17}$ and P-450 aromatase as well as serum E2 concentration were significantly increased in the infected group compared to control. These findings show that chronic infection with *Taenia crassiceps* WFU may alter the reproductive functions of female mice.

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P58 CAGE ENRICHMENTS NEGATIVELY IMPACT THE REPRODUCTIVE BRAIN IN MALE MICE

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The vertebrate reproductive brain comprises neurons that secrete gonadotropin-releasing hormone (GnRH) as well as their afferents/efferents. Increasing evidence suggests that the reproductive brain, like the cognitive brain, can respond to various forms of enrichment cues to alter its functions. Since enrichment cues provide sensory cues that may be processed differently by different neurocircuits, we hypothesize that the same enrichment may have highly divergent effects on different brain functions. The objective of this study was to examine if cage enrichments known to benefit the cognitive brain were also beneficial to the reproductive brain and downstream gonadal function in male mice. To test this, male mice were treated at the time of weaning on postnatal day (PN) 20 with either (1) no cage enrichments or (2) combined cage enrichments of nestlets, egg cartons, and igloos. Animals were sacrificed on PN35 and PN50 and assessed for reproductive hormone levels and gene expression as well as testicular histology. Cage enrichments did not significantly affect the expression of *GnRH* and *KiSS1,* an upstream stimulator of GnRH neurons, on PN35, but significantly decreased the expression of both genes on PN50. The expression of fibroblast growth factor 2, a factor known to enhance neural plasticity, in the preoptic area was also significantly decreased by enrichments. However, hypothalamic GnRH and pituitary luteinizing hormone (LH) stores were not significantly altered. Serum LH content as well as testicular morphology and spermatogenic function were also not altered by cage enrichments. These results suggest that environmental enrichments may not consistently have positive effects on all brain functions. Further, the negative impact of cage enrichments on the reproductive brain was manifested only in older animals with longer enrichment exposure, suggesting this is a slow process that may need more time to secondarily inhibit pituitary and testicular functions. Overall, the same enrichment cues may exert neurocircuit-specific effects, leading to highly variable outcomes depending on the brain region examined.

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P60 AEROBIC METABOLISM IN NEURONS AND ASTROCYTES UNDERGOING CELLULAR STRESS: MODULATION BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE-1 (TCAP-1)

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Glial cell regulation is critical to normal neuronal activity *in vivo,* yet in many *in vitro* models of cell activity only neurons are typically investigated. Such studies provide only a partial understanding of how any given *in vitro* treatments can be evaluated *in vivo*. The teneurin C-terminal associated peptides (TCAP), along with their teneurin proproteins, evolved before the divergence of neurons and glial cells. Thus, TCAP may provide an understanding of an ancient peptidergic regulatory mechanism of both neurons and glia. Upon binding to its receptor, adhesion G-protein-coupled receptor subfamily latrophilin (ADGRL), we have shown that TCAP potentiates metabolic activity in both neurons and astrocytes. *In vitro*, TCAP-1 rescues neuronal viability and proliferative capacity in cells undergoing pH-induced alkalosis. Similarly, growth parameters are significantly improved in oxygen-deprived immortalized hypothalamic neurons treated with TCAP-1. The mechanism by which this occurs is not clear but appears to be associated with cytosolic calcium flux. Interestingly, the actions of TCAP on calcium flux differs among immortalized neurons, astrocytes, and mixed co-cultures of neurons and astrocytes. TCAP-mediated astrocytic calcium flux is inhibited by blockers to glutamate receptors. We postulate that TCAP acts to regulate aerobic metabolism in neurons and astrocytes by a calcium-mediated signal transduction system that may involve glutamate.

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Explore Gainesville

The University of Florida: A Preeminent Academic Institution

Albert and Alberta, the Florida Gator Mascots

In 1858, James Henry Roper, an educator from North Carolina and a state senator from Alachua County, opened a school in Gainesville: the Gainesville Academy. In 1866, Roper offered his land and school to the State of Florida in exchange for the relocation of the East Florida Seminary to Gainesville and the University of Florida (UF) was born. Since the founding of UF over 160 years ago, it has become the state of Florida's largest university and is currently the home for more than 60,000 undergraduate and graduate students, and over 5000 faculty. The UF is the largest and oldest university in the state and is the 6th largest university campus by enrollment in the USA. The UF includes 16 colleges that include the Colleges of Medicine, Dentistry, Veterinary Medicine, Food and Agricultural Science, Liberal Arts and Sciences, Law, and Business among others. There are more than 150 research centers and institutes associated with UF, including the McKnight Brain Institute, Cancer and Genetics Institute, Emerging Pathogens Institute, Center for Environmental and Human Toxicology (CEHT), and the Clinical and Translational Science Institute. It is a dynamic and thriving academic institution that is driving to be ranked one of the top 10 Universities in the country. To achieve this goal, the University has undergone Preeminent Recruitment, an initiative that has enticed some of the top researchers nationally and internationally to the university (>250 Preeminent Scholars) http://ufpreeminence.org/focus-areas/. The University is also home to approximately 40 National Academy of Science Fellows and is a hub for research excellence and leadership across a number of disciplines.

Ben Hill Griffin Stadium (top photo), the Clinical and Translational Science Institute (left photo) and the Century Tower on campus (right photo).

The campus is large and diverse, with a perfect blend of new and old. There are many historic buildings on campus that give it an "ivy league" campus feel, intermingled with modern. Ben Hill Griffin Stadium is the home of the Florida Gator football team, a 95,000 seat football stadium on campus within walking distance to the conference location. It is an impressive site to see. There are also a number of trails on campus, and Lake Alice is at the center of campus and is a very pleasant wildlife area. The dirt path and short boardwalk on the lake's north side meander through the woods, where a variety of wetland plants, birds and alligators can be seen. Other trails on campus

include the Upland Pine Trail (0.3-mile) that includes pines and wildflowers in spring; the Old Field Trail (0.3-mile) with abandoned agricultural fields, and the Hammock Trail (0.25-mile) with a variety of flowering plants. Although large and bustling with students, the campus retains that "Natural Florida" feel that the state is well known for (i.e. extensive wetlands and forest). The University of Florida Bat Barn also sits close to Lake Alice. The Bat House and Bat Barn were built to relocate bats from other structures on the UF campus. The Bat House was constructed in 1991 and rebuilt internally in 2009; the Bat Barn was added in 2010. The bat house contains species that include the Brazilian free-tailed bat, Tadarida brasiliensis but also is home to The Southeastern bat, Myotis austroriparius, and Evening bat, Nycticeius humeralis. Each evening, one can sit and watch 300,000 bats swoop out of their home and consume nearly 2.5 billion insects in the warm evening light. Conference attendees will enjoy this experience on campus. There is an excellent public transport system that runs throughout campus, making all of these locations accessible. Walking from one end of campus to another is also feasible and would take approximately 45 min. if conference-goers preferred to explore the many trails and sites on campus.

UF Bat Barn that houses 300,000 bats.

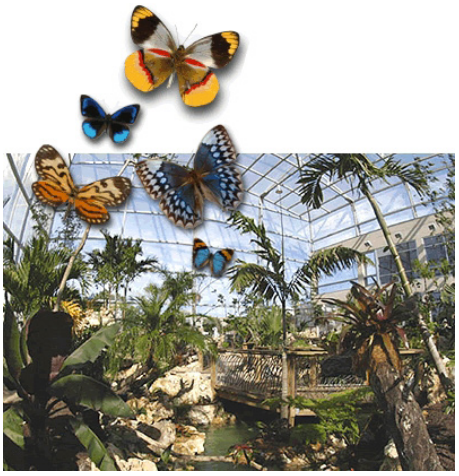
Gainesville: A Multi-Cultural City with Southern Charm

Gainesville is the largest city and the county seat in Alachua County, and has a population of approximately 130,000. It is the quintessential "College Town" with a dynamic and growing population. Downtown is very quaint and historic (approx. 5 min cab ride from campus), and is home to the Hippodrome (Performing Arts Center) and a number of unique shops and cafes. There are many options for restaurant dining and to enjoy warm evening weather on a patio. Downtown Gainesville offers a multicultural array of dining experiences, including Dragonfly Sushi & Sake Company, serving contemporary izakaya and tapas-style Japanese pub fare, the OAK (aka Original American Kitchen) for those seeking American-style cuisine and assortment of micro-brewed products, Emiliano's Cafe serving Latin fusion fare ranging from Mexican to Spanish, as well as specialty beverages such as mojitos, and Harry's Seafood bar and Grille which features Cajun, Creole & Southern cuisine in a friendly atmosphere. Conference attendees will enjoy the evening in downtown Gainesville, and may be fortunate enough to catch one of the many free music concerts at Bo Diddley Plaza, which features local blues and jazz artists.

Hippodrome (left photo), evening in Gainesville (center photo), and Bo Diddley Square (right photo)

Additional Sites in Gainesville.

Florida Museum of Natural History: One of the nation's largest and fastest-growing natural history museums on the UF campus in Gainesville. But the Museum is more than facilities and a repository for millions of specimens and artifacts. Our faculty, staff, part-time employees and volunteers are passionately committed to learning and communicating to others the fascination we share for our natural and cultural worlds. Museum researchers investigate bird extinctions on Pacific islands, excavate shell mounds on the Southwest Florida coast, document shark attacks worldwide, monitor endangered and threatened species such as the Florida panther and the manatee, and explore the genetic codes relating families of tropical orchids. Their findings are shared through scholarly publications, university courses, public lectures, museum exhibits and K-12 education programs.



The Butterfly Exhibit, features dozens of species both exotic and rare

The Samul P. Harns Museum of Art on campus contains an impressive and diverse collection of art that includes the African Collection and Wood Sculptures, and the Asian Collection with Jades, Metalwork, and Stone Sculptures. The Modern Collection includes paintings, sculpture, and drawings featuring painting from Monet and Van Gogh. The Contemporary Collection presents work form a number of internationally acclaimed artists. Also presented are Multi-media, Painting, Photography, Installation and Film

Samul P. Harns Museum of Art

Home of Gatorade and the Cade Museum The Cade Museum for Creativity + Invention is named for Dr. James Robert Cade, a professor of renal (kidney) medicine at the University of Florida and the lead inventor of the sports drink Gatorade. This museum is 5 minutes from campus and offers interactive science and art activities for childer. http://www.cademuseum.org/

The Cade Museum, an excellant visit for family activities

Thank you for attending the

5th Biennial Conference of the North American Society for Comparative Endocrinology (NASCE)

held jointly with the

10th International Symposium on Amphibian and Reptilian Endocrinology and Neurobiology (ISAREN)