Meeting Program and Abstract Book

NASCE
2011

The inaugural meeting of the North American Society for Comparative Endocrinology

University of Michigan, Ann Arbor
July 13-16, 2011

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Welcome to Ann Arbor, home of the University of Michigan. We are glad that you have come to attend the inaugural meeting of the North American Society for Comparative Endocrinology (NASCE 2011). This is an exciting time for comparative endocrinology in North America. We have over three hundred registrants, with seven plenary, ninety eight oral and one hundred twenty two poster presentations. We hope that you will enjoy the scientific and social programs. And don't forget to get outside to enjoy the warm summer days and mild summer evenings in our beautiful city. Welcome!

Bob Denver, Chair of the NASCE 2011 Local Organizing Committee

Please note that beginning July 1, 2011 the University of Michigan will be a smoke-free campus. All University of Michigan facilities, buildings and grounds should be smoke free. Smoking is allowed on sidewalks adjacent to public thoroughfares on the Ann Arbor campuses (e.g., North University Avenue in front of the Michigan League; State Street).

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North American Society for Comparative Endocrinology (NASCE; La Societé Nord-Americaine d'Endocrinologie Comparée; La Sociedad Norteamericana de Endocrinologia Comparada)

Purpose and Mission

The North American Society for Comparative Endocrinology (NASCE) is an interdisciplinary scientific organization dedicated to the study of comparative, evolutionary, ecological and model systems endocrinology. The NASCE fosters the study of diverse species, and of non-traditional animal model systems (i.e., invertebrate and vertebrate) to elucidate basic mechanisms of hormone action, the evolution of animal endocrine systems, and factors affecting the health of human and wildlife populations. Biennial meetings of the NASCE, and its official journal, *General and Comparative Endocrinology,* provide essential forums for the communication and exchange of discoveries and ideas in the field of comparative endocrinology.

Comparative endocrinologists work across disciplinary boundaries, from molecular to ecological. Members of the NASCE conduct basic research in diverse areas of the life sciences. Investigations by comparative endocrinologists lead to the development of alternative animal model systems for discovery of novel hormones and hormone-signaling pathways; the discovery of new pharmaceuticals to treat human disease; the design of hormonally-based strategies for pest control; the development of sensitive, representative and high-throughput endocrine-screening assays for EDCs; the analysis of the impact of global climatic change on animal populations; the elucidation of pathways and mechanisms of evolution through the study of endocrine genes and structures; and the development of more efficient means for the production of animal protein to feed the world's growing human population.

Comparative endocrinology in the 21st century

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Synopsis Hormones coordinate developmental, physiological, and behavioral processes within and between all living organisms. They orchestrate and shape organogenesis from early in development, regulate the acquisition, assimilation, and utilization of nutrients to support growth and metabolism, control gamete production and sexual behavior, mediate organismal responses to environmental change, and allow for communication of information between organisms. Genes that code for hormones; the enzymes that synthesize, metabolize, and transport hormones; and hormone receptors are important targets for natural selection, and variation in their expression and function is a major driving force for the evolution of morphology and life history. Hormones coordinate physiology and behavior of populations of organisms, and thus play key roles in determining the structure of populations, communities, and ecosystems. The field of endocrinology is concerned with the study of hormones and their actions. This field is rooted in the comparative study of hormones in diverse species, which has provided the foundation for the modern fields of evolutionary, environmental, and biomedical endocrinology. Comparative endocrinologists work at the cutting edge of the life sciences. They identify new hormones, hormone receptors and mechanisms of hormone action applicable to diverse species, including humans; study the impact of habitat destruction, pollution, and climatic change on populations of organisms; establish novel model systems for studying hormones and their functions; and develop new genetic strains and husbandry practices for efficient production of animal protein. While the model system approach has dominated biomedical research in recent years, and has provided extraordinary insight into many basic cellular and molecular processes, this approach is limited to investigating a small minority of organisms. Animals exhibit tremendous diversity in form and function, life-history strategies, and responses to the environment. A major challenge for life scientists in the 21st century is to understand how a changing environment impacts all life on earth. A full understanding of the capabilities of organisms to respond to environmental variation, and the resilience of organisms challenged by environmental changes and extremes, is necessary for understanding the impact of pollution and climatic change on the viability of populations. Comparative endocrinologists have a key role to play in these efforts.

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The science of chemical mediation

Chemical mediators signal between cells within an organism, or between organisms, and function by binding to proteins expressed on the surface of, or within, target cells to elicit a change in cell physiology. They first evolved in single-celled organisms for communiation among individuals (e.g. quorum sensing in bacteria; Miller and Bassler, 2001). In metazoans, they play pivotal roles in coordinating development, physiology, and behavior, and the interactions among individuals within populations and communities. Chemical mediators influence how individuals develop, function, and interact with their environment, but they also underlie population-level responses to environmental change. Knowledge of these actions is essential to understand and predict how habitat fragmentation, environmental contamination by industrially-derived compounds, and climatic change may impact the viability of populations, communities, and ecosystems.

The general term ''chemical mediator'', as used here, encompasses several terms that are used within specific scientific disciplines. Hormones, the focus of the science of endocrinology, are a type of chemical mediators, originally defined as organic chemicals that are released from living cells into the blood or interstitial fluid and that travel via the bloodstream some distance from their site of production to target tissues where they coordinate physiological processes (Gorbman and Bern, 1962). As our knowledge of hormones and hormone receptors increases, the definition of a hormone continues to evolve. For example, the classical view of a hormone has changed in recent years to include actions on the cell producing the hormone (autocrine), on adjacent cells within tissues through cell–cell communication (paracrine), or on other individuals of the same or a different species (ectocrine). Chemical mediators acting as hormones are often distinguished from neurotransmitters in that the latter are released at synapses to allow for propagation of electrical signals among nerve cells, and hormones and neurotransmitters are distinguished from cytokines which function in cell-to-cell signaling in defense of the body against invading pathogens. Despite these operational definitions that distinguish among hormones, neurotransmitters, and cytokines, there are many examples in which a chemical functions in more than one role; i.e. a hormone may also function as a neurotransmitter, and vice versa. Furthermore, the endocrine, nervous, and immune systems interact at many levels in the maintenance of homeostasis and survival.

The comparative study of hormones in diverse species dates to the early part of the 20th century, when the field of endocrinology first developed. Prior to 1940, research in endocrinology was almost exclusively associated with medical schools (Kobayashi, 1983). The comparative study of animal hormones developed with the expansion of the field of Zoology during the 1940s and 1950s, and the formal discipline of comparative endocrinology was ''born'' in 1954 with the First International Symposium on Comparative Endocrinology, held at Liverpool, England. Recent decades have brought startling advances in our understanding of animal hormones and their actions, in large part due to advances in biochemistry, molecular biology, and genetics. Hundreds of hormones have now been identified, and new medical therapies, means for enhancing the production of animal protein for food, and strategies for biological control have emerged. The fields of comparative endocrinology and biomedical endocrinology continue to be closely associated, and because hormones play central roles in so many aspects of life, endocrinologists will continue to make seminal contributions that impact all disciplines of the life sciences. In this review, we discuss some of the major contributions that comparative endocrinologists have made to the science of endocrinology, and we highlight the emerging areas of research and how endocrinologists can contribute to the study of organismal biology in the 21st century. Comparative endocrinology has a rich history, and cutting-edge research in the field is now being conducted in laboratories throughout the world.

Structure and function of animal endocrine systems—the contributions of comparative endocrinologists to biomedical research

In 1849, Arnold Adolph Berthold of the University of Göttingen reported the first endocrinological experiment in which he castrated cockerels and found that this caused regression of secondary sex characters, such as the wattles and comb, and the loss of male-typical sexual behavior (Berthold, 1849). The term ''hormone'' was first coined by Ernest Starling, who together with his brother-in-law, William Bayliss, found that the upper part of the dog's small intestine, the duodenum, produced a substance (secretin) that caused secretion of pancreatic juice into the small intestine. This was the first demonstration that factors transported via the bloodstream could act on other tissues and coordinate physiological functions (Henderson, 2005). In the 19th century, the role of the pituitary gland in growth of the body was suggested by post-mortem observations of humans that suffered from acromegaly, but the first experimental evidence that the pituitary produced a substance that influenced bodily functions was the discovery made in the early 20th century that pituitary extract caused growth of the gonads of frogs (Greep, 1988).

The discovery of neurosecretion and neuropeptides marked a revolution in physiology, which led to the integration of endocrinology, neurobiology and behavior, and later immunology. Comparative studies played a pivotal role in the development of the concept of neurosecretion and of the field of neuroendocrinology. The earliest work on neurosecretion and neurohemal structures was carried out in insects (see Kopec, 1922). Ernst and Berta Sharrer, and Wolfgang Bargmann, working from the 1930s to the 1960s,

are credited with having established the intellectual basis for the field of neuroendocrinology. Ernst Sharrer first developed the concept of neurosecretion based on his work with the minnow, *Phoxinus laevis*, in which he postulated that specific neurons in the preoptic nucleus of the hypothalamus possessed endocrine activity related to pituitary function (Klavdieva, 1995). He and his wife Berta conducted comparative studies on animal neurosecretory systems, dividing their efforts, with Berta studying invertebrates (Scharrer, 1941) and Ernst studying vertebrates (Scharrer and Scharrer, 1937). Wolfgang Bargmann is credited with having firmly established the existence and functional role of neurosecretion in vertebrates (Klavdieva, 1995). Bebnado Houssay, working with toads, was the first to show that blood flowed from the hypothalamus to the pituitary gland (Houssay et al. 1935a, 1935b), and Geoffrey Harris later showed, in studies conducted with rats, that the functioning of the nervous and the endocrine systems were linked through neurohormones produced in the hypothalamus that controlled pituitary hormone secretion (Harris and Jacobsohn, 1952).

The contribution of comparative studies to the field of neuroendocrinology continues today. Many new neuropeptides were originally discovered in invertebrates and nonmammalian vertebrates, and their orthologs were subsequently found in mammals. For example, a cardioexcitatory peptide with a characterisitic FMRFamide C-terminal sequence was first isolated in 1977 by Price and Greenberg from the ganglia of the clam, *Macrocallista nimbosa* (Price and Greenberg, 1977). Recently, RFamide peptides were discovered in mammals and found to play critical roles in controlling pituitary hormone secretion, reproduction, appetite and pain, among other functions (Chartrel et al. 2003; Fukusumi et al. 2006; Tsutsui, 2009). The concentration of neuropeptides in the frog brain is estimated to be an order of magnitude greater than that of mammalian brain, which has facilitated the discovery of novel vertebrate neuropeptides (Chartrel et al. 2006).

There are many examples of neuropeptides and neuropeptide actions first discovered in nonmammalian species that were subsequently found to play important roles in human physiology and disease states. For example, the neuropeptide arginine vasotocin (the mammalian homolog is arginine vasopressin–AVP) was first found to influence reproductive behavior in amphibians, and is now known to control social behavior in diverse vertebrate species (Goodson and Bass, 2001). Recent discoveries implicate AVP in human pair-bonding behavior (Walum et al. 2008), and mental health disorders such as autism (Wassink et al. 2004; Egashira et al. 2007). The isolation of urotensin peptides from the fish caudal neurosecretory system (the urophysis) is another example of how comparative endocrinology has laid the foundation for understanding human physiology. The recently discovered human homolog of fish urotensin II is now implicated in human cardiovascular function and heart disease, and may also function as a neurotransmitter/neuromodulator in the brain (Maguire and Davenport, 2002).

The nuclear mechanisms of action of steroid hormones were first discovered by comparative biologists working with insects, and these and other nonmammalian model species continue to play a central role in the study of steroid hormone action in development, physiology, and disease. Steroid hormones bind to nuclear receptors to regulate gene expression. This concept first came from studies of the insect steroid ecdysone that was found to induce "puffing" of the giant polytene chromosomes in the salivary glands of midges and flies. This phenomenon was first observed by Clever and Karlson (1960) in the midge *Chironomus* and later expanded into a theory of a transcriptional cascade of hormone action by Ashburner et al. (1974). This theory, which has had broad impact in biology and medicine, described a gene-regulation cascade directly induced by the hormone ecdysone and that led to the tissue-specific activation or suppression of genes. Work done in the early 1990s in which ecdysone target genes were cloned showed that most of the direct-response genes were transcription factors (King-Jones and Thummel, 2005). Recent studies with insect, crustacean, and amphibian nuclear hormone receptors are helping to unravel the complexities of receptor dimerization (Kozlova et al. 2009), transcriptional regulation (King-Jones and Thummel, 2005; Buchholz et al. 2006; Hopkins et al. 2008), and the roles of nuclear hormone receptors in animal development (King-Jones and Thummel, 2005; Buchholz et al. 2006).

In addition to the well-known genomic actions of steroid hormones, rapid, nongenomic actions are now known to be mediated by the receptors located in the plasma membrane. Rapid actions of steroids were first discovered in the 1970s by Godeau et al. (1978) who showed rapid, membrane-mediated effects of progesterone on frog oocyte maturation. The first discovery and pharmacological characterization of a membrane steroid receptor located in neuronal membranes was carried out in the male rough-skinned newt in which the stress hormone corticosterone causes rapid inhibition of males' clasping behavior (Orchinik et al. 1991). In 2003 Peter Thomas and colleagues, working with ovaries of the spotted sea trout, isolated and characterized the first G protein-coupled receptor (GPR) that mediates rapid steroid actions. The fish receptor was activated by progestins (Zhu et al. 2003b), and subsequently orthologous genes were identified in mice and humans (Zhu et al. 2003a). These findings have set the stage for the discovery of other GPR steroid receptors, and the expansion of the field of nongenomic steroid hormone actions.

The invertebrates have played a major role in the development of the field of comparative endocrinology, and the findings of invertebrate endocrinologists have had far-reaching impact on the life sciences as a whole. For example, studies by Michael Berridge in the 1970s on the blowfly led to the discovery of the phosphatidylinositol signaling pathway, its role in mobilization of intracellular calcium, and more generally the role of calcium in intracellular signaling

(Berridge, 1993). The role of neuropeptides acting on the central nervous system to elicit discrete behaviors was first discovered in the mollusk, *Aplysia californica*, in which egg-laying hormone was shown to act on the nervous system to elicit stereotypical oviposition behavior (Strumwasser, 1984; Smock and Arch, 1986). Another well-characterized example of hormonal control of behavior is ecdysis in insects, in which ecdysis-triggering hormone and eclosion hormone cooperate to activate neuropeptidergic pathways in the nervous system leading to ecdysis (Truman, 2005; Zitnan et al. 2007). More recently, studies of insects are leading the way in linking control of growth and body size, and its hormonal regulation, to nutrient intake and insulin signaling (Nijhout, 2003a, 2003b; Mirth and Riddiford, 2007; Shingleton et al. 2007).

The study of mammalian model organisms such as the rat and the mouse have provided extraordinary insight into the molecular and cellular mechanisms of hormone biosynthesis and action, but relying on one or a few species for research has important limitations. The model systems approach assumes that the findings from a handful of model organisms (now primarily the mouse) can be extrapolated broadly to other species, most importantly to humans. However, these animal models may not be ideal for some basic research questions such as the roles of hormones in development, for which invertebrate or nonmammalian vertebrate models may be better suited. Importantly, model systems cannot represent the diversity of structure and function, and life-history strategy among animals. This is of particular concern for conservation biology, in which species use different physiological and behavioral strategies to survive, and may show differential susceptibility to environmental contaminants and to environmental degradation (environmental stressors). The study of one or a few model species may not provide relevant information for the species of concern, and inbreeding of model species in the laboratory reduces interindividual variation and plasticity that are critical for population sustainability in the wild. The comparative study of animal endocrine systems can lead to the development of new model systems for biomedical research, and can provide a rational basis for the development of strategies for wildlife conservation.

Evolutionary endocrinology

Variation in Darwinian fitness results from variation in organismal form, function and life-history traits. Hormones have widespread and diverse actions in coordinating the expression of animal form and function, and are thus key players in determining fitness. Natural selection acts on genes that code for hormones, hormone synthesizing or metabolizing enzymes, hormone binding proteins and receptors, and hormone signaling pathways that influence the evolution of animal diversity. Evolutionary endocrinology is a subdiscipline of evolutionary physiology (Garland and Carter, 1994), whose broad goal is to understand the manner and mechanism by which organismal function has responded to natural selection (Zera et al. 2007). Specifically, it is the study of how animal hormones and their signaling pathways have evolved to control diverse developmental, physiological, and behavioral processes; of evolutionary relationships among animal species by comparing endocrine organs, processes and genes; and of how hormone systems underlie adaptation to diverse environments and the evolution of new traits and formation of new species.

Hormones influence virtually every morphological, physiological, and life-history trait of an animal. Understanding the physiological/endocrinological mechanisms is essential to our understanding of the mechanistic underpinnings for evolutionary correlations and constraints commonly observed at higher levels of biological organization (e.g. animal form and physiological performance) (Husak et al. 2009). The actions of hormones represent a complex network of interactions, and selection may act at any point within these networks. Hormones mediate trade-offs among life-history traits (e.g. development versus growth; growth versus reproduction), the interactions between the environment and genes, and the establishment of constraints on phenotypic expression (the range and limits of phenotypic plasticity) and phenotypic evolution (e.g. maximum, species-specific body size or allometric relationships among organs/body structures). Hormones also play a key roles in the evolution of development (e.g. heterochrony developmental plasticity, polyphenisms) and the evolution of life histories (e.g. timing of metamorphosis or birth, survivorship, age at first reproduction, clutch or litter size, and frequency of reproductive cycles) (Zera et al. 2007).

Variation in endocrine function underlies variation in animal morphologies and life-history patterns. For example, components of thyroid physiology determine variation in metamorphic timing among frog species, and this timing correlates with the relative permanence of the larval habitat (Buchholz and Hayes, 2005). Thus, evolution of the length of the larval period, which is a central amphibian life-history trait, is governed by changes in the endocrine system that controls metamorphosis. The evolution of paedomorphic life histories among salamander species likely depended, in part, on mutations in genes that control production or action of thyroid hormone (Voss et al. 2000; Voss et al. 2003; Safi et al. 2006). Size-dependent, photoperiodic stimulation of growth hormone and cortisol both control development of salinity tolerance that occurs during downstream migration of juvenile salmon, and this endocrine response is reduced in landlocked salmon that have abandoned seaward migration. (McCormick, 2009). These examples show how the study of hormone-dependent phenomena in a developmental and ecological context can contribute to an understanding of the mechanistic basis for the evolution of animal diversity, and provide an intellectual basis for the development of a subfield of comparative endocrinology, evolutionary developmental endocrinology.

Variation in nucleotide sequence in hormone and hormone-receptor genes are linked to developmental, physiological, morphological, and behavioral diversity among species. For example, changes in the melanocortin receptor type 1 (MC1R) gene, which mediates actions of hormones, such as α-MSH, on pigmentation, are linked to variation in melanin-based, dark plumage color in birds (Mundy, 2005; Pointer and Mundy, 2008), and in coat color in mammals (Nachman et al. 2003). Changes in the coding sequence of the MC1R underlie the evolution of pigmentation loss in cave-dwelling fish (Gross et al. 2009). Interestingly, the de-pigmented phenotype has arisen independently in geographically separate caves through different mutations of the MC1R. Genes like the MC1R, and other hormone or hormone-receptor genes, may be frequent targets for mutation in the repeated evolution of similar phenotypes, owing to the central roles they play in development, physiology, and morphology. Insulin-like growth factor 1 (IGF-1) plays a key role in controlling body growth, and variation in the IGF-1 gene is linked to variation in body size in dogs, suggesting that this locus is a target for both artificial and natural selection (Sutter et al. 2007). The scaling of body parts (allometric scaling) is a fundamental feature of animal form and function, and findings in insects point to a key role for insulin/IGF signaling in controlling allometric relationships among body parts (i.e. body shape; Emlen et al. 2006; Shingleton et al. 2007). Recent work in invertebrates and vertebrates implicate insulin/IGF signaling in the control and evolution of lifespan (Partridge, 2008).

Hormones are key mediators of phenotypic plasticity (the property of individual genotypes to produce different phenotypes under different environmental conditions) (Pigliucci, 2001). Phenotypic plasticity may be an important driver of evolutionary change (e.g. through genetic assimilation) (Pigliucci et al. 2006), and may influence the evolution of animal life histories. For example, the neurohormone arginine vasotocin (AVT) causes shifts in sex-typical behavior in reef fish that change sex (Semsar and Godwin, 2003), and in behavioral diversification in pupfishes found in Death Valley (Lema, 2006, 2008). The neural/neuroendocrine pathways in which AVT functions as a neurotransmitter, and that mediate sex-typical behaviors, show plasticity in response to a changing social environment (Semsar and Godwin, 2003; Lema, 2006, 2008). Steroid hormones play central roles in sexual and stress-related behaviors, and modulation of their production and actions plays a key role in behavioral plasticity and in the evolution of behavioral modes and social structures (Adkins-Regan, 2005). Neurohormones of the corticotropin-releasing hormone family mediate environmental effects on the timing of amphibian metamorphosis and on the timing of birth in mammals (Denver, 2009). These are just a few examples of the many ways in which hormones mediate environmental effects on development, physiology, and behavior and provide the mechanistic basis for the evolution of diversity in morphology and life history.

The application of molecular biology to the function and evolution of the endocrine system has revolutionized comparative and evolutionary endocrinology. The mapping of genomes from species in key phylogenetic positions, such as the cephalochordate amphioxus (*Branchiostoma floridae)*, the urochordate sea squirt (*Ciona intestinalis)*, and the vertebrate sea lamprey (*Petromyzon marinus)*, is allowing comparative endocrinologists to understand the evolutionary history of vertebrate endocrine systems at the molecular level (Sherwood et al. 2005; Holland et al. 2008; Kavanaugh et al. 2008; Paris et al. 2008; Sower et al. 2009; Tello and Sherwood, 2009). Molecular phylogenetic analyses of the neurohypophysial nonpeptides (Acher et al. 1997), gonadotropin-releasing hormone (Kavanaugh et al. 2008; Okubo and Nagahama, 2008; Tsai and Zhang, 2008; Sower et al. 2009), and proopiomelanocortin (Dores and Lecaude, 2005), to name just a few, have helped to clarify phylogenetic relationships, structure/function associations, and the evolution of diversity in physiological control.

The nuclear receptor superfamily evolved over 500 million years ago, and represents a fascinating case study of molecular evolution. Joe Thornton and colleagues used phylogenetic reconstruction to ''resurrect'' the predicted ancestral steroid receptors, and then they tested the functional characteristics of these receptors using techniques of modern molecular endocrinology. This allowed the discovery that the ancestral steroid receptor of vertebrates was an estrogen receptor-like protein that first evolved in invertebrates (Thornton, 2001; Thornton et al. 2003).

Environmental endocrinology, global change, and conservation

One of the greatest challenges to biologists in the 21st century is to understand the molecular and cellular mechanisms underlying how organisms perceive environmental change, and then transduce that information into neural and neuroendocrine secretions that orchestrate morphological, physiological, and behavioral responses. The bewildering array of potential cues from the physical and social environments can actually be simplified into two major groups (or types). First, environmental information can be used for the predictable environment such as day and night, high tide/low tide, and the seasons. Therefore, organisms can use environmental cues to time and prepare for future events such as breeding, migration and hibernation. Hormones thus transduce predictive environmental signals, such as annual change in day length (or photoperiod), temperature, rainfall, or abundance of food into developmental, morphological, physiological, and behavioral responses. While mechanisms underlying photoperiodic responses have received extensive attention, mechanisms whereby organisms respond to other predictive environmental cues remain much less studied.

Second, organisms must respond appropriately to unpredictable events

in the environment, including potential stressors such as storms, predators, drought, and floods. In recent decades, human disturbance (loss of habitat, urbanization, pollution, recreational disturbance, invasive species, and spread of disease) has exacerbated how animals cope with the unpredictable environment (e.g. Travis, 2003). In contrast to hormonal responses to the predictable life cycle, animals must respond to unpredictable events during, or very soon after, the perturbation. This is a fundamentally different suite of mechanisms from responses to the predictable. Thus, although hormones mediate the interaction between the environment and the genotype, the mechanisms involved can be very different depending upon context and predictability. Understanding these two major types of response to the environment, and interactions between them, is crucial for an understanding of how, and whether, organisms will cope with global change (Wingfield, 2008).

Endocrine disruption

Disruption of hormone signaling by industrially derived chemicals [endocrine disrupter compounds (EDCs)] may compromise organismal function, and is now recognized as a significant threat to the health of human and wildlife populations. A recent position paper published by the Endocrine Society highlights the growing evidence and concern for EDC impacts on humans and wildlife (Diamanti-Kandarakis et al. 2009). The potential for endocrine disruption was first recognized when wildlife populations began to experience reproductive problems; e.g. decline of the bald eagle population on the Gulf Coast of Florida in the late 1940s, of the river-otter population in England in the 1950s, of the herring-gull population of Lake Ontario, of the western-gull population of the Channel Islands of California in the 1970s, and population decline and male reproductive deformities in alligators living in Lake Apopka, Florida, in the 1980s, as well as limb deformities and altered sex ratios of frogs in the 1990s (Colburn et al. 1996; Guillette and Guillette, 1996; Taylor et al. 2005; Hogan et al. 2008; Iguchi and Katsu, 2008). Some of the earliest indications that chemicals in the environment could mimic endogenous hormones came from studies of invertebrates in the 1960s and 1970s that showed that chemicals derived from newspaper could mimic insect juvenile hormone (Slama and Williams, 1966) and that water-borne chemicals could disrupt crustacean life cycles (Bookhout and Costlow, 1970). The study by Slama and Williams (1966) led to the development of Insect Growth Regulators (hormonal mimics) for the selective control of insect pests (Dhadialla et al. 1998). In the 1980s, several investigators discovered that tributyltin from marine paints acts as a hormonal mimic that induces intersexes (imposexes) in mollusks (Spence et al. 1990; Alzieu, 1998). Numerous studies of vertebrate wildlife and experimental animals have since shown that EDCs can have estrogenic, anti-androgenic, and anti-thyroid effects (Diamanti-Kandarakis et al. 2009). In 2008, the United States Environmental Protection Agency (EPA) established an Endocrine Disrupter Screening Program comprised of a battery of tests to evaluate the potential for industrially derived chemicals to alter androgen, estrogen, or thyroid-hormone signaling (US EPA, 2008). Several of the assays use nonmammalian species (e.g. the amphibian metamorphosis assay; the fish reproduction assay) for which knowledge of the biology and endocrinology of these animals was derived from basic research conducted by comparative endocrinologists, and upon which future development of these and other assays will depend.

While some populations, or individuals within populations appear to be unaffected by EDCs, others may be much more sensitive and show increased mortality or reduced reproductive success (e.g. Norris, 2000; Norris, 2006). What are the mechanisms for these differences? In addition to a need for knowledge of the molecular and physiological pathways that are altered by EDCs, much more information is needed concerning the effects of endocrine disrupting chemicals on free-living populations in which subtle effects on development, physiology and behavior may have far reaching, long-term effects not necessarily apparent from studies of captive animals. Basic knowledge of how animals perceive and transduce environmental information will thus be fundamental to understanding how, and whether, they can cope with EDCs (Wingfield and Mukai, 2009).

Conservation endocrinology

Endocrinologists can make significant contributions to conservation biology by helping to understand the mechanisms by which organisms cope with changing environments. In recent years physiologists and endocrinologists have provided approaches to address conservation issues relevant to land managers who make decisions on how to conserve habitat as well as protect specific populations (e.g. Cockrem, 2005; Wikelski and Cooke, 2006). For example, a given population may be impacted by environmental stress, which can often be detected by measuring a number of endocrine-related endpoints (Cyr and Romero, 2009). Endocrine biomarkers may also be useful in detecting EDCs and other lethal and sublethal contaminants. Alternatively, changes in the environment such as climate change may lead to inappropriate timing of endocrine-controlled life history events, the phenology of which can be determined by examining altered patterns of circulating hormones. Field endocrine techniques can provide substantial information on the growth, stress and reproductive status of individual animals, thereby providing insight into current and future responses of populations to changes in the environment. In addition, basic information on the environmental requirements of individual species for normal growth and development will provide critical information for species and ecosystem conservation.

Comparative endocrinology and food in the 21st century

Hormones are critical control elements of growth and reproduction and have long been targeted to increase animal food production. Knowledge of the endocrine control of growth, fat content, and appetitive behavior has led to improvements in husbandry methods in many species. Sex steroids are used to increase protein and to decrease fat content in most of the beef production in the USA (Raloff, 2002). Since 1994, growth hormone has been approved and widely used in the USA to improve milk yield in cattle. Gonadotropin releasing hormone is widely used to induce mating and spawning in many cultured fish species, especially when initial domestication is occurring (Mylonas and Zohar, 2000). Growth hormone-transgenic salmon have increased growth rates and conversion efficiency (Devlin et al. 2000). Thus, there is an opportunity for use of hormone supplements and transgenic animals to increase the efficiency, total productivity and profitability of farming operations.

There are also potential negative effects of these approaches. Hormone treatments can alter the composition of food destined for human consumption, such as the IGF-I content of milk or steroid content of meat, with possible impacts on human health. Animals themselves may be negatively influenced by hormone treatments; growth hormone treatment of cattle has been shown to result in increased mastitis, infertility, and lameness (Dohoo et al. 2003). Broader environmental impacts are also of concern. Natural and synthetic hormones may be released into the soils and waterways from concentrated animal-feeding operations (CAFOs) with potential impact on animal and human health (Jensen et al. 2006). Inadvertent release of transgenic animals could result in their interaction, including breeding, with wild animals (Muir and Howard, 2002). Both the reality and perception of these impacts has the capacity to influence consumers' responses and eventual acceptability of these treatments. Research by comparative endocrinologists can contribute in a substantive way to providing information and the clarity necessary for decisions on these trade-offs.

Domestication of new species, especially in aquaculture, can bring protein production to areas with otherwise limited production capacity and reduce pressure to harvest natural populations. Knowledge of the basic environmental requirements for the proper endocrine control of growth and reproduction will be critical for rearing of these newly domesticated species and for improving traditional approaches to husbandry of established species. Innovative techniques in animal husbandry, such as the use of altered photoperiod to improve animal growth or the timing or reproduction, are often determined or enlightened by previous understanding of basic endocrinology (Bjornsson, 1997). Hormones and receptors can act as endpoints for selection of desirable traits (Oksbjerg et al. 2004). There is increasing interest in understanding and promoting animal welfare in farming, and endocrinology can contribute by determining the factors that impose stress or compromise the health of domesticated animals.

Frontiers in comparative endocrinology

The scope of comparative endocrinology has expanded dramatically since its formal origins over 50 years ago. Studies of hormones and their actions impact virtually every field of the life sciences, and the importance of work by comparative endocrinologists for the study of organismal biology in the 21st century will only continue to increase. The neuroendocrine system transduces environmental signals into developmental, physiological, and behavioral responses, and knowledge of these mechanisms is essential for understanding how organisms interact with their environment and how the environment influences organismal form, function, and survival. Some important unanswered questions include: By what sensory modalities do organisms perceive environmental change? How is this sensory information transduced into neuroendocrine and endocrine secretions (stimulatory and inhibitory)? In what ways will global change (climate and human disturbance) affect organisms in relation to their ''perception/transduction systems?'' For example, an organism whose life cycle is driven by photoperiod may become mismatched with other changes in its environment (e.g., global warming); whereas, other organisms that respond to multiple environmental signals such as temperature and photoperiod will be more likely to adjust. What is the potential for new and existing chemicals to affect neuroendocrine systems? Why are some individuals or species less susceptible to the impacts of exposure to EDCs while others are greatly affected? Can we develop sensitive, high-throughput assays for EDCs that will be representative of endocrine disruption in a broad range of species? Only comparative studies of diverse species will allow us to address such questions.

Comparative endocrinologists have important roles to play in many areas of the life sciences, such as the development of alternative animal model systems for discovery of novel hormones and hormone-signaling pathways; the discovery of new pharmaceuticals to treat human disease; the design of hormonally-based strategies for pest control; the development of sensitive, representative and high-throughput endocrine-screening assays for EDCs; the analysis of the impact of global climatic change on animal populations; the elucidation of pathways and mechanisms of evolution through the study of endocrine genes and structures; and the development of more efficient means for the production of animal protein to feed the world's growing human population. This is not intended to be a comprehensive list, or to limit research in this field, but rather to serve as a stimulus for further thought and discussion. Critical to these efforts is the recruitment and broad training of young

scientists, continued and expanded support for their research, and the coordination of efforts among scientists in diverse areas of the life sciences who have a common interest in chemical mediation.

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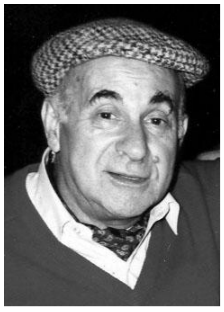

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Table #2 – Elsevier

NASCE 2011
Meeting Highlights

Plenary Lectures

P1. Louis Guillette Jr. Professor, Medical University of South Carolina and Hollings Marine Laboratory, SC, U.S.A.
"Scrambled genes and ovaries: environmental contaminants and the developing gonad"
P2. Carmen Clapp Professor, National University of Mexico, Queretaro, Mexico.
"Prolactin: new functions for an old hormone"
P3. Michael O'Connor Professor, University of Minnesota, MN, U.S.A.
"Vesicle mediated secretion of ecdysone from the drosophila prothoracic gland"
P4. Tony Williams Professor, Simon Fraser University, BC, Canada
"Hormones, life-history, and phenotypic variation"
P5. Vincent Laudet Professor, Ecole Normale Supérieure de Lyon, France
"Origin and evolution of metamorphosis and thyroid hormones"
P6. Elizabeth Adkins-Regan Professor, Cornell University, Ithaca, New York, U.S.A.
"Hormonal organization and activation: evolutionary implications and questions"
P7. Robert Millar Professor, University of Edinburgh, Scotland
"The evolution and function of GnRH and GnRH-like ligands and receptors: a model of biomedical translation of comparative endocrinology"

Symposia

S1. Endocrine disruption: Impacts on wildlife
S2. Insulin/IGF signaling in longevity regulation: New insights from animal models
S3. Tick, tock, setting the clock: Hormones and biological rhythms
S4. Endocrine-immune system interactions
S5. Evolution of polypeptide hormones and their receptors
S6. Endocrine control of growth, body size and allometric scaling
S7. Sex determination and differentiation (cosponsored by ISAREN and NASCE)
S8. Hormone action in neural development and plasticity
S9. Extreme endocrinology: Physiological and behavioral adaptation to extreme environments
S10. Iodothyronine actions throughout the life cycle
S11. Nuclear hormone receptors: evolution and roles in development and physiology
S12. Neuroendocrine control of reproduction
S13. Ion and water balance
S14. Coping with environmental change: adaptive roles for neuroendocrine stress pathways
S15. Ectohormones, environmental chemicals and their perception
S16. Advances in comparative neuroendocrinology
S17. Hormones and behavior
S18. Feeding and metabolism
S19. Regulatory pathways controlling gonadal development and gamete maturation
S20. Special symposium: *General and Comparative Endocrinology* 50th Anniversary Symposium (sponsored by Elsevier and the Division of Comparative Endocrinology, Society for Integrative and Comparative Biology)

Panels and Workshops

P1. Career opportunities and survival strategies for new scientists
P2. Publishing in Elsevier journals
P3a. Funding opportunities in comparative endocrinology – US National Science Foundation
P3b. Funding opportunities in comparative endocrinology – National Sciences and Engineering Research Council of Canada
W1. Model systems and emerging technologies for endocrine disrupter research and screening
W2. Proteopedia and other tools for easily accessing structural biological information
W3. Genomic tools and applications in comparative endocrinology

NASCE 2011
General Information

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Registration: The meeting registration desk will be located in the **Concourse** on the second floor of the Michigan League. Open registration hours are:

Wednesday, July 13, 10:00am – 4:30pm
Thursday, July 14, 7:30am – 12:00pm
Friday, July 15, 7:30am – 12:00pm

The registration desk will be staffed during these times. Additional registration support will be provided by request to members of the local organizing committee (identified by ribbons on their nametags).

Official Language: All events at NASCE 2011 will use English.

NASCE 2011

Conference Social Events

Poster Session 1 and Reception, July 13, 4:30pm – 6:30pm, Michigan League Ballroom (open to all registrants)
Excursion to Henry Ford Museum, Greenfield Village, July 14, 12:30pm - 4:30pm (open to ticket holders)
NASCE 2011 Banquet, July 15, 7:00pm - 10:00pm, Michigan League Ballroom (open to ticket holders)

Presenter Information

Posters - Poster boards for NASCE 2011 are **3 feet WIDE X 4 feet HIGH**. Posters must fit within this space. Push pins will be provided. You do not need to include the poster number on your poster. **All posters** should be set up and remain on display on July 13 and July 14. The ballroom will be open for poster setup at 11am on Wednesday, July 13; please set up your poster between 11am - 1pm on this day. **Odd numbered posters** will be presented during **Poster Session 1** on July 13, 4:30-6:00pm, **even numbered posters** will be presented during **Poster Session 2** on July 14, 5:00-6:30 pm. Posters should be taken down by 7pm on July 14.

Plenary, Symposium and Oral Presentations - Plenary lectures will be 50 minutes.
Symposium presentations will be 25 minutes (20 minutes with 5 minutes for questions). Oral presentations will be 15 minutes (10 minutes with 5 minutes for questions). Please prepare your presentation in Powerpoint and bring it to the meeting on a flash drive or CD-ROM. Unfortunately, we cannot allow speakers to present using their own laptops unless there is a compelling reason to do so (e.g., showing movies that only work on Mac) and prior approval has been requested.

Speaker Ready Room - During the meeting there will be a Speaker Ready Room (**Room 4** on the first floor of the Michigan League) for you to preview your presentation and upload it to the meeting computers. Assistants will be on hand to help you. Please visit the Speaker Ready Room and upload your presentation on the morning of the day of your session, but no later than one hour before the session begins.

Speaker Ready Room Hours:

Wednesday, July 13, 7:30am – 4:30pm
Thursday, July 14, 7:30–9:30am; 4:30-6:30pm
Friday, July 15, 7:30am – 3:30pm
Saturday, July 16, 8:30am – 1:30pm

Coffee Breaks

Coffee break service will be provided each day of the meeting. Morning and afternoon coffee breaks will be held in the **Concourse** on the second floor of the Michigan League.

NASCE 2011

Final Program

The Local Organizing Committee does not assume responsibility for any inconsistencies or errors in the abstracts for contributed paper and poster presentations. We regret any possible omissions, changes and/or additions not reflected in this final program book.

Publication

Accepted abstracts will be published in the online, open access journal ***Frontiers in Endocrinology***. The corresponding author will be contacted with instructions for submitting the abstract for publication in ***Frontiers in Endocrinology***.

The proceedings of NASCE 2011 will be published in a special issue (or issues) of ***General and Comparative Endocrinology.*** Plenary and symposium speakers are invited to submit original articles for consideration for publication in the special issue. The submission date for papers is **September 1, 2011** with publication expected in early to mid-2012. All manuscripts will be peer reviewed.

Plenary speakers are invited to submit an original review or mini-review article. For a full-length review there are no limits on page number, references or figures. Mini-reviews are limited to 12 double-spaced pages. References, figures and figure legends are not included in this page limit. Mini-reviews typically have four or fewer figures.

Invited symposium speakers may submit an original mini-review or a regular research article. Regular research articles are typically 20 double-spaced pages in length. Speakers selected for oral presentations from the submitted abstracts are evaluated by the meeting Program Committee and then selected for invitation to submit an original mini-review or a regular research article.

Papers should be submitted online at:

http://www.elsevier.com/wps/find/journaldescription.cws_home/622837/description#description

When submitting your paper you will be asked to select that the paper is for the special issue of the Proceedings of NASCE 2011.

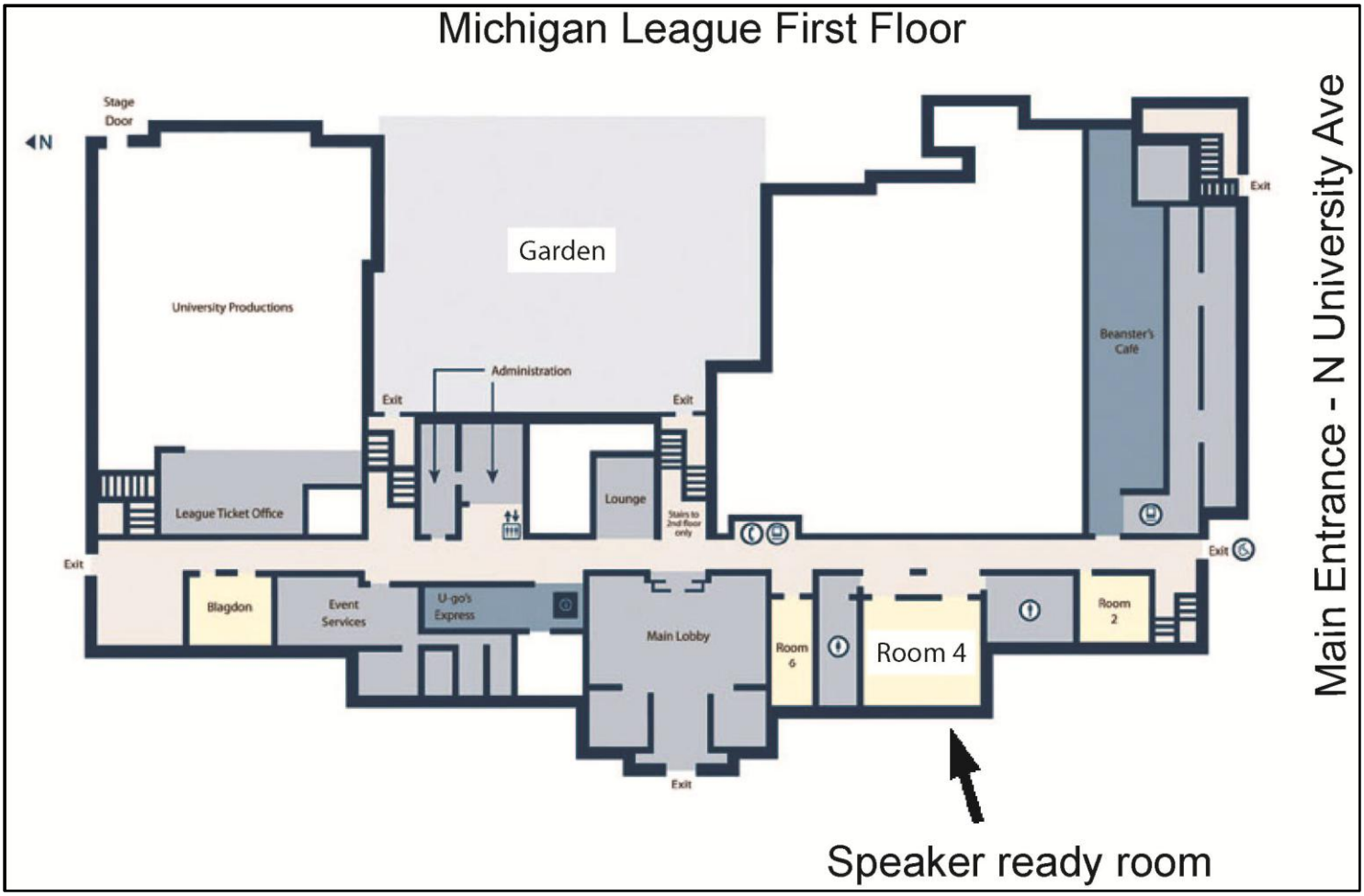
There will be no charge for the first four color figures in the printed version of the paper. There is no limit to the number of color figures in the online version. Authors will receive a complimentary copy of the issue in which their paper appears. Additional copies may be ordered in advance of publication (estimate $30 to $40 per copy).

THE UNIVERSITY OF MICHIGAN – MAPS

CENTRAL CAMPUS

THE UNIVERSITY OF MICHIGAN – MAPS

MICHIGAN LEAGUE – 1st Floor

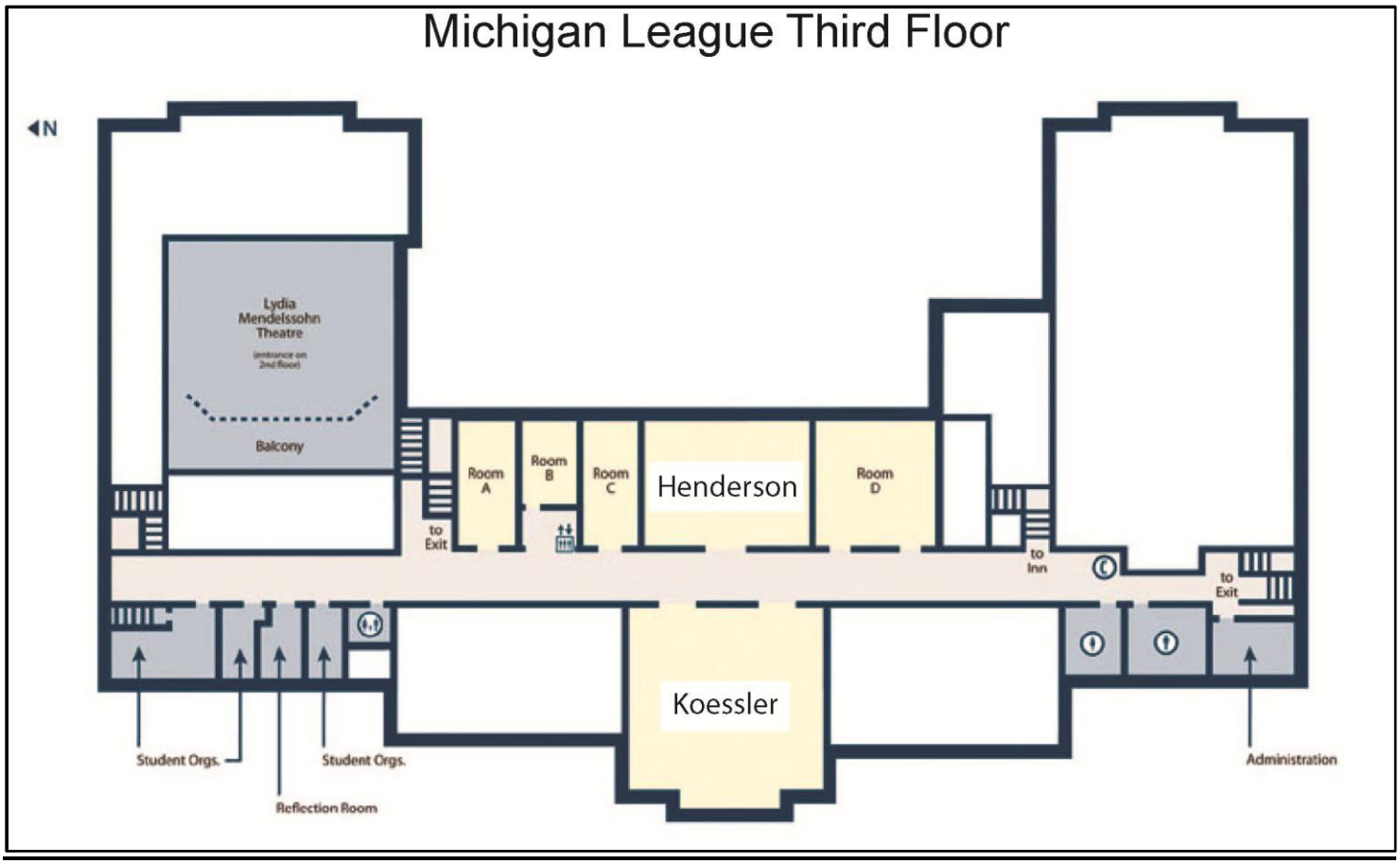


THE UNIVERSITY OF MICHIGAN – MAPS

MICHIGAN LEAGUE – 2nd Floor

THE UNIVERSITY OF MICHIGAN – MAPS

MICHIGAN LEAGUE – 3rd Floor



NASCE 2011

Program Grid

13-Jul	14-Jul	15-Jul	16-Jul
Wednesday	**Thursday**	**Friday**	**Saturday**
Registration: 10:00 a.m.- 4:30 p.m.	**Registration***: 7:30 a.m.- 9:30 a.m. and 4:00 - 6:00 p.m.	**Registration***: 7:30 a.m.- 9:30 a.m.	
ISAREN MEETING	**Plenary: Carmen Clapp** 8:00-8:50 a.m.	**Plenary: Tony Williams** 8:00-8:50 a.m.	**Plenary: Elizabeth Adkins-Regan** 9:00-9:50 a.m.
	Coffee break 8:50-9:20 a.m.	**Coffee break** 8:50-9:20 a.m.	
	Morning Symposia (S5-S7) 9:20-11:10 a.m.	**Morning Symposia (S8-10)** 9:20-11:10 a.m.	**Coffee break** 9:50-10:20
	Plenary: Michael O'Connor 11:20 a.m.-12:10 p.m.	**Plenary: Vincent Laudet** 11:20 a.m.-12:10 p.m.	**Morning Symposia (S17-19)** 10:20 a.m.-12:10 p.m.
		Lunch time 12:10-1:30 p.m.	**Lunch time** 12:10-1:30 p.m.
NASCE Opening Ceremony 1:15-1:30 p.m.	**Free Time/Excursion** 12:10–4:30 p.m.	***NASCE Council Meeting**	
Plenary: Lou Guillette (Joint NASCE/ISAREN) 1:30-2:20 p.m.		**Afternoon Symposia (S11-13)** 1:30-3:20 p.m.	**GCE 50th Anniversary Plenary: Robert Millar** 1:30-2:20 p.m.
Afternoon Symposia (S1-S4) 2:30-4:20 p.m.		**Coffee break** 3:20-3:40 p.m.	**GCE 50th Anniversary Symposium** 2:30-4:00 p.m.
NASCE Poster Session 1 (Odd #s) 4:30-6:30 p.m.	**NASCE Poster Session 2 (Even #s)** 4:30-6:30 p.m.	**Afternoon Symposia (S14-16)** 3:40-5:30 p.m.	**NASCE Closing** 4:00 p.m.
Panels/workshops (P1, P2, W1, W2) 6:30-8:00 p.m.	**Panels/workshops (P3a, P3b, W3)** 6:30-8:00 p.m.	**NASCE Banquet** 7:00-10:00 p.m.	
GCE Editorial Board Meeting (Directly Following P2)			

*The Registration Desk (located in the Concourse of the Michigan League) will be staffed during this time. Additional registration support will be provided during Coffee Breaks, and by request to members of the local organizing committee.
**The height of the pictured table blocks are NOT proportional to the actual time duration of the events.

Wednesday, July 13th:

1:15pm – 1:30pm

NASCE 2011 Opening Ceremony

Mendelssohn Theater

1:30pm – 2:20pm

Plenary Session 1 (Joint NASCE/ISAREN)

Louis Guillette, Jr.

"SCRAMBLED GENES AND OVARIES: ENVIRONMENTAL CONTAMINANTS AND THE DEVELOPING GONAD."

Chairperson: Taisen Iguchi

Mendelssohn Theater

2:30pm – 4:20pm

Afternoon Symposia (S1 – S4):

	Symposium 1: **Endocrine Disruption: Impacts on Wildlife** Chairpersons: Mary Ann Ottinger and Peter Thomas	**Symposium 2:** **Insulin/IGF Signaling in Longevity Regulation: New insights From Animal Models** Chairpersons: Cunming Duan and Patrick Hu	**Symposium 3:** **Tick, Tock, Setting the Clock: Hormones and Biological Rhythms** Chairpersons: Theresa Lee and Orie Shafer	**Symposium 4:** **Endocrine-Immune System Interactions** Chairpersons: Susannah French and Gregory Demas
	Mendelssohn Theater	***Vandenberg Room***	***Michigan Room***	***Hussey Room***
Symposium Speakers:				
	S1-1. **Ottinger, M. A.** *"Neuroendocrine measures of endocrine disruptor chemical impact in birds."*	S2-1. **Hu, P.** *"The C. Elegans serine/threonine kinase SGK-1 promotes longevity by activating the FOXO transcription factor DAF-16."*	S3-1. **De la Iglesia, H.** *"The master clock and its timing of hormonal release."*	S4-1. **Klein, S.** *"Hormonal mechanisms of hantavirus transmission, persistence, and disease in rodent reservoirs and human hosts."*
	S1-2. **Helbing, C.** *"Determining endocrine disruptive potential of environmental contaminants in frogs."*	S2-2. **Bartke, A.** *"Somatotropic signaling, adipose tissue and longevity."*	S3-2. **Sellix, M.** *"The ovulation clock: How the hands of the circadian oscillator time reproduction."*	S4-2. **French, S.** *"Side-blotched lizards, stress and immunity across an urban gradient."*
	S1-3. **Patino, R.** *"Indices of endocrine disruption and reproductive dysfunction in fish populations from lake mead and the Lower Colorado River."*	S2-3. **Miller, R.** *"Stress resistance in mutant mice and long-lived species: Clues to evolutionary mechanism?"*	S3-3. **Hagenauer, M.** *"Adolescent circadian timekeeping in two rodent models, Rattus norvegicus and Octodon degus"*	S4-3. **Ashley, N.** *"Testosterone-induced immunosuppression in songbirds: Trouble or triumph?"*
Oral Presentations:				
	OR1-1. **Vajda, A.** *"Reproductive disruption by estrogenic wastewater effluents."*	OR2-1. **Duan, C.** *"Identification and functional analysis of an ancient IGF binding protein in the most primitive chordate, amphioxus."*	OR3-1. **Cable, E.** *"Effects of circadian arrhythmia on reproductive function in female siberian hamsters."*	OR4-1. **Demas, G.** *"Leptin: A neuroendocrine mediator of immune function in vertebrates."*
	OR1-2. **Hoffman, F.** *"Calling behavior of male south african clawed frogs (Xenopus laevis) is affected by the estrogen 17α-ethinyl estradiol."*	OR2-2. **Rahman, M.** *"Molecular characterization of three IGFBPs in atlantic croaker and their regulation during hypoxic stress: Potential mechanisms of their upregulation by hypoxia."*	OR3-2. **Shafer, O.** *"The anatomical and physiological basis of pigment dispersing factor's circadian functions in Drosophila."*	OR4-2. **Philip, A.** *"Cortisol modulates LPS-mediated immune responses in rainbow trout hepatocytes."*

4:30pm – 6:30pm

NASCE Poster Session I – Odd numbered posters

Ballroom

6:30pm – 8:00pm

Panels/Workshops:

	P1. Career Opportunities and Survival Strategies Panelists: Lynn Riddiford, Vance Trudeau, David Lovejoy, Yun-Bo Shi	**P2. Publishing in Elsevier Journals** Panelists: Shamus O'Reilly, Robert Dores & Elizabeth Adkins-Regan **GCE Editorial Board Meeting following panel**	**W1. Model Systems & Emerging Technologies for Endocrine Disrupter Research and Screening** Chairpersons: Daniel Villaneuve and Markus Hecker Speakers: Dalma Martinovic, Steven Wiseman, Nil Basu, Cheryl Murphy & Theresa Kissane	**W2. Proteopedia and Other Tools for Easily Accessing Structural Biological Information** Chairperson: Wayne Decatur
	Vandenberg Room	***Hussey Room***	***Michigan Room***	***Room D***

Thursday, July 14th:

8:00am – 8:50am

Plenary Session 2 – Carmen Clapp

"PROLACTIN: NEW FUNCTIONS FOR AN OLD HORMONE."

Chairperson: Carlos Arámburo

Mendelssohn Theater

8:50am – 9:20am

Coffee Break

9:20am – 11:10am

Morning Symposia (S5 – S7):

	Symposium 5: **Evolution of Polypeptide Hormones and Their Receptors** Chairpersons: William Bendena and David Lovejoy	**Symposium 6:** **Endocrine Control of Growth, Body Size and Allometric Scaling** Chairpersons: Alexander Shingleton and Russell Borski	**Symposium 7:** **Sex Determination and Differentiation** Chairpersons: Louis Guillette Jr. and Taisen Iguchi
	Mendelssohn Theater	***Vandenberg Room***	***Hussey Room***
Symposium Speakers:			
	S5-1. **Sherwood, N.** *"Evolution of superfamilies for GnRH and its receptor."*	S6-1. **Mirth, C.** "*Ecdysone regulates size-dependent development."*	S7-1. **Nagahama, Y.** "*Sex determination /differentiation and sexual plasticity in fish."*
	S5-2. **Larhammar, D.** *"Complex evolution of somatostatin and urotensin II receptors: Eleven ancestral gnathostome genes of which only six remain in mammals."*	S6-2. **Shingleton, A.** "*Keeping things in proportion: The coordination of organ growth in Drosophila."*	S7-2. **Kohno, S.** "*Which estrogen receptor (ESR1; ERA or ESR2; ERB) is involved in sex determination and gonadal differentiation?*"
	S5-3. **Bendena, W.** "*Allatostatin-like receptors influence behaviour in Drosophila melanogaster and Caenorhabiditis elegans."*	S6-3. **Borski, R.** "***Leptin stimulates hepatic growth hormone receptor expression: Possible role in enhancing GH-mediated anabolic processes in fish."***	S7-3. **Ito, M.** "*Opposite roles of DMRT1 and its W-linked paralogue, DM-W, in the ZZ/ZW-type sex determination in Xenopus laevis."*
Oral Presentations:			
	OR5-1. **Roch, G.** "*Stanniocalcin: A calcium regulator with an ancient origin in unicellular eukaryotes."*	OR6-1. **Kamei, H.** "*Regulation of allometry by modulating local IGF actions: Role of IGF binding proteins."*	OR7-1. **Olmstead, A.** "*ZWY sex determination in Xenopus tropicalis."*
	OR5-2. **Kosugi, T.** "*Identification of ancestral vertebrate glycoprotein hormones from a basal vertebrate, the sea lamprey."*	OR6-2. **Kubicka, L.** "*The role of testosterone in development of sexual size dimorphism in the tropical lizard Paroedura picta (squamata: gekkonidae)."*	OR7-2. **Paitz, R.** *"The decline in yolk progesterone concentrations during development is dependent on the presence of a developing embryo in the European starling"*

11:20am – 12:10pm

Plenary Session 3 – Michael B. O'Connor

"VESICLE MEDIATED RELEASE OF ECDYSONE FROM THE DROSOPHILA PROTHORACIC GLAND."

Chairperson: Lynn Riddiford

Mendelssohn Theater

12:15pm – 4:30pm

Free Time/Excursions to Henry Ford Museum and Greenfield Village

Buses depart from Michigan League a 12:30pm

4:30pm – 6:30pm

NASCE Poster Session II – Even #s

Ballroom

Thursday, July 14th continued:

6:30pm – 8:00pm

Panels/Workshops:

6:30pm – 8:00pm Panels & Workshops		
P3a. Funding Opportunities in Comparative Endocrinology – US National Science Foundation Panelists: John Wingfield and Hannah Carey	**P3b. Funding Opportunities in Comparative Endocrinology – National Sciences and Engineering Council of Canada** Panelist: Mario Lamarca	**W3. Genomic Tools and Applications in Comparative Endocrinology** Chairpersons: Jeramiah Smith, Caren Helbing, & Laurent Sachs
Henderson Room	***Koessler Room***	***Michigan Room***

Friday, July 15th:

Session 4 – Tony Williams
"HORMONES, LIFE-HISTORY, AND PHENOTYPIC VARIATION."
Chairperson: John Wingfield
Mendelssohn Theater

8:50am – 9:20am

Coffee Break

9:20am – 11:10am

Morning Symposia (S8 – S10):

	Symposium 8: Hormone Action in Neural Development and Plasticity Chairpersons: Susan Fahrbach and Stephen Harvey	**Symposium 9: Extreme Endocrinology: Physiological and Behavioral Adaptation to Extreme Environments** Chairpersons: Brian Barnes and John Wingfield	**Symposium 10: Iodothyronine Actions Throughout the Life Cycle** Chairpersons: Andreas Heyland and Carlos Valverde Rodríguez
	Vandenberg Room	***Mendelssohn Theater***	***Hussey Room***
Symposium Speakers:			
	S8-1. **Fahrbach, S.** *"Does the molting hormone shape the adult bee brain?"*	S9-1. **Denlinger, D.** "*Shutting down for the winter: A role for insulin signaling in insect diapauses.*"	S10-1. **Shi, Y.** "*Molecular and genetic studies of histone-modifying complexes in thyroid hormone action during Xenopus development.*"
	S5-2. **Kah, O.** *"Hormone actions in neural development and plasticity of teleost fish."*	S9-2. **Thomas, P.** "*Widespread reproductive disruption, masculinization and endocrine imbalance in croaker exposed to environmental hypoxia."*	S10-2. **Visser, T.** "*Regulation of thyroid hormone bioactivity by deiodinases; from prevertebrates to humans."*
	S5-3. **Harvey, S.** "*Growth hormone and retinal neurogenesis during chick embryo development."*	S9-3. **Wingfield, J.** "*What are extreme environmental conditions and organisms cope with them?"*	S10-3. **Heyland, A.** "*Iodine uptake and thyroid-like function in sea urchins."*
Oral Presentations:			
	OR5-1. **Pang, Y.** "*Characterization of mammalian recombinant PAQR6 and PAQR9 (mPRδ and mPRε) and their potential involvement in mediating antiapoptotic effects of neurosteroids in neuronal cells."*	OR6-1. **Boonstra, R.** "*Preparing to hibernate in a deep freeze: adrenal androgen production in summer linked to environmental severity in winter in arctic ground squirrels."*	OR10-1. **Fujimoto, K.** "*Transcription of thyroid hormone co-activator PRMT1 is regulated by a thyroid hormone-induced transcription factor C-MYC during Xenopus intestinal remodeling."*
	OR5-2. **Chand, D.** *"C-Terminal region of the evolutionary conserved teneurin-1 in mouse hippocampus interacts with the dystroglycan complex and regulates ERK-dependent stathmin modulation of the cytoskeleton"*	OR6-2. **Love, O.** "*Do state-mediated hormones predict reproductive decisions in arctic-nesting common eiders?"*	OR7-2. **Demeneix, B.** "Thyroid hormone metabolism is active in the developing *Xenopus* brain"

Friday, July 15th continued:

11:20am – 12:10pm

Plenary Session 5 – Vincent Laudet

"ORIGIN AND EVOLUTION OF METAMORPHOSIS AND THYROID HORMONES."

Chairperson: Barbara Demeneix

Mendelssohn Theater

12:10pm – 1:30pm

Lunch time

NASCE Council Meeting

Koessler Room

1:30pm – 3:20pm

Afternoon Symposia (S11 – S13):

		Symposium 11: **Nuclear Hormone Receptors: Evolution and Roles in Development and Physiology** Chairpersons: Penny Hopkins and Yun-Bo Shi	**Symposium 12:** **Neuroendocrine Control of Reproduction** Chairperons: Stacia Sower and Pei-San Tsai	**Symposium 13:** **Ion and Water Balance** Chairpersons: Ian Orchard and Stephen McCormick
		Vandenberg Room	***Mendelssohn Theater***	***Hussey Room***
	Symposium Speakers:			
		S11-1. **Hopkins, P.** *"Ecdysteroids and their receptors in the crustacean, Uca pugilator."*	S12-1. **Kauffman, A.** *"Development and hormonal regulation of reproductive neural circuits in rodents."*	S13-1. **Paluzzi, J.** *"Neuroendocrine regulation of diuresis and anti-diuresis in the Chagas' disease vector, Rhodnius prolixus."*
		S11-2. **Riddiford, L.** *"Role of juvenile hormone and its receptor in Drosophila metamorphosis and reproduction."*	S12-2. **Tsai, P.** *"Aplysia GnRH: A role in reproduction?"*	S13-2. **Tanaka, S.** *"Molecular diversity of aquaporin for water adaptation strategy in anuran amphibians."*
		S11-3. **Sachs, L.** *"Whole genome mapping of thyroid hormone receptor in Xenopus tropicalis."*	S12-3. **Joseph, N.** *"Agnatha and Avian: Evolutionary Recruitment of GnRH systems."*	S13-3. **Grau, E.** *"Osmoreception and endocrine responses in the euryhaline tilapia, Oreochromis mossambicus"*
	Oral Presentations:			
		OR11-1. **Bagamasbad, P.** *"Discovering the regulatory logic of an ancient, evolutionarily conserved nuclear receptor enhancer module."*	OR12-1. **Grey, C.** *"Differential involvement of PKC and PKA in ghrelin-induced growth hormone and gonadotropin release from primary cultures of dispersed goldfish pituitary cells."*	OR13-1. **McCormick, S.** *"Hormonal and developmental regulation of salinity-dependent isoforms of the branchial sodium pump in Atlantic salmon."*
		OR11-2. **Mendoza Cisneros, A.** *"3,5-T_2 and T3 activate different isoforms of the thyroid hormone receptor B1 in Fundulus heteroclitus."*	OR12-2. **Onuma, T.** *"The duplicated zebrafish kisspeptin receptor (KISSR) genes exhibit non-overlapping expression patterns and functions: the identification of a nuclear KISSR variant that has transactivating activity."*	OR13-2. **Baltzegar, D.** *"Characterization of leptin and its putative receptor (LEPR) in euryhaline tilapia: A novel link between energy status and osmoregulatory function?"*

3:20pm – 3:40pm

Coffee Break

Friday, July 15th continued:

3:40pm – 5:30pm

Afternoon Symposia (S14 – S16):

		Symposium 14: Coping with Environmental Change: Adaptive Roles for Neuroendocrine Stress Pathways	Symposium 15: Ectohormones, Environmental Chemicals, and Their Perception	Symposium 16: Advances in Comparative Neuroendocrinology
		Chairpersons: L. Michael Romero and Matt Vijayan	Chairpersons: Nicholas Johnson and Jeremy McNeil	Chairpersons: Angela Lange and Vance Trudeau
		Mendelssohn Theater	***Hussey Room***	***Vandenberg Room***
	Symposium Speakers:			
		S14-1. **Langkilde, T.** *"Stress and invasion: Elevated levels of corticosterone may facilitate survival-enhancing behavior of native lizards."*	S15-1. **McNeil, J.** **"***Pheromone mediated mating in migratory moths.***"**	S16-1. **Adams, M.** "*Peptidergic signaling cascades in the regulation of insect ecdysis."*
		S14-2. **Romero, L..** *"Using Reactive Scope to understand physiological responses during stress."*	S15-2. **Johnson, N.** "*Identity, function and application of a male pheromone in sea lamprey.***"**	S16-2. **Lovejoy, D.** "*Teneurin C-terminal Associated Peptides (TCAP): New peptides involved in the neural regulation of corticotropin-releasing factor (CRF)."*
		S14-3. **Vijayan, M.** "*The role of cortisol in teleosts: Stress adaptation and early development.*"	S15-3. **Mason, R.** "*Chemical ecology of snakes: from pheromones to receptors.*"	S16-3. **Martyniuk, C.** "*Quantitative proteomics for fish neuroendocrinology and neurotoxicology.*"
	Oral Presentations:			
		OR14-1. **Dantzer, B.** "*Adaptive hormone-mediated maternal effects in free-ranging red squirrels."*	OR15-1. **Dittman, A.** "*Changes in odorant receptor messenger rna expression associated with maturation, reproductive hormones, and homing in sockeye salmon (Oncorhynchus nerka)."*	OR16-1. **Eppler, E.** "*New insights into the pituitary GH/IGF-I system in ontogeny and phylogeny."*
		OR14-2. **Romero, M.** "*Taenia crassiceps WFU cysticerci synthetize glucocorticoids in vitro: Metirapone regulates the steroid production."*	OR15-2. **Eisthen, H.** "*Odorant responses depend on physiological state in axolotls (Ambystoma mexicanum)."*	OR16-2. **da Silva, R.** "*The regulation of insect cardiac activity and a frank-starling-like mechanism."*

7:00pm – 10:00pm

NASCE Banquet

Ballroom

Saturday, July 16th:

9:00am – 9:50am

Plenary Session 6 – Elizabeth Adkins-Regan

"HORMONAL ORGANIZATION AND ACTIVATION: EVOLUTIONARY IMPLICATIONS AND QUESTIONS."

Chairperson: Juli Wade

Mendelssohn Theater

9:50am – 10:20am

Coffee Break

Saturday, July 16th continued:

10:20am – 12:10pm

Morning Symposia (S17 – S19):

	Symposium 17: Hormones and Behavior	Symposium 18: Feeding and Metabolism	Symposium 19: Regulatory Pathways Controlling Gonadal Development and Gamete Maturation
	Chairpersons: Rosemary Knapp and Juli Wade	Chairpersons: J. Sook Chung and Suraj Unniappan	Chairpersons: Alexander Reikhel and Glen Van Der Kraak
	Mendelssohn Theater	***Vandenberg Room***	***Hussey Room***
Symposium Speakers:			
	S17-1. **Ball, G.** *"Hormone-induced adult neuroplasticity and the activation of behavior in birds."*	S18-1. **Brown, M.** "*Neuropeptides and amino acid sensing coordinately regulate blood digestion and reproduction in the mosquito Aedes aegypti."*	S19-1. **Ge, W.** "*Intrafollicular communication network in the zebrafish ovary – What have we learned from the zebrafish model?"*
	S17-2. **Trainor, B.** *"Effects of the environment on estrogen-dependent mechanisms of aggressive behavior."*	S18-2. **Volkoff, H.** "*Comparative aspects of the endocrine regulation of feeding in fish models."*	S19-2. **Raikhel, A.** "*Regulatory pathways controlling mosquito reproduction."*
	S14-3. **Knapp, R.** "*Cross-talk between endocrine stress and reproductive axes in a livebearing fish."*	S18-3. **Sheridan, M.** "*Resolving the growth-promoting and lipid catabolic actions of growth hormone."*	S19-3. **Yao, H.** "*Sex, survival, and hedgehog: a story of how mammalian embryos make their testes and adrenal glands."*
Oral Presentations:			
	OR17-1. **McEvoy, E.** "*Endocannabinoids regulate vasotocin signaling using a novel neuromodulatory mechanism."*	OR18-1. **Cui, M.** "*Ontogeny of leptin signaling in hypothalamic feeding control centers in the frog Xenopus laevis."*	OR19-1. **Mita, M.** "*Regulatory mechanism in starfish reproduction by a relaxin-like gonad-stimulating substance (GSS)."*
	OR17-2. **van Anders, S.** "*Androgen responsiveness to parental behavior: Nurturant contexts modulate effects of infant cues on human male testosterone."*	OR18-2. **Gonzalez, R.** "*Nesfatin-1: A novel metabolic hormone in fish and rodents."*	OR19-2. **Shepperd, E.** "*Ghrelin regulates reproductive physiology in fish."*

12:10pm – 1:30pm

Lunch time

1:30pm – 2:20pm

GCE 50th Anniversary Plenary Session – Robert Millar

"THE EVOLUTION AND FUNCTION OF GNRH AND GNRH-LIKE LIGANDS AND RECEPTORS: A MODEL OF BIOMEDICAL TRANSLATION OF COMPARATIVE ENDOCRINOLOGY."

Chairperson: Robert Dores

Mendelssohn Theater

2:30pm – 4:00pm

GCE 50th Anniversary Symposium:

S20. *General and Comparative Endocrinology* 50th Anniversary Symposium (Sponsored by Elsevier and the Division of Comparative Endocrinology, Society for Integrative and Comparative Biology) Chairperson: Robert Dores
Mendelssohn Theater
Symposium Speakers:
S20-1. **Habibi, H.** *"Thyroid hormone and control of reproduction in goldfish."*
S20-2. **Trudeau, V.** *"Peptide Identity Crisis: Is secretoneurin a new hormone?"*
S20-3. **Deviche, P.** "*Plasma testosterone and behavior in free-ranging vertebrates: Evolving views on temporal dynamics, control mechanisms, and significance."*

4:00pm

NASCE 2011 Closing

Mendelssohn Theater

NASCE 2011 Panels and Workshops

Panels

P1: Career opportunities and survival strategies for new scientists. This session is intended for graduate students, postdocs and new faculty. The panel will focus on skills important for early career development such as how and when to publish your research; grant proposal writing; navigating federal science funding agencies; the job search; interviewing skills; project management; peer review; mentoring; how to manage a research group, etc. Opportunities and challenges that are perhaps unique to the field of comparative endocrinology will be addressed.
Panelists: Lynn Riddiford, Howard Hughes Institute for Medical Research; Vance Trudeau, University of Ottawa; David Lovejoy, University of Toronto; Yun-Bo Shi, National Institutes of Health
Date and time: July 13, 6:30-8:00 pm
Location: Vandenberg Room

P2: Publishing in Elsevier journals. This panel will offer an opportunity to discuss with the publisher and editors-in-chief of the journals *General and Comparative Endocrinology* and *Hormones and Behavior* about the publication process. Topics will include: how to get published, how to review a paper, measuring journal prestige, author's rights and responsibilities, etc.
Panelists: Shamus O'Reilly, Publisher, *General and Comparative Endocrinology*, *Hormones and Behavior*; Robert Dores, Editor-in-Chief, *General and Comparative Endocrinology*; Elizabeth Adkins-Regan, Editor-in-Chief, *Hormones and Behavior*
Date and time: July 13, 6:30-8:00 pm
Location: Hussey Room

GCE Editorial Board Meeting will be held immediately after P2 in the Hussey Room

P3a: Funding opportunities in comparative endocrinology - NSF. Representatives from the National Science Foundation of the United States will discuss funding opportunities in the life sciences and answer questions about proposal preparation, proposal review, etc.
Panelists: John Wingfield, Interim Director, Integrative and Organismal Systems (IOS), NSF; Hannah Carey, Program Director, Physiological and Structural Systems (PSS) Cluster, NSF
Date and time: July 14, 6:30-8:00 pm
Location: Koessler Room

P3b: Funding opportunities in comparative endocrinology - NSERC. Representatives from the National Sciences and Engineering Research Council of Canada will discuss funding opportunities in the life sciences and answer questions about proposal preparation, proposal review, etc.
Panelist: Mario Lamarca, Program Director, NSERC
Date and time: July 14, 6:30-8:00 pm
Location: Henderson Room

NASCE 2011 Panels and Workshops

Workshops

W1: Model systems and emerging technologies for endocrine disrupter research and screening
Chairpersons: Dan Villeneuve, US EPA, Mid-Continent Ecology Division, Duluth, MN; Markus Hecker, University of Saskatchewan, Saskatoon, SK, Canada; Nil Basu, University of Michigan, Ann Arbor, MI
Speakers
Dalma Martinovic, University of St. Thomas, St. Paul, MN USA ***Application of transcriptomics and metabolomics to study endocrine mediated dominance behaviors in fish***
Steven Wiseman; University of Saskatchewan, Saskatoon, Canada ***Use of whole transcriptome sequencing in fish to characterize endocrine activity associated with oil sands process water***
Nil Basu, University of Michigan, MI USA ***Development of a cell-free neurochemical screen to predict adverse outcomes in mammals, fish, and birds.***
Cheryl Murphy, Michigan State University, MI USA ***Linking endocrine disruption to population level impacts via computational modeling***
Theresa Kissane, University of Illinois, and US Army Corps of Engineers, CERL, Champaign, IL USA ***Hypothalamic-pituitary-gonadal axis on a chip***
Date and time: July 13, 6:30-8:00 pm
Location: Michigan Room

W2: Proteopedia and other tools for easily accessing structural biological information
Chairperson: Wayne Decatur, University of New Hampshire (w.decatur@unh.edu)
Proteopedia is an online 3D encyclopedia of proteins, nucleic acids and other macromolecules, as well as a structural biology resource. Wayne Decatur, a member of the editorial board of Proteopedia, will present an overview of Proteopedia and several related tools. The workshop is designed for scientists at all levels interested in macromolecules. No computer is necessary for the workshop; however, participants should feel free to bring their own laptops for exploring some of the content and getting technical guidance following the formal presentation.
Date and time: July 13, 6:30-8:00 pm
Location: Room D

W3: Genomic tools and applications in comparative endocrinology
Co-chairs: Caren Helbing, Univ. Victoria, BC, Canada; Jeramiah Smith, University of Kentucky, Lexington, Kentucky, USA; Laurent Sachs, Museum National d'Histoire Naturelle, CNRS, Paris, France
This workshop will explore state-of-the-art genomic tools and resources for comparative endocrinologists including genome-wide association and gene expression analysis using high throughput sequencing and the bioinformatic tools necessary for analyzing the massive amounts of data generated by such methods, and the means for annotating and analyzing recently sequenced genomes of diverse organisms. This workshop will also consider genomic resource needs for studies of diverse species.

Schedule:
6:30 – 6:45 Caren Helbing, ***Opportunities and challenges in genomics: A lesson from frogs***
6:45 - 7:00 Jeramiah Smith, ***Development and analysis of lamprey genome assembly: challenges and insights***
7:00-7:15 Laurent Sachs, ***The Xenopus tropicalis genome: where are we at and what does the future hold for functional genomics?***
7:15-8:00 Open discussion

Date and time: July 14, 6:30-8:00 pm
Location: Michigan Room

List of abstracts

Plenary Lecture Abstracts

PL1 L. J. Guillette Jr.
Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, U.S.A. and Hollings Marine Laboratory, Charleston, SC, U.S.A.
Scrambled genes and ovaries: environmental contaminants and the developing gonad

PL2 C. Clapp, E. Arnold, M. Ramirez, N. Adan, C. Vega, G. Ledesma-Colunga, Y. Macotela, G. Martínez de la Escalera, and S. Thebault
Institute of Neurobiology, National University of Mexico (UNAM), Queretaro 76230, Mexico.
Prolactin: new functions for an old hormone

PL3 M. B. O'Connor and N. Yamanaka
Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN, 55455, U.S.A.
Vesicle mediated secretion of ecdysone from the drosophila prothoracic gland

PL4 T. D. Williams
Dept. of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 4H8, Canada.
Hormones, life-history, and phenotypic variation

PL5 V. Laudet
Institut de Genomique Fonctionnelle de Lyon (IGFL), CNRS-UMR 5242, Ecole Normale Supérieure de Lyon, Allée d'Italie, 69364 Lyon CEDEX 07, France.
Origin and evolution of metamorphosis and thyroid hormones

PL6 E. Adkins-Regan
Departments of Psychology/Neurobiology & Behavior, Cornell University, Ithaca, New York, U.S.A.
Hormonal organization and activation: evolutionary implications and questions

PL7 R. P. Millar
Mammal Research Institute, University of Pretoria, Pretoria, South Africa; UCT/MRC Receptor Biology Group, University of Cape Town, Cape Town, South Africa; Centre of Integrative Physiology, University of Edinburgh, Scotland.
The evolution and function of GnRH and GnRH-like ligands and receptors: a model of biomedical translation of comparative endocrinology

Invited Symposium Abstracts

S1-1 M. A. Ottinger, M. Bohannon, T. Carro, L. Carpenter, and K. Dean
Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 20742, U.S.A.
Neuroendocrine measures of endocrine disruptor chemical impact in birds

S1-2 C. C. Helbing
Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., Canada.
Determining endocrine disruptive potential of environmental contaminants in frogs

S1-3 R. Patiño(1), S. L. Goodbred(2), E. Orsak(3), J. A. Jenkins(4), M. R. Rosen(5), L. Torres(6), P. Sharma(6), C. Wieser(7) and S. Ruessler(7)
(1) U.S. Geological Survey, Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409-2120, U.S.A.; (2) U.S. Geological Survey (Emeritus), High Point, NC 27262, U.S.A.; (3) U.S. Fish and Wildlife Service, Arlington Ecological Services Field Office, Arlington, TX 76011, U.S.A.; (4) U.S. Geological Survey, National Wetlands Research Center, 700 Cajundome Blvd., Lafayette, Louisiana 70506, U.S.A.; (5) U.S. Geological Survey, Nevada Water Science Center, Carson City, NV 89701, U.S.A.; (6) Dept. of Biological Sciences and Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409, U.S.A.; (7) U. S. Geological Survey, Southeast Ecological Science Center, Gainesville, FL 32653, U.S.A.
Indices of endocrine disruption and reproductive dysfunction in fish populations from lake mead and the lower Colorado river

S2-1 C. Guo(1), K. Dumas(1)(2), T. Williams(1), K. Ashrafi(3), and P. J. Hu(1)(4)
(1) Life Sciences Institute, University of Michigan, Ann Arbor, MI, U.S.A.; (2) Cellular and Molecular Biology Graduate Program, University of Michigan Medical School, Ann Arbor, MI U.S.A.; (3) Department of Physiology, University of California, San Francisco School of Medicine, San Francisco, CA, U.S.A.; (4) Departments of Internal Medicine and Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI U.S.A.
The C. elegans serine/threonine kinase SGK-1 promotes longevity by activating the foxo transcription factor DAF-16

S2-2 A. Bartke
Department of Physiology and Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL, U.S.A.
Somatotropic signaling, adipose tissue and longevity

S2-3 R. A. Miller
Department of Pathology and Geriatrics Center, University of Michigan, and Ann Arbor VA GRECC, Ann Arbor, MI, U.S.A.
Stress resistance in mutant mice and long-lived species: clues to evolutionary mechanism?

S3-1 H. de la Iglesia, T. Lilley, M. Schwartz, B. Smarr, and C.Wotus
University of Washington, Department of Biology and Program of Neurobiology and Behavior, Seattle, WA, U.S.A.
The master clock and its timing of hormonal release

S3-2 M. T. Sellix, A.Tao and M. Menaker
Department of Biology, University of Virginia, Charlottesville, VA, U.S.A.
The ovulation clock: how the hands of the circadian oscillator time reproduction

S3-3 Megan H. Hagenauer and Theresa M. Lee
Department of Psychology, University of Michigan, Ann Arbor, MI, U.S.A.
Adolescent circadian timekeeping in two rodent models, Rattus norvegicus and Octodon degus

S4-1 S. L. Klein
Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, U.S.A.
Hormonal mechanisms of hantavirus transmission, persistence, and disease in rodent reservoirs and human hosts

S4-2 S. S. French and L. D. Lucas
Department of Biology, Utah State University, Logan UT, 84322, U.S.A.
Side-blotched lizards, stress, and immunity across an urban landscape

List of abstracts

S4-3 N. T. Ashley
Department of Neuroscience, Ohio State University College of Medicine, Columbus, OH, U.S.A.
Testosterone-induced immunosuppression in songbirds: trouble or triumph?

S5-1 N. M. Sherwood, G. J. Roch, and E. R. Busby
Department of Biology, University of Victoria, Victoria, BC, Canada.
Evolution of superfamilies for GnRH and its receptor

S5-2 D. Larhammar, Christina Bergqvist, Görel Sundström and Daniel Ocampo Daza
Dept of Neuroscience, Uppsala University, Uppsala, Sweden.
Complex evolution of somatostatin and urotensin ii receptors: eleven ancestral gnathostome genes of which only six remain in mammals

S5-3 W. G. Bendena, C. Wang and I. Chin-Sang
Dept of Biology, Queen's University, Kingston On, K7L 3N6, Canada.
Allatostatin-like receptors influence behaviour in drosophila melanogaster and C. elegans

S6-1 T. Koyama, M. Oliveira and C. Mirth
Instituto Gulbenkian de Ciência, Fundação Calouste Gulbenkian, Oeiras, Portugal.
Ecdysone regulates size-dependent development

S6-2 N. F. Parker and A. W. Shingleton
Department of Zoology, Michigan State University, East Lansing, MI, 48824, U.S.A.
Keeping things in proportion: the coordination of organ growth in Drosophila

S6-3 R. J. Borski, E. T. Won, and D. A. Baltzegar
Department of Biology, North Carolina State University, Raleigh, NC, U.S.A.
Leptin stimulates hepatic growth hormone receptor expression: possible role in enhancing GH-mediated anabolic processes in fish

S7-1 Y. Nagahama
Institute for Collaborative Relations, Ehime University, Matsuyama 790-8577, Japan and Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan.
Sex determination / differentiation and sexual plasticity in fish

S7-2 S. Kohno(1)(2), Y. Katsu(3), Y. Ohta(4), T. Iguchi(5)(6), L. J. Guillette Jr(1)(2)
(1) Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, U.S.A.; (2) Marine Biomedicine and Environmental Science Center, Hollings Marine Laboratory, Charleston, SC, U.S.A.; (3) Graduate School of Life Science and Department of Biological Sciences, Hokkaido University, Sapporo, Japan; (4) Laboratory of Experimental Animals, Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan; (5) Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan; (6) Department of Basic Biology, The Graduate School for Advanced Studies (SOKENDAI), Okazaki, Japan.
Which estrogen receptor (ESR1; ERα or ESR2; ERβ) is involved in sex determination and gonadal differentiation?

S7-3 M. Ito, S. Yoshimoto, S. Mawaribuchi, N. Ikeda, K. Fujitani, K. Tamura, and N. Takamatsu
School of Science, Kitasato University, Sagamihara, Japan.
Opposite roles of DMRT1 and its W-linked paralogue, DM-W, in the ZZ/ZW-type sex determination in Xenopus laevis

S8-1 S. Fahrbach and R. A. Velarde
Department of Biology, Wake Forest University, Winston-Salem, NC, U.S.A.
Does the molting hormone shape the adult bee brain?

S8-2 O. Kah(1), N. Diotel(1), J. D. Rego(2), I. Anglade(1), C. Vaillant(1), E. Pellegrini(1), and H. Vaudry(2)
(1) Neurogenesis And Œstrogens, UMR CNRS 6026, Campus de Beaulieu, Université de Rennes 1, 35042 Rennes cedex, France.; (2) Différenciation et Communication Neuronale et Neuroendocrine, INSERM U982, PRIMACEN, IFRMP 23, Université de Rouen, Mont-Saint-Aignan, France.
Hormone actions in neural development and plasticity of teleost fish

S8-3 S. Harvey and E. J. Sanders
Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.
Growth hormone and retinal neurogenesis during chick embryo development

S9-1 D. L. Denlinger(1) and C. Sim(1,2)
(1) Department of Evolution, Ecology and Organismal Biology; Department of Entomology, Ohio State University, Columbus, OH, U.S.A.; (2) Department of Biology, Baylor University, Waco, TX, U.S.A.
Shutting down for the winter: a role for insulin signaling in insect diapauses

S9-2 P. Thomas and M. S. Rahman
University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX78373, U.S.A.
Widespread reproductive disruption, masculinization and endocrine imbalance in croaker exposed to environmental hypoxia

S9-3 J. C. Wingfield
Department of Neurobiology, Physiology and Behavior, University of California, One Shields Avenue, Davis, CA 95616, U.S.A.
What are extreme environmental conditions and how do organisms cope with them?

S10-1 Y. Shi
Laboratory of Gene Regulation and Development, NICHD, NIH, Bethesda, MD 20892, U.S.A.
Molecular and genetic studies of histone-modifying complexes in thyroid hormone action during Xenopus development

S10-2 T. J. Visser
Dept Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands
Regulation of thyroid hormone bioactivity by deiodinases; from invertebrates to humans

S10-3 A. Heyland, B. Lautens, and A. E. M. Miller
Integrative Biology, University of Guelph, ON, Canada.
Iodine metabolism and thyroid-like function in sea urchins

S11-1 P. M. Hopkins
Department of Zoology, University of Oklahoma, U.S.A.
Ecdysteroids and their receptors in the crustacean, Uca pugilator

S11-2 L. M. Riddiford, J. Bilen and J. W. Truman
Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, U.S.A.
Role of juvenile hormone and its receptor in drosophila metamorphosis and reproduction

S11-3 N. Buisine(1), P. Bilesimo(1), G. Alfama(1), A. Grimaldi(1), X. Ruan(2), E. Liu(2), B. A. Demeneix(1), Y. Ruan(2) and L. M. Sachs(1)
(1) CNRS UMR 7221, Muséum National d'Histoire Naturelle, 7 rue Cuvier F75231 Paris cedex 05, France; (2) Genome Institute of Singapore, 60 Biopolis street, 138672, Singapore.
Whole genome mapping of thyroid hormone receptor in Xenopus tropicalis

S12-1 A. S. Kauffman
Department of Reproductive Medicine, University of California San Diego, La Jolla, CA, U.S.A.
Development and hormonal regulation of reproductive neural circuits in rodents

List of abstracts

S12-2 P. Tsai, S. I. Kavanaugh and B. Sun
Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO 80301-0354, U.S.A.
Aplysia GnRH: a role in reproduction?

S12-3 N. Joseph(1)(2)(3)(4), K. Morgan(2), R. Millar(2), I. Dunn(3), G. Bedecarrats(4), and S. Sower(1)
(1) Center for Molecular and Comparative Endocrinology, 46 College Road, University of New Hampshire, Durham NH 03824, U.S.A.; (2) MRC Human Reproductive Sciences Unit, The Queens Medical Research Institute, Edinburgh, EH16 4TJ, U.K.; (3) The Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, Edinburgh, EH25 9RG, U.K.; (4) Animal and Poultry Science, University of Guelph, 50 Stone Road East, Guelph, N1G 2W1, Canada.
Agnatha and avian: evolutionary recruitment of GnRH systems

S13-1 J. P. Paluzzi and M. J. O'Donnell
Department of Biology, McMaster University, Hamilton, Ontario, Canada.
Neuroendocrine regulation of diuresis and anti-diuresis in the chagas' disease vector, Rhodnius prolixus

S13-2 S. Tanaka(1,2) and M. Suzuki(2)
(1) Integrated Bioscience, Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8522, Japan; (2) Department of Biology, Faculty of Science, Shizuoka University, Shizuoka 422-8522, Japan.
Molecular diversity of aquaporin for water adaptation strategy in anuran amphibians

S13-3 A. P. Seale(1), J. P. Breves(2), S. Watanabe(3), T. Kaneko (3), D. T. Lerner(1)(4), T. Hirano (1), E. G. Grau(1)
(1) Hawai'i Institute of Marine Biology, University of Hawaii, Kaneohe, HI, U.S.A.; (2) Department of Biology & Center for Neuroendocrine Studies, University of Massachusetts, Amherst, MA, U.S.A.; (3) Department of Aquatic Bioscience, University of Tokyo, Tokyo, Japan; (4) Sea Grant College Program, University of Hawai'i, Honolulu, HI, U.S.A.
Osmoreception and endocrine responses in the euryhaline tilapia, Oreochromis mossambicus

S14-1 T. Langkilde, N. A. Freidenfelds and T. Robbins
Department of Biology, Pennsylvania State University, State College, PA 16802, U.S.A.
Stress and invasion: elevated levels of corticosterone may facilitate survival-enhancing behavior of native lizards

S14-2 L. M. Romero
Dept. of Biology, Tufts University, Medford, MA, U.S.A.
Using reactive scope to understand physiological responses during stress

S14-3 D. Nesan and M. M. Vijayan
Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada.
The role of cortisol in teleosts: stress adaptation and early development

S15-1 J. N. McNeil
Department of Biology, The University of Western Ontario, London, ON, N6A 5B, Canada.
Pheromone mediated mating in migratory moths

S15-2 N. S. Johnson(1), M. J. Siefkes (2), C. O. Brant(3) and W. Li(3)
(1) United States Geological Survey, Great Lakes Science Center, Hammond Bay Biological Station, Millersburg, MI, U.S.A.; (2) Great Lakes Fishery Commission, Ann Arbor, MI, U.S.A.; (3) Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, U.S.A.
Identity, function and application of a male pheromone in sea lamprey

S15-3 R. T. Mason(1) and M. Halpern(2)
(1) Department of Zoology, Oregon State University, Corvallis, OR 97331, U.S.A.; (2) Department of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, NY 11203, U.S.A.
Chemical ecology of snakes: from pheromones to receptors

S16-1 M. E. Adams(1), D. Kim(1), Y. J. Kim(1), and D. Zitnan(2)
(1) Department of Entomology, University of California, Riverside, CA, U.S.A.; (2) Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia.
Peptidergic signaling cascades in the regulation of insect ecdysis

S16-2 D. A. Lovejoy(1), D. Chand(1), L. A. Tan(1), R. De Almeida(1), T. G. Nock(1), M. Xu(1), T. Ng(1), C. Yeung(1), L. Song(1), and D. Barsyte-Lovejoy(2)
(1) Dept. of Cell and Systems Biology, University of Toronto, Toronto ON, Canada; (2) Structural Genomics Consortium, University of Toronto, Toronto ON, Canada.
Teneurin C-terminal associated peptides (TCAP): new peptides involved in the neural regulation of corticotropin-releasing factor (CRF)

S16-3 C. J. Martyniuk(1), S. Alvarez(2), B. Chown(1), J. T. Popesku(3), V. L. Trudeau(4), and N. D. Denslow(5)
(1) Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, NB, Canada; (2) Donald Danforth Plant Science Center, St Louis, MO, U.S.A.; (3) Child & Family Research Institute, University of British Columbia, Vancouver, BC, Canada; (4) Department of Biology, University of Ottawa, Ottawa, ON, Canada; (5) Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, U.S.A.
Quantitative proteomics for fish neuroendocrinology and neurotoxicology

S17-1 G. F. Ball
Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, 21218, U.S.A.
Hormone-induced adult neuroplasticity and the activation of behavior in birds

S17-2 Brian C. Trainor
Department of Psychology, University of California, Davis, CA 95616, U.S.A.
Effects of the environment on estrogen-dependent mechanisms of aggressive behavior

S17-3 R. Knapp(1) and E.Marsh-Matthews(1)(2)
(1) Department of Zoology, University of Oklahoma, Norman, OK, U.S.A.; (2) Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK, U.S.A.
Cross-talk between endocrine stress and reproductive axes in a livebearing fish

S18-1 M. R. Brown, M. Gulia-Nuss, A. E. Robertson, and M. R. Strand
Department of Entomology, University of Georgia, Athens, GA, 30602, U.S.A.
Neuropeptides and amino acid sensing coordinately regulate blood digestion and reproduction in the mosquito Aedes aegypti

S18-2 H.Volkoff
Department of Biology, Memorial University of Newfoundland, St. John's, NL, Canada.
Comparative aspects of the endocrine regulation of feeding in fish models

S18-3 M.A. Sheridan
Department of Biological Sciences, North Dakota State University, Fargo, ND, U.S.A.
Resolving the growth-promoting and lipid-catabolic actions of growth hormone

List of abstracts

S19-1 W. Ge
School of Life Sciences and Centre for Cell and Developmental Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.
Intrafollicular communication network in the zebrafish ovary – what have we learned from the zebrafish model?

S19-2 A. S. Raikhel
Department of Entomology and Institute of Integrative Genome Biology, University of California Riverside, Riverside California, U.S.A.
Regulatory pathways controlling mosquito reproduction

S19-3 H. Yao(1), I. Barsoum(2), and J. Chen-Che Huang(3)
(1) Reproductive Developmental Biology Group, Laboratory of Reproduction and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC, U.S.A.; (2) Department of Cell & Developmental Biology, University of Illinois, Urbana, U.S.A.; (3) Department of Comparative Biosciences, University of Illinois, Urbana, U.S.A.
Sex, survival, and hedgehog: a story of how mouse embryos make their testes and adrenal glands

S20-1 H. R. Habibi, E. R. Nelson, E. R. O. Allan, and F. Y. Pang
Institute of Environmental Toxicology and Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.
Thyroid hormone control of reproduction in goldfish

S20-2 V. L. Trudeau(1), E. Zhao(1), A. Basak(2), G. C. López(3), L. F. Canosa(3), G. M. Somoza(3), P. Pouso(4), and A. Silva(4)
(1) Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, Canada; (2) Diseases Program, Ottawa Health Research Institute, Ottawa, ON, Canada; (3) Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico Chascomús, Argentina; (4) Laboratorio de Neurociencias, Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay.
Peptide identity crisis: is secretoneurin a new hormone?

S20-3 P. Deviche
School of Life Sciences, Arizona State University, Tempe, AZ U.S.A.
Plasma testosterone and behavior in free-ranging vertebrates: evolving views on temporal dynamics, control mechanisms, and significance

Oral Presentation Abstracts

OR1-1 A. M. Vajda(1), L. B. Barber(2), H. L. Schoenfuss(3), and D. O. Norris(4)
(1) Department of Integrative Biology, University of Colorado at Denver, CO, U.S.A.; (2) United States Geological Survey, Water Resources Division, Boulder, CO, U.S.A.; (3) Aquatic Toxicology Laboratory, St. Cloud University, St. Cloud, MN, U.S.A.; (4) Department of Integrative Physiology, University of Colorado at Boulder, CO, U.S.A.
Reproductive disruption by estrogenic wastewater effluents

OR1-2 F. Hoffmann(1)(2) and W. Kloas(1)(2)
(1) Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany; (2) Department of Endocrinology, Humboldt University, Berlin, Germany.
Calling behavior of male South African clawed frogs (Xenopus laevis) is affected by the estrogen 17α-ethinyl estradiol

OR2-1 J. Zhou(1)(2), J. Xiang(3), S. Zhang(2), and C. Duan(1)
(1) MCDB, Univ. of Michigan, Ann Arbor, MI48109; (2) Ocean University of China, Qingdao, China; (3) Institute of Oceanology, CAS, Qingdao, China.
Identification and functional analysis of an ancient IGF binding protein in the most primitive chordate, amphioxus

OR2-2 M. S. Rahman and P. Thomas
University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373, U.S.A.
Molecular characterization of three IGFBPs in Atlantic croaker and their regulation during hypoxic stress: potential mechanisms of their upregulation by hypoxia

OR3-1 E. J. Cable(1), D. C. Eckhoff(1), R. Narvaez(1), and B. J. Prendergast(1,2)
(1) Department of Psychology, Integrative Neuroscience Program, University of Chicago, Chicago, IL 60637, U.S.A.; (2) Committee on Neurobiology, University of Chicago, Chicago, IL 60637, U.S.A.
Effects of circadian arrhythmia on reproductive function in female siberian hamsters

OR3-2 Orie Shafer, Katherine Lelito, Ann Marie Macara, Tamara Minosyan, and Zepeng Yao
Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, U.S.A
The anatomical and physiological basis of pigment dispersing factor's circadian functions in Drosophila

OR4-1 G. E. Demas
Department of Biology, Indiana University, Bloomington, IN, U.S.A.
Leptin: a neuroendocrine mediator of immune function in vertebrates

OR4-2 A. M. Philip, S. Kim and M. M. Vijayan
Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.
Cortisol modulates LPS-mediated immune responses in rainbow trout hepatocytes

OR5-1 G. J. Roch and N. M. Sherwood
Department of Biology, University of Victoria, Victoria, BC, Canada.
Stanniocalcin: a calcium regulator with an ancient origin in unicellular eukaryotes

OR5-2 T. Kosugi, M. T. Wilmot and S. A. Sower
Center for Molecular and Comparative Endocrinology, University of New Hampshire, Durham, NH, U.S.A.
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OR6-1 H. Kamei and C. Duan
Department of Molecular, Cellular, & Developmental Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.
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OR6-2 Z. Starostova(1), L. Kubicka(2), A. Golinski(3), and L. Kratochvil(2)
(1) Department of Zoology, Charles University in Prague, Prague, Czech Republic; (2) Department of Ecology, Charles University in Prague, Prague, Czech Republic; (3) Graduate Program in Endocrinology and Animal Biosciences, Rutgers University, New Brunswick, NJ 08901, U.S.A.
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OR8-2 D. Chand(1), L. Song(1), D.Barsyte-Lovejoy(2) and D. A. Lovejoy(1)
(1) Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada; (2) Structural Genomics Consortium, University of Toronto, Toronto ON, Canada.
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(1) Department of Biological Sciences, University of Toronto Scarborough, Toronto, Ontario, Canada; (2) Centre for the Neurobiology of Stress Scarborough, University of Toronto Scarborough, Toronto, Ontario, Canada; (3) School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia.
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OR9-2 H. L. Hennin(1), J. Bêty(2), H. G. Gilchrist(3) and O. P. Love(1)
(1) Department of Biological Sciences, University of Windsor, Windsor, ON, Canada; (2) Département de Biologie et Centre d'études nordiques, Université du Québec à Rimouski, Rimouski, QC, Canada; (3) National Wildlife Research Centre, Environment Canada, Ottawa, ON, Canada.
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OR10-1 K. Fujimoto and Y. Shi
Section on Molecular Morphogenesis, Laboratory of Gene Regulation and Development, Program in Cellular Regulation and Metabolism (PCRM), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD 20892, U.S.A.
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OR10-2 G. Morvan-Dubois, J. Fini, K. Le Blay, and B. Demeneix
UMR CNRS/MNHN 7122, Evolution des Régulations Endocriniennes, Département Régulations, Développement et Diversité Moléculaire, 75231 Paris, France.
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OR11-1 P. Bagamasbad(1), R. M. Bonett(2) and R. J. Denver(1)(3)
(1) Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, U.S.A; (2) Department of Biological Science, The University of Tulsa, Tulsa, OK; (3) Neuroscience Program, University of Michigan, Ann Arbor, MI, U.S.A.
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OR11-2 A. Mendoza-Cisneros, P. Villalobos, C. Valverde-R, and A. Orozco
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla Querétaro, Querétaro México.
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Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.
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OR12-2 T. A. Onuma and C. Duan
Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.
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(1) USGS, Conte Anadromous Fish Research Center, Turners Falls, MA, U.S.A.; (2) Department of Biology, University of Massachusetts, Amherst, MA, U.S.A.; (3) Department of Biology, University of Bergen, High Technology Centre, Bergen N-5000, Norway; (4) Fish Endocrinology Laboratory, Department of Zoology/Zoophysiology, Göteborg University, Sweden.
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Department of Biology, North Carolina State University, Raleigh, NC, U.S.A.
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(1) Department of Zoology, Michigan State University, MI 48823, U.S.A.; (2) Centre for the Neurobiology of Stress, University of Toronto Scarborough, Scarborough, Ontario, M1C 1A4, Canada; (3) Stan Boutin, Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada; (4) Department of Biomedical Sciences/Biochemistry, University of Veterinary Medicine, A-1210 Vienna, Austria; (5) Department of Natural Resource Sciences, Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, Québec, H9X 3V9, Canada; (6) Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.
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OR14-2 M. C. Romano(1), R. A. Valdez(1), L. Hinojosa(1), and K. Willms(2).
(1) Dpto. Fisiología, Biofísica y Neurociencias, Cinvestav, Apdo. Postal 14-740, 07000, México D.F.; (2) Depto. de Microbiología y Parasitología, Facultad de Medicina, UNAM, México 04510, D.F.
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(1) Environmental Physiology Program, Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA 98112, U.S.A.; (2) School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105, U.S.A.
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Research Group Neuro-Endocrine-Immune Interactions, Institute of Anatomy, University of Zürich, Zürich, Switzerland.
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OR16-2 R. da Silva(1), S. R. da Silva(2) and A. B. Lange(1)
(1) Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada; (2) Department of Chemical and Physical Sciences, University of Toronto Mississauga, Mississauga, ON, Canada.
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OR17-1 E. McEvoy(1), S. Sonnenfeld(1), K. Dolence(2), and E. J. Coddington(1)(3)
(1) Department of Biology, Willamette University, 900 State St., Salem, OR, 97301, U.S.A.; (2) Department of Pharmacology, University of Wyoming, Laramie, WY, 83071, U.S.A.; (3) Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA, 02543, U.S.A.
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OR17-2 S. M. van Anders(1)(2), R. M. Tolman(3), and B. L. Volling(1)
(1) Department of Psychology, University of Michigan, Ann Arbor, MI, U.S.A; (2) Department of Women's Studies, University of Michigan, Ann Arbor, MI, U.S.A.; (3) School of Social Work, University of Michigan, Ann Arbor, MI, U.S.A.
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OR18-1 M. Cui, Caroline Hu, C. Pelletier and R. J. Denver
Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, U.S.A.
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Laboratory of Integrative Neuroendocrinology, (1) Department of Biology and (2)School of Kinesiology and Health Sciences, York University, Toronto, Ontario, Canada.
Nesfatin-1: a novel metabolic hormone in fish and rodents

OR19-1 M. Mita(1), K. Yamamoto(2), M. Nakamura(3) and Y. Nagahama(4)
(1) Department of Biology, Faculty of Education, Tokyo Gakugei University, Tokyo, Japan; (2) Department of Biology, School of Education, Waseda University, Tokyo, Japan; (3) Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan; (4) Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki, Japan.
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OR19-2 E. Shepperd and S. Unniappan
Laboratory of Integrative Neuroendocrinology, Department of Biology, York University, Toronto, ON, Canada.
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SCRAMBLED GENES AND OVARIES: ENVIRONMENTAL CONTAMINANTS AND THE DEVELOPING GONAD
Louis J. Guillette Jr.
Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, U.S.A. and Hollings Marine Laboratory, Charleston, SC, U.S.A.

Many chemicals introduced into the environment adversely affect embryonic development of animals, including humans. It has been documented that developmental alterations can be induced by the endocrine disruptive effects of environmental contaminants as the endocrine system exhibits organizational effects on the developing embryo. Thus, a disruption of normal hormonal signals can permanently modify the organization and future functioning of the reproductive system. We have examined the health of wildlife species, especially the American alligator, in wetland systems, including freshwater and coastal ecosystems in Florida, USA with various pollutant histories (e.g., Lake Apopka – agricultural chemicals with high pesticide and nutrient levels; Kennedy Space Center, with industrial chemicals and metals). Using the American alligator (*Alligator mississippiensis*), wild caught animals and egg dosing studies have produced data indicating alterations in gonadal hormone production, secondary sex characteristics and gonadal anatomy. We have found alterations in the structure of the developing ovarian follicle that are associated with high infertility and early embryonic loss. These experimental studies provide the causal relationships between embryonic pesticide exposure and reproductive abnormalities that have been lacking in pure field studies. Moreover, at the gene expression level, the changes observed in wildlife are similar to changes described in the human ovary that are associated with several prevalent diseases (e.g., premature ovarian failure, polycystic ovary syndrome, polyovular follicle syndrome). An understanding of the developmental consequences of endocrine disruption in aquatic wildlife can lead to new indicators of exposure and a better understanding of the most sensitive life stages and the consequences of exposure during these periods for wildlife and humans. [Supported by NIEHS R21 ES014053-01 and Howard Hughes Medical Institute.]

PL2.
PROLACTIN: NEW FUNCTIONS FOR AN OLD HORMONE
Carmen Clapp, Edith Arnold, Mayda Ramirez, Norma Adan, Claudia Vega, Guadalupe Ledesma-Colunga, Yazmin Macotela, Gonzalo Martínez de la Escalera, and StephanieThebault
Institute of Neurobiology, National University of Mexico (UNAM), Queretaro 76230, Mexico.

Prolactin (PRL), the hormone fundamental for lactation, has a wide spectrum of biological effects within and beyond reproduction. Recent work has further extended the scope of PRL actions to protection of retinal function, survival of articular cartilage, and regulation of neurohypophyseal hormone release. For these functions, PRL serves as both a circulating hormone and a locally produced cytokine able to regulate cell survival, vascular function, and nitric oxide production. Notably, the proteolytic cleavage of PRL to vasoinhibins, a family of peptides with potent antiangiogenic and proapoptotic effects, critically influences the outcome of these actions. Attention is focused on the roles of PRL and vasoinhibins in the retina, cartilage, and hypothalamo-neurohypophyseal system under physiological conditions and diseased states that are characterized by altered angiogenesis and tissue degradation, such as diabetic retinopathy and rheumatoid arthritis. (Supported by CONACYT grant SALUD-2008-CO1-87015 and UNAM grant IN200509).

PL3.
VESICLE MEDIATED SECRETION OF ECDYSONE FROM THE DROSOPHILA PROTHORACIC GLAND
Michael B. O'Connor and Naoki Yamanaka
Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN, 55455, U.S.A.

The insect prothoracic gland (PG) is an ideal model system for analyzing regulatory mechanisms that control steroid hormone biosynthesis and release. Through application of *Drosophila* molecular genetics, we have identified many of the ecdysone biosynthetic enzymes and now know that the activities of these enzymes are regulated at multiple levels (transcription, translation, phosphorylation) in the PG through the action of several neuropeptides including prothoracicotropic hormone (PTTH) and insulin. In contrast to the knowledge concerning biosynthetic enzyme regulation, almost nothing is known about control of gland activity at the level of hormone secretion. Using Drosophila genetics, we have uncovered several components that appear to be required for efficient secretion of ecdysone from the PG. These include a secretory Rab, components of the SNARE complex, and at least one ABC-type transporter. We propose that steroid hormone release from the PG is through a regulated vesicle-mediated process and not by simple diffusion as is generally assumed for many steroid hormones. The implications of our findings and possible signaling systems involved in triggering hormone release will be discussed.

PL4.
HORMONES, LIFE-HISTORY, AND PHENOTYPIC VARIATION

Tony D. Williams
Dept. of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 4H8, Canada.
In this talk I will argue that life-histories provide a powerful, conceptual framework for integration of endocrinology, evolutionary biology and ecology. This has been a commonly articulated statement (e.g. *Ann. Rev. Ecol. Evol. System*. 38:793; *TREE* 22:80) but I will show, in the context of **avian reproduction**, that true integration of ultimate and proximate approaches has been slow. We have only a very rudimentary understanding of the physiological and hormonal basis of *phenotypic* variation in reproductive traits that contribute to

individual variation in lifetime fitness in birds (e.g. laying date, clutch size, parental effort) and of trade-offs that link these traits or that link reproduction to other life stages (e.g. costs of reproduction). I will explore some of the reasons for this including, a) an increasingly reductionist and centralist focus which is more and more removed from ecological/evolutionary 'context' and from 'peripheral' physiological mechanisms that actually determine how phenotypes work, b) a long-standing male-bias in experimental studies, even though the key reproductive traits which contribute most to variation in fitness are female-specific traits (e.g. egg size or number). Endocrine systems provide strong candidate mechanisms for generation of phenotypic variation in single traits as well as trade-offs between traits due to, a) hormonal 'pleiotropy': single hormones having both positive and negative effects on multiple physiological systems and, b) hormonal or physiological 'conflict' between regulatory systems required for different but over-lapping or linked life-history stages. I will illustrate this with examples involving reproductive anemia in egg-producing females, parental care in starlings, migration-reproduction interactions in penguins and albatrosses, and moult-breeding overlap in petrels (Supported by NSERC Discovery Grant).

PL5.
ORIGIN AND EVOLUTION OF METAMORPHOSIS AND THYROID HORMONES
Vincent Laudet
Institut de Genomique Fonctionnelle de Lyon (IGFL), CNRS-UMR 5242, Ecole Normale Supérieure de Lyon, 46, Allée d'Italie, 69364 Lyon CEDEX 07, France.

Vertebrate metamorphosis is a spectacular post-embryonic process exemplified by the transformation of a tadpole into a frog. This transition implies the appearance of new body parts (e.g. the limbs), the resorption of larval features (e.g. the tail) and the remodelling of many organs (e.g. the skin or the intestine). Metamorphosis has been well characterized in anuran amphibians, where thyroid hormone (TH) orchestrates the intricate and seemingly contradictory changes observed at the cellular and tissue levels. TH controls a complex hierarchical cascade of target genes via binding to specific receptors, TRα and TRβ, which are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Metamorphosis is in fact widespread throughout vertebrates, which exhibit an impressive diversity in this regard. In addition, variations of this key event (from paedomorphosis, the absence of metamorphosis, to direct development which actually corresponds to precocious metamorphosis) provides a unique opportunity to illustrate how the tinkering of the cascade controlling metamorphosis can lead to divergent life histories. The study of invertebrate chordates has also shed light on the origin of metamorphosis. The data converge towards a model suggesting that post-embryonic remodelling governed by TH is a common feature of all chordates. According to this view, metamorphosis of the anurans is an extreme example of a widespread life history transition.

PL6.
HORMONAL ORGANIZATION AND ACTIVATION: EVOLUTIONARY IMPLICATIONS AND QUESTIONS
Elizabeth Adkins-Regan
Departments of Psychology/Neurobiology & Behavior, Cornell University, Ithaca, New York, U.S.A.

Vertebrate comparative endocrinologists have made many important discoveries about the role of sex steroid hormones in the organization and activation of sexually differentiated behavior, brain function, anatomy and physiology. Such research has also addressed the organization/activation conceptual framework itself, providing important extensions and modifications. In additional to general principles and conserved features, there is also striking diversity among vertebrates in organization and activation by sex steroids. The evolution of this diversity (both phylogeny and function) is poorly understood. A set of largely unanswered questions will be raised to illustrate this point, including taxonomic differences in patterns of sexual differentiation. This last example raises a further evolutionary puzzle: how to explain the phylogeny of vertebrate sex determining mechanisms. Results of a new study will be presented that combines modeling with a comparative analysis to conclude that body size sexual dimorphism has been an important factor in the evolution of XX/XY and ZZ/ZW systems from environmental sex determination.

PL7.
THE EVOLUTION AND FUNCTION OF GNRH AND GNRH-LIKE LIGANDS AND RECEPTORS: A MODEL OF BIOMEDICAL TRANSLATION OF COMPARATIVE ENDOCRINOLOGY
Robert P. Millar(1)(2)(3)
(1) Mammal Research Institute, University of Pretoria, Pretoria, South Africa; (2) UCT/MRC Receptor Biology Group, University of Cape Town, Cape Town, South Africa; (3) Centre of Integrative Physiology, University of Edinburgh, Scotland.

Comparative endocrinology has made numerous contributions to biomedicine.The current emphasis on seeking translational relevance of fundamental discovery poses challenges to comparative endocrinologists. The comprehensive elucidation of GnRH and GnRH-like ligands and their cognate receptors spanning many million years of evolution has provided structural and functional insights which has translated into drug discovery and development. The findings have revealed ligand features for conformation restriction, super agonism, antagonism and ligand-selective signalling. The presence of related GnRH-like ligands and receptors in insects and nematodes suggests that our findings of structurally important features from comparative studies in vertebrates and chordates may be further exploited to develop new classes of analogs to regulate insect pests and pathogenic parasites. Overall, several decades of research on the comparative and evolutionary biology of GnRHs and their receptors has provided a substrate for the elaboration of s suite of drugs which are extensively used in medical therapies and generated billions of dollars.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Wednesday, July 13th
2:30 – 4:20 p.m.
Mendelssohn Theater

NASCE 2011 Symposium 1:
Endocrine Disruption: Impacts on Wildlife

Chairpersons:
Mary Ann Ottinger, University of Maryland, USA
Peter Thomas, University of Texas, Austin, USA

S1-1.
NEUROENDOCRINE MEASURES OF ENDOCRINE DISRUPTOR CHEMICAL IMPACT IN BIRDS
M. A. Ottinger, M. Bohannon, T. Carro, L. Carpenter, and K. Dean
Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 20742, U.S.A.

Assessing potential risk associated with exposure to endocrine disrupting chemicals (EDCs) has been difficult in birds due to variations in reproductive strategies, life span, and steroid action in precocial and altricial species. In precocial birds, embryonic exposure to EDCs impacts sexual differentiation of neuroendocrine systems and behavior. Often, detectable non-lethal effects of EDCs diminish as the organism matures so that the long term impact of EDCs may appear relatively innocuous by the time an individual is sexually mature. Few studies have considered lifetime effects of EDC exposure in birds, especially in field populations. Assessing the consequences of EDC exposure in field birds is often to complex mixtures rather than to lone compounds. Our studies have been designed to examine effects of individual EDCs administered to the embryo as well as in a multi-generational dietary study in which birds received low doses of the pesticide, methoxychlor (MXC). We also compared effects of dietary methoxychlor exposure in Japanese quail and northern bobwhite quail to compare effects of dietary exposures (0.5, 5 and 10ppm) that are relatively low. Finally, we have considered the comparative effects of a complex mixture. Our data will be discussed in the context of applicability of toxicological yardsticks, including the Toxic Equivalent (TEQ) and Neurotoxic Equivalent (NEQ) as predictive indices for short and long term outcomes to non-lethal concentrations of EDCs. Other approaches have been developed to address inconsistencies in effects and to incorporate diverse data into the potency estimates. Perhaps it is time to develop a more inclusive estimation method for endocrine and neuroendocrine effects. An Endocrine Disruption Index (EDI) would complement other indices, and focus on endocrine disruption and includes effects beyond those mediated by the aryl hydrocarbon receptor (AhR) for a comparative assessment of non-lethal EDC effects. [Research supported by EPA grants # R826134010 (Star Grant) and R-82877801; Battelle contract for EPA-EDSTAC validation studies, NRI #92-37203 and NSF #9817024; MAES, University of Maryland, College Park; and U.S. Fish and Wildlife Service.]

S1-2.
DETERMINING ENDOCRINE DISRUPTIVE POTENTIAL OF ENVIRONMENTAL CONTAMINANTS IN FROGS
Caren C. Helbing
Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., Canada.

Thyroid hormones (THs) are important in the regulation of growth, development, and metabolism in all vertebrates. In frogs, THs are required for the metamorphosis of a tadpole into a frog. The best-understood mechanism of action is through highly-conserved TH receptors (TRs) and modulation of gene transcription. The effect of THs on gene expression varies from tissue to tissue and these differences in effect can translate into differential sensitivities to environmental contaminants capable of disrupting TH action. We have been developing various molecular methods in combination with whole animal and organ culture exposures to identify such disruption. In order to understand what constitutes disruption, a better understanding of what is considered "normal" is required. Using transcriptomics, proteomics, and metabolomics approaches, we have taken an integrated approach to defining a TH response in bullfrog (*Rana catesbeiana*) tadpoles. Studies involving nanoparticles and substances used in personal care products will be highlighted. (This work was supported by NSERC.)

S1-3.
INDICES OF ENDOCRINE DISRUPTION AND REPRODUCTIVE DYSFUNCTION IN FISH POPULATIONS FROM LAKE MEAD AND THE LOWER COLORADO RIVER
Reynaldo Patiño(1), Steven L. Goodbred(2), Erik Orsak(3), Jill A. Jenkins(4), Michael R. Rosen(5), Leticia Torres(6), Prakash Sharma(6), Carla Wieser(7) and Shane Ruessler(7)
(1) U.S. Geological Survey, Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409-2120, U.S.A.; (2) U.S. Geological Survey (Emeritus), High Point, NC 27262, U.S.A.; (3) U.S. Fish and Wildlife Service, Arlington Ecological Services Field Office, Arlington, TX 76011, U.S.A.; (4) U.S. Geological Survey, National Wetlands Research Center, 700 Cajundome Blvd., Lafayette, Louisiana 70506, U.S.A.; (5) U.S. Geological Survey, Nevada Water Science Center, Carson City, NV 89701, U.S.A.; (6) Department of Biological Sciences and Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409-2120, U.S.A.; (7) U. S. Geological Survey, Southeast Ecological Science Center, Gainesville, FL 32653, U.S.A.

Municipal and industrial runoff and wastewater inputs to Lake Mead reservoir via Las Vegas Wash (LVW) generate an environmental contaminant gradient within the reservoir. We used multivariate (discriminant function) analysis to examine spatiotemporal differences in overall health of adult male common carp (*Cyprinus carpio*) from Lake Mead and downstream of Hoover Dam. Fish were collected from LVW, Las Vegas Bay (LVB, mixing zone between LVW and the reservoir), Overton Arm (OA, reference site), and Willow Beach (WB, downstream of the dam) in 2007-2008. We examined total length, Fulton's condition factor, gonadosomatic index (GSI), hematocrit, plasma 11-ketotestosterone (11kt), estradiol-17β (E2), and vitellogenin (Vtg). The influence of season on morpho-physiological condition was greatest at OA followed by LVB, LVW and WB. This observation indicates the occurrence of phenological (including endocrine) variability among the target fish populations. Also, OA fish generally had higher GSI, higher 11kt and E2, and lower E2/KT ratio (except for WB, with ratios similar to OA); and the overall status of LVB fish seemed to fall between OA and LVW fish. WB fish generally deviated from the reference condition (OA fish) in similar manner as LVW fish, an anomaly perhaps explained by unique water quality conditions at WB. Vtg was low or undetectable at all sites. Preliminary results of canonical correlation analysis indicated an association between the biological endpoints examined and whole-body burdens of organic contaminants and metals. In conclusion, the morpho-physiological condition of male carp can be ranked in

descending order according to study site as follows: OA>LVB>LVW/WB; which generally reflects the contaminant gradient in the system. Results of the multivariate-statistical approach used in this study also suggest that changes in phenology or the strength of phenological events should be considered when assessing reproductive and endocrine disruption in wild fishes.

OR-1.
REPRODUCTIVE DISRUPTION BY ESTROGENIC WASTEWATER EFFLUENTS
A. M. Vajda(1), L. B. Barber(2), H. L. Schoenfuss(3), and D. O. Norris(4)
(1) Department of Integrative Biology, University of Colorado at Denver, CO, U.S.A.; (2) United States Geological Survey, Water Resources Division, Boulder, CO, U.S.A.; (3) Aquatic Toxicology Laboratory, St. Cloud University, St. Cloud, MN, U.S.A.; (4) Department of Integrative Physiology, University of Colorado at Boulder, CO, U.S.A.

The reproductive potential of native fishes may be compromised in stream reaches of western states where large volumes of treated wastewater are discharged into relatively small-sized streams. We investigated the impact of City of Boulder wastewater treatment plant (WWTP) effluent on fish reproduction. This effluent contains endocrine-active compounds including nonylphenol, bisphenol A, and synthetic and natural reproductive steroids. We have identified female biased sex ratios, gonadal intersex, asynchronous ovarian development, elevated vitellogenin, and other forms of reproductive disruption in feral white suckers (*Catostomus commersoni*) collected downstream of WWTP effluent but not at reference sites. Analysis of museum specimens collected between 50 and 100 years ago from these sites reveals no evidence of reproductive disruption. We conducted on-site controlled exposure experiments using a mobile flow-through laboratory, exposing adult male fathead minnows (*Pimephales promelas*) to either WWTP effluent, reference water from Boulder Creek upstream of the WWTP, or mixtures of reference water and WWTP effluent (containing an average of 29 ng/L estradiol-equivalents of estrogenicity) for up to 56 days. Exposure to diluted WWTP effluent significantly elevated vitellogenin and suppressed male sex characters after 7 days of exposure. A similar study of possible effects of wastewater effluent from a small mountain community on juvenile brown trout (*Salmo trutta*) showed no increase in vitellogenin. A re-evaluation conducted following a major upgrading of the Boulder WWTP revealed diminished responses to wastewater effluent in adult male fathead minnows.

OR-2.
CALLING BEHAVIOR OF MALE SOUTH AFRICAN CLAWED FROGS (*XENOPUS LAEVIS)* IS AFFECTED BY THE ESTROGEN 17A-ETHINYL ESTRADIOL
Frauke Hoffmann(1)(2) and Werner Kloas(1)(2)
(1) Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany; (2) Department of Endocrinology, Humboldt University, Berlin, Germany.

17α-ethinyl estradiol (EE2), a main component of many contraceptive pills, is an environmentally relevant model substance for estrogenic endocrine disrupting compounds (EDC). In this study, effects of EE2 on male mate calling behaviour of *Xenopus laevis* induced by human chorionic gonadotropin treatment were investigated. Adult males were exposed to EE2 at five different concentrations (296 µg/l, 2.96 µg/l, 29.6 ng/l, 2.96 ng/l, and 0.296 ng/) for 96 h. Effects of the treatments were analyzed by determining the total calling activity as well as the preferred utilization of different call types used in their vocalizations. Additionally, effects of EE2 exposures on temporal and spectral parameters of the advertisement calls (AC) were analyzed. While the absolute calling activity was not affected, the relative proportions of AC, calls indicating a sexually aroused state of the male, were diminished even by the lowest investigated EE2 concentration, 0.296 ng/l. The proportions of produced call types signalling a sexually unaroused state of the male, like rasping, on the other hand increased significantly in all EE2 treatment groups. EE2 exposure also altered several temporal and spectral features of the AC, per se; click durations decreased and accentuation of clicks within the calls diminished. The results of additional playback experiments examining the ecological relevance of these alterations of call features suggest that exposures to environmentally relevant concentrations of EE2 lower the chances of exposed male *X. laevis* to mate and reproduce successfully. In a supplemental experiment we also show that the alterations of the male advertisement call features are reversible over time. To our knowledge this study provides for the first time evidence that estrogens affect parameters of male calling behaviour of *X. laevis* and that calling behaviour can be used as highly sensitive non-invasive biomarker for the detection of estrogenic EDC.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Wednesday, July 13th
2:30 – 4:20 p.m.
Vandenberg Room

NASCE 2011 Symposium 2:
Insulin/IGF Signaling in Longevity Regulation: New Insights from Animal Models

Chairpersons:
Cunming Duan, University of Michigan, USA
Patrick Hu, University of Michigan, USA

S2-1.
THE *C. ELEGANS* SERINE/THREONINE KINASE SGK-1 PROMOTES LONGEVITY BY ACTIVATING THE FOXO TRANSCRIPTION FACTOR DAF-16
Chunfang Guo(1), Kathleen Dumas(1)(2), Travis Williams(1), Kaveh Ashrafi(3), and Patrick J. Hu(1)(4)
(1) Life Sciences Institute, University of Michigan, Ann Arbor, MI, U.S.A.; (2) Cellular and Molecular Biology Graduate Program, University of Michigan Medica
-*2*, which extend lifespan, null mutations in *sgk-1* shorten lifespan. Conversely, a gain-of-function (gf) mutation in *sgk-1* extends mean lifespan by ~18% in a DAF-16/FoxO-dependent manner. Knockdown of each of the two distinct isoforms *daf-16a* and *daf-16d/f* redul School, Ann Arbor, MI U.S.A.; (3) Department of Physiology, University of California, San Francisco School of Medicine, San Francisco, CA, U.S.A.; (4) Departments of Internal Medicine and Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI U.S.A.

Insulin/insulin-like growth factor signaling (IIS) has an evolutionarily conserved role in regulating longevity. In invertebrates, the increase in lifespan due to reduction of IIS requires FoxO transcription factor activity, indicating that FoxO transcription factors promote longevity and are inhibited by IIS. Therefore, understanding the molecular basis for FoxO regulation by IIS promises to illuminate general mechanisms underlying longevity control by IIS. IIS inhibits FoxO activity via the serine/threonine kinase Akt, which promotes FoxO phosphorylation at three conserved sites that lie within consensus motifs recognized by the AGC (cAMP-dependent, cGMP-dependent, and protein kinase C) family of serine/threonine kinases. The conserved AGC family member Sgk is thought to act in concert with Akt to regulate longevity by directly phosphorylating and inhibiting FoxO activity. This is based in large part on the finding that in the nematode *Caenorhabditis elegans*, knockdown of *sgk-1* by RNAi extends lifespan in a manner dependent upon the FoxO transcription factor DAF-16. We have recently shown that, in contrast to null mutations in *akt-1* and *akt*ces lifespan in *sgk-1(gf)* animals, suggesting that SGK-1 extends lifespan by activating both DAF-16A and DAF-16D/F isoforms. Taken together, our data suggest that, in contrast to AKT-1 and AKT-2, which control lifespan by inhibiting DAF-16/FoxO activity, SGK-1 promotes longevity by activating DAF-16/FoxO. These findings highlight a complexity to FoxO regulation that is not captured by prevailing models of concordant FoxO regulation by Akt and Sgk.

S2-2.
SOMATOTROPIC SIGNALING, ADIPOSE TISSUE AND LONGEVITY
Andrzej Bartke
Department of Physiology and Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL, U.S.A.

Mutant mice lacking growth hormone (GH) or GH receptor live longer than their normal siblings. This somewhat counterintuitive finding is reproducible and robust with significant increases of average and maximal longevity in both females and males and is not limited to a particular genetic background or diet composition. Increased longevity was reported also in mice with deletion of genes regulating levels and principal signaling pathway of insulin-like growth factor 1(IGF-1) an important mediator of GH action. Multiple mechanisms are involved in linking GH resistance in GH receptor deleted (Ghr-/-) mice and GH deficiency in hypopituitary Ames dwarf and Snell dwarf mice with their extended longevity. These mechanisms include partial protection from cancer, reduced activity of mammalian target of rapamycin (mTOR) signaling pathway, hypoinsulinemia combined with greatly improved insulin signaling, enhanced stress resistance and reduced oxidative damage. Unexpectedly, improved insulin sensitivity in these long-lived mutants is associated with increased rather than reduced adiposity. The likely reasons why in the absence of GH signaling, increased adiposity does not promote insulin resistance include different (preferentially subcutaneous rather than intra-abdominal) distribution of adipose tissue and different secretory profiles of intra-abdominal adipocytes and associated macrophages. Of particular significance are increased levels of adiponectin which exerts anti-inflammatory and anti-atherogenic effects and promotes insulin sensitivity, and reduced levels of pro-inflammatory cytokines. There is increasing evidence that relationships of GH signaling and somatic growth to healthspan and life span discovered in mutant mice apply also to genetically normal animals and to other species, including the human. (Supported by NIA.)

S2-3.
STRESS RESISTANCE IN MUTANT MICE AND LONG-LIVED SPECIES: CLUES TO EVOLUTIONARY MECHANISM?
Richard A. Miller
Department of Pathology and Geriatrics Center, University of Michigan, and Ann Arbor VA GRECC, Ann Arbor, MI, U.S.A.

Mutations that extend lifespan in C. elegans usually render the worms resistant to multiple forms of stress, including oxidative stress, heat, heavy metals, and agents that damage DNA, suggesting that stress resistance may be the cause of the deceleration of aging. To test the link between aging and cellular stress resistance, we made fibroblasts from three long-lived mouse endocrine mutants (Snell and Ames dwarf, and growth hormone receptor KO), and found that the cells were resistant to lethal injury caused by UV light, H_2O_2, cadmium, paraquat, heat, and the DNA alkylating agent MMS. To see if evolution of long-lived species also involved modulation of cellular stress resistance, we made primary fibroblasts from 8 species of rodents. Cells from the species with longest lifespans were resistant to cadmium and H_2O_2, with suggestive trends for resistance to heat and MMS, but we saw no relationship to UV and paraquat resistance. Cells from a long-lived bat (Little Brown Bat) were more resistant than rat and mouse cells to all four forms of stress. Because birds live longer than comparably sized rodents, we tested the hypothesis that bird-derived fibroblasts would be particularly resistant to multiple forms of stress, and found elevated resistance of bird cells to cadmium, paraquat, H_2O_2, MMS, and UV light (35 bird species vs. 9 rodent species, weight adjusted). Among bird species, maximal longevity was associated with resistance to cadmium, paraquat, H_2O_2, and MMS (but not UV). Lastly, a study of genetically heterogeneous mice showed that those individuals whose cultured skin fibroblasts were relatively resistant to H_2O_2 or UV tended to retain hearing into old

age. These data suggest that extended longevity is accompanied by, and could perhaps be caused by, resistance to multiple forms of cellular injury, both within a species and across vertebrate species at various evolutionary scales. (Supported by the National Institute on Aging.)

OR2-1.
IDENTIFICATION AND FUNCTIONAL ANALYSIS OF AN ANCIENT IGF BINDING PROTEIN IN THE MOST PRIMITIVE CHORDATE, AMPHIOXUS

J. Zhou(1)(2), J. Xiang(3), S. Zhang(2), and C. Duan(1)
(1) MCDB, Univ. of Michigan, Ann Arbor, MI48109; (2) Ocean University of China, Qingdao, China; (3) Institute of Oceanology, CAS, Qingdao, China.

Insulin-like growth factor binding proteins (IGFBPs) are high affinity IGF binders and play important roles in controlling IGF availability and bioactivity. All known vertebrate IGFBPs are secreted proteins but some can also be found in the nucleus and possess IGF-independent actions. To understand the evolution origin of this gene family, we cloned and characterized an IGFBP gene from Amphioxus, the most basal lineage of chordates. In contrast to the presence of 6 IGFBP genes in mammal genomes and about a dozen IGFBP genes in teleost genomes, only a single IGFBP gene is found in Amphioxus genome. The genomic and protein structure of Amphioxus IGFBP (AmiBP) is similar to its vertebrate counterparts and it encodes a mature protein with a N-, L-, and C-domain. There is an IGFBP motif in the N-domain and a thyroglobulin type-1 motif in its C-domain. Like human IGFBP-3, -5, and -6, AmBP is not only secreted but also found in the nucleus when transfected into human cells. Its N-domain had transactivation activity when tested in a one-hybrid assay. Western immunoblot and Ligand blot analysis indicated that AmBP is secreted protein but it has low binding activity to human IGFs. A single amino acid change, however, converted AmBP into a high-affinity IGFBP. To examine its biological activity, AmBP mRNA was injected into zebrafish embryos along with human IGFBP-5 and -2 mRNA. Forced expression of IGFBP-5 in developing zebrafish embryos resulted in reduced trunk and tail, while forced expression IGFBP-2 caused no morphological abnormality but significantly decreased the growth and developmental rates. Forced expression of AmBP resulted in a phenotype very similar that of IGFBP-5. These results suggest that IGFBPs are ancient molecules present in all chordates. The AmBP shares many similar structural and biological properties with modern IGFBP-3 and -5. These results provide novel insights into the structural and functional evolution of the IGFBP family.

OR2-2.
MOLECULAR CHARACTERIZATION OF THREE IGFBPs IN ATLANTIC CROAKER AND THEIR REGULATION DURING HYPOXIC STRESS: POTENTIAL MECHANISMS OF THEIR UPREGULATION BY HYPOXIA

Md. Saydur Rahman and Peter Thomas
University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373, U.S.A.

Insulin-like growth factor binding proteins (IGFBPs) play important roles in down-regulating IGF activity and growth and development in vertebrates under hypoxic stress. However, the mechanisms of hypoxia regulation of IGFBPs in teleost fishes are unknown. The involvement of reactive oxygen species (ROS) and hypoxia-inducible factors (HIFs) in hypoxia upregulation of IGFBPs in Atlantic croaker were investigated. Three croaker IGFBPs, IGFBP-1, IGFBP-2, and IGFBP-5 were cloned and characterized. Chronic hypoxia exposure (dissolved oxygen, DO: 1.7 mg/L for 2 to 4 weeks) caused significant increases in hepatic and neural IGFBP-1 mRNA expression compared to tissue mRNA levels in fish held under normoxic conditions (6.5 mg DO/L). Moreover, longer-term chronic hypoxia exposure (2-2.7 mg DO/L for 15 to 20 weeks) caused significant increases in mRNA levels of all 3 IGFBPs in both liver and brain tissues. Hypoxia exposure also markedly increased superoxide radical ($O_2^{\bullet -}$, an index of ROS) production and HIF-1α mRNA and HIF-2α protein expression in croaker livers. Pharmacological treatment with an antioxidant attenuated the hypoxia-induced increases in $O_2^{\bullet -}$ production and HIFαs mRNA and protein expression, as well as the elevation of IGFBP-1 mRNA levels. These results suggest that the upregulation of IGFBP expression under hypoxia stress is due, in part, to alterations in the antioxidant status, which may involve ROS and HIFs. (Supported by NOAA Coastal Ocean Program Gulf of Mexico GOMEX grant NA06NOS4780131 to PT).

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Wednesday, July 13th
2:30 – 4:20 p.m.
Michigan Room

NASCE 2011 Symposium 3:
Tick, Tock, Setting the Clock: Hormones and Biological Rhythms

Chairpersons:
Teresa Lee, University of Michigan, USA
Orie Shafer, University of Michigan, USA

S3-1.
THE MASTER CLOCK AND ITS TIMING OF HORMONAL RELEASE
Horacio de la Iglesia, Travis Lilley, Michael Schwartz, Benjamin Smarr, and Cheryl Wotus
University of Washington, Department of Biology and Program of Neurobiology and Behavior, Seattle, WA, U.S.A.

The hypothalamic suprachiasmatic nucleus (SCN) contains a master circadian clock that regulates the timing of physiological and behavioral processes. This regulation is achieved through neural and humoral input to other brain centers that directly regulate these processes, as well as through input to extra-SCN and peripheral circadian oscillators that can presumably regulate physiology and behavior locally. The release of hormones is under striking circadian regulation; yet, the pathways by which the SCN times the release of hormones and the specific SCN neurons that are critical for this regulation are not completely clear. We will present data on the regulation of three key endocrine circadian rhythms, the release of melatonin, glucocorticoids and luteinizing hormone in females. To further understand the pathways through which the SCN governs the release of these hormones we exploit the independent oscillation of neuronal oscillators in the forced desynchronized rat and the split hamster, as unique neurologically, genetically and pharmacologically intact animal models.

S3-2.
THE OVULATION CLOCK: HOW THE HANDS OF THE CIRCADIAN OSCILLATOR TIME REPRODUCTION
Michael T. Sellix, Albert Tao and Michael Menaker
Department of Biology, University of Virginia, Charlottesville, VA, U.S.A.

Circadian clocks play an established role in the timing of reproductive physiology. Circadian rhythms of hormone secretion depend on the central circadian clock in the suprachiasmatic nucleus of the hypothalamus (SCN). It is widely accepted that the timing of events in female reproductive physiology, such as ovulation, depends entirely on the timing of hierarchical control by the hypothalamo-pituitary-gonadal axis. At the cellular level the molecular clock, an autoregulatory transcriptional feedback loop, drives circadian rhythms of physiology and behavior. Though first described in SCN neurons, rhythmic activity of this oscillator has been reported in most organs, including tissues of the reproductive system like the uterus, oviduct and ovary. However, it remains to be determined if the circadian clocks in the ovary, specifically the granulosa cells lining the ovarian follicle, play a significant role in the timing of ovulation. We have examined the role of the clock in the timing of ovulation by measuring the rhythmic sensitivity of the ovary to exogenous luteinizing hormone (LH) following suppression of endogenous gonadotrophin secretion. Adult cycling and juvenile gonadotrophin-primed (GP) female C57BL/6 mice were injected with a selective GnRH receptor antagonist to suppress endogenous LH secretion followed by timed injections of equine LH (eLH). Both adult and juvenile GP mice displayed circadian rhythms of ovarian sensitivity to eLH with greater ovulation during the night or subjective night. These data suggest that the circadian clock in the ovary, or some as yet unidentified rhythmic humoral or neural input, mediates the timing of ovulation by establishing a window of ovarian sensitivity to gonadotrophins. These data support transition to a new conceptual framework wherein oscillators at each level of the HPG axis contribute to the timing of critical events in reproductive physiology. (This work was partially supported by NIH P50 MH074924 (to MM) and a Harrison Undergraduate Research Award (to AT).
Acknowledgements: The authors acknowledge the technical assistance of Gwendolyn Yao, Ilia Iliev, Hanting Feng, Jordan Davis and Denise T. Holmes.

S3-3.
ADOLESCENT CIRCADIAN TIMEKEEPING IN TWO RODENT MODELS, RATTUS NORVEGICUS AND OCTODON DEGUS
Megan H. Hagenauer and Theresa M. Lee, Department of Psychology, University of Michigan, Ann Arbor, MI, USA 48105

Despite overwhelming evidence that the circadian timekeeping system is sensitive to gonadal hormones during perinatal development and adulthood, there has been a noticeable lack of research focusing on circadian rhythms during puberty, a developmental window of intense hormonal change. We addressed this research gap using behavioral and physiological experiments in both fast developing and slow developing rodent species (*Rattus norvegicus* and *Octodon degus*). Our results indicate that both species show large changes in chronotype during puberty similar to human adolescents, exhibiting an altered timing of rest and activity. These pubertal changes are particularly robust in males, involving a switch from bimodal to unimodal activity patterns as well as a 3 – 5hr magnitude phase advance of daily activity rhythms. Prepubertal gonadectomy diminished these changes in both species, indicating that pubertal increases in gonadal hormones drive chronotype changes. To determine the location of hormone action within the circadian timekeeping system, we used behavioral tests to rule out the possibility that chronotype changes during puberty were due to an alteration in passive responses to rhythmic environmental stimuli, such as the laboratory light-dark cycle. Then we characterized the phasing of "clock gene" rhythms within the central circadian regulator in the suprachiasmatic nucleus, as well as in downstream "slave" oscillators in other brain regions. Surprisingly, the phasing of these rhythms remained unchanged during puberty, despite previous evidence showing a tight correlation between these rhythms and behavioral chronotype in adults. These results suggest a striking desynchrony between adolescent rest/activity patterns and the underlying circadian physiology. We discuss these findings in the context of adolescent sleep studies and explain the relevance of our results to the debate regarding teenage chronotype and high school start times.

OR3-1.

EFFECTS OF CIRCADIAN ARRHYTHMIA ON REPRODUCTIVE FUNCTION IN FEMALE SIBERIAN HAMSTERS

Erin J. Cable(1), David C. Eckhoff(1), Rayna Narvaez(1), and Brian J. Prendergast(1,2)
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Circadian timing of behavior has profound implications for reproduction and survival. A circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) times the preovulatory LH surge and the onset of behavioral estrus to occur shortly before the onset of the active phase. Several studies have established that the LH surge requires a functional SCN. A SCN time-of-day signal dictates a circadian gate during which LH surges may occur. Virtually everything known about the necessity of circadian rhythms for female reproduction is derived from lesion studies or inbred/transgenic mice. To circumvent limitations inherent in such preparations, we used a hamster model of SCN arrhythmia generated noninvasively to investigate consequences of complete circadian desynchrony on aspects of female reproduction. In Siberian hamsters (Phodopus sungorus), precisely-timed light treatments permanently eliminate circadian rhythms in sleep/wake and locomotor activity. Initial studies determined whether deficits in fertility are evident in arrhythmic females. Control (entrained; "ENTR") and arrhythmic ("ARR") females were paired with males for 5 or 90 days, and fecundity was examined. In both experiments, fertility was comparable in ARR and ENTR females. Follow-up studies identified whether ARR females exhibit deficits in reproductive neuroendocrine function. Estradiol concentrations were comparable between ARR and ENTR females over a 4 day estrous cycle. In steroid-clamped, ovariectomized hamsters, we investigated whether ARR hamsters exhibit deficits in the timing or amplitude of LH surges. Data to be presented will identify whether ARR females exhibit LH surges comparable to those of ENTR females, and whether arrhythmia alters responsiveness of the HPG axis to steroid feedback. Persistence of breeding and accurate timing of daily LH surges in hamsters lacking coherent behavioral circadian rhythms would suggest a novel, circadian-independent, control of reproductive function. (Supported by NIH grant AI67406, and a Mellon Mays Fellowship from the University of Chicago).

OR3-2.

THE ANATOMICAL AND PHYSIOLOGICAL BASIS OF PIGMENT DISPERSING FACTOR'S CIRCADIAN FUNCTIONS IN DROSOPHILA

Orie Shafer, Katherine Lelito, Ann Marie Macara, Tamara Minosyan, and Zepeng Yao
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The sleep/activity rhythms of *Drosophila* require the presence of the pigment-dispersing factor (PDF)-expressing neurons of the central brain. PDF is an 18 amino acid peptide that regulates circadian rhythms in a manner highly similar to that of vasoactive intestinal peptide (VIP) in the mammalian suprachiasmatic nuclei. PDF is thought to coordinate the molecular rhythms of the network of ~150 clock neurons in the fly's central brain and exerts its circadian function through PDFr, a secretin-like G-protein-coupled-receptor. Here we present anatomical and live imaging experiments addressing the physiological and anatomical basis of PDF's circadian functions. We show that bath-applied PDF has little apparent effects on calcium dynamics and acts largely as a modulator of cyclic AMP in its clock neuron targets. Finally, we present a new set of circuit interrogation techniques to test current models of peptide signaling within the clock neuron network and to address the ways by which input from external photoreceptors modulates the physiology of the PDF-expressing clock neurons.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Wednesday, July 13th
2:30 – 4:20 p.m.
Hussey Room

NASCE 2011 Symposium 4:
Endocrine-Immune System Interactions

Chairpersons:
Gregory Demas, Indiana University, USA
Susannah French, Utah State University, USA

S4-1.
HORMONAL MECHANISMS OF HANTAVIRUS TRANSMISSION, PERSISTENCE, AND DISEASE IN RODENT RESERVOIRS AND HUMAN HOSTS
Sabra L. Klein
Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, U.S.A.

Steroid hormones, including androgens, estrogens, and corticosteroids, have profound effects on both behavior and immunity in vertebrates. Hantaviruses that cause known human disease are maintained in the environment by rodent reservoirs and are primarily transmitted in saliva during aggressive encounters. In natural populations of rodent reservoirs, males are more frequently infected with hantaviruses and are more likely to engage in aggressive encounters than are females, which increases exposure risk for males. There also are significant differences in the dynamics of within-host infection that depend on sex. Among Norway rats and deer mice, males have higher hantavirus RNA loads than females, which can be experimentally modulated by altering androgen and estrogen levels. Consistent with sex differences in hantavirus load, the expression of innate antiviral, proinflammatory, and regulatory factors differ between the sexes and are related to the concentrations of steroids, including androgens and corticosteroids in males and estrogens in females. There is a bidirectional relationship between hormones and hantavirus infection as growing evidence also indicates that hantavirus infection alters concentrations of hormones differentially in males and females, which may further affect transmission of and susceptibility to hantaviruses. Sex differences in the incidence and outcome of hantaviral disease also exist in human populations, which may be mediated by endocrine-immune interactions.

S4-2.
SIDE-BLOTCHED LIZARDS, STRESS, AND IMMUNITY ACROSS AN URBAN LANDSCAPE
Susannah S. French and Leilani D. Lucas
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Urbanization results in significant landscape changes, presenting organisms with different challenges and novel stimuli unlike those which would be typically experienced by their non-urban counterparts. Prior work has helped to elucidate how urbanization can affect organisms on an individual level, revealing mechanisms that may initiate observed population trends in response to such perturbations. One key physiological pathway, indicative of an individual's ability to cope with environmental stressors, such as those imposed by urbanization, is the vertebrate stress response. By increasing circulating glucocorticoids (i.e. corticosterone in lizards) the stress response can exert a suite of physiological effects, such as by altering immune function. We investigated the relationship between stress and immunity across an urban landscape in St. George, Utah, examining corticosterone in response to restraint stress, as well as two innate immune measure, bactericidal ability and cutaneous wound healing in populations of common side-blotched lizards (*Uta stansburiana*). We sampled four populations (two urban, one semi-rural, and one rural), and found elevated stress responsivity in urban populations corresponding with suppressed immunity, relative to more rural populations. Interesting sex and reproductive stage also significantly influenced immune function in all populations. Investigating individual physiological effects is an important first step to understanding how urbanization may contribute to observed population level changes.

S4-3.
TESTOSTERONE-INDUCED IMMUNOSUPPRESSION IN SONGBIRDS: TROUBLE OR TRIUMPH?
Noah T. Ashley
Department of Neuroscience, Ohio State University College of Medicine, Columbus, OH, U.S.A.

Nearly two decades has elapsed since the Immunocompetence Handicap Hypothesis (ICHH) was introduced to provide a proximate explanation for honest signaling of sexually-selected traits in male vertebrates. Folstad and Karter (1992) proposed that the steroid hormone testosterone (T) acts as a "double-edged sword" to promote the development and maintenance of androgen-regulated traits important for female choice while categorically suppressing the immune system. In effect, this immune system "handicap" would enforce honesty by preventing low-quality males from displaying a high-quality trait. After recognizing that obligate immunosuppression was evolutionarily unstable, the ICHH was later modified to incorporate resource allocation as an adaptive explanation underlying T-induced immunosuppresion. To date, a number of empirical studies have provided mixed support for the ICHH. Experimental elevation of testosterone suppresses immune function in some species, but not others. The use of chronic T administration in these studies confounds interpretation because glucocorticosteroid concentrations tend to increase in the circulation and likely act to suppress immune function independent of T. Furthermore, the density and distribution of androgen receptors on immune cells is poorly characterized, especially in non-model species. Most studies have framed their findings in the context of testosterone as a handicap ("trouble"), rather than as an adaptation ("triumph"). Recent studies in seasonally-breeding songbirds are discussed to highlight the possibility that T-induced immunosuppression could be an adaptive phenomenon.
Acknowledgements: I thank Greg Demas and Susannah French for organizing this symposium and Randy Nelson and John Wingfield for providing helpful comments.

OR4-1.
LEPTIN: A NEUROENDOCRINE MEDIATOR OF IMMUNE FUNCTION IN VERTEBRATES
Gregory E. Demas
Department of Biology, Indiana University, Bloomington, IN, U.S.A.

Immunity, like all other physiological processes, requires adequate energy to sustain optimal function. Despite this fact, the role of energy balance has only recently been considered in the context of immune function and disease. Energy is not limitless; finite energy reserves must serve all physiological processes and thus energy must be allocated to a wide variety of often competing physiological functions. At times of reduced energy availability, trade-offs occur in which energy must be re-allocated from less critical physiological functions to those most important for immediate survival. Despite the clear link between energy availability and immunity, relatively little is known regarding the physiological mechanisms by which energy regulates immune function. In the past few years alone, however, a variety of endocrine factors have been identified as potential candidates for providing biochemical signals of current energy availability. One obvious endocrine candidate linking available energy stores with immune function is leptin. Leptin, a peptide hormone synthesized and secreted almost exclusively by adipose tissue, is directly proportional to adipose tissue mass. Importantly, a wide variety of diverse actions within the immune system are influenced by leptin. Recent finding in support of leptin as a key regulator of energetic trade-offs in both laboratory and field studies and across several vertebrate taxa will be presented. Taken together, these studies provide compelling support for the hypothesis that leptin provides a neuroendocrine signal from body fat to the immune system indicating current energy reserves.

OR4-2.
CORTISOL MODULATES LPS-MEDIATED IMMUNE RESPONSES IN RAINBOW TROUT HEPATOCYTES
Anju M. Philip, Sangsoo Kim and Mathilakath M. Vijayan
Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.

The objective of this study was to examine whether cortisol, the major corticosteroid in teleosts, affects the molecular response of liver to an immune challenge. Trout hepatocytes in primary culture were exposed to lipopolysaccharides (LPS; as an immune stimulant) either in the presence or absence of cortisol along with a glucocorticoid receptor (GR) antagonist (RU 486). Exposure to LPS elevated the mRNA abundance of key mediators of the innate immune response, including interleukin-1β (IL-1β), tumour necrosis factor α2 (TNF α2), interleukin-8 (IL-8) and serum amyloid protein A (SAA). LPS downregulated suppressor of cytokine signalling (SOCS) 3, but did not modify either SOCS 1 or SOCS 2 transcript levels. Cortisol had an inhibitory effect on TNF α2, IL-8 and SAA transcript levels, whereas it stimulated the transcript levels of IL-1β, SOCS 1 and SOCS 2. Cortisol also modulated the LPS-induced transcript levels of TNF α2, IL-8 and SAA. The effects of cortisol on IL-1β, TNF α2, IL-8, SOCS 1 and SOCS 2 mRNA abundances were reversed in the presence of RU 486 suggesting a direct role for GR signalling in the stress-immune interaction in fish liver. LPS had no effect on GR protein content while cortisol, either in the presence or absence of LPS, downregulated GR protein expression in trout hepatocytes. LPS treatment also induced heat shock protein 70 (HSP70) expression and altered glucose metabolism. Altogether, LPS induces immune-related gene mRNA abundances and this corresponded with an elevated hepatocyte stress response and altered metabolic capacity in trout hepatocytes. Our results suggest that stress induced elevation in cortisol concentration modulates the immune response in trout liver to infection. (This study was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada discovery grant.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Thursday, July 14th
9:20 – 11:10 a.m.
Mendelssohn Theater

NASCE 2011 Symposium 5:
Evolution of Polypeptide Hormones and their Receptors

Chairpersons:
William Bendena, Queens University, Canada
David Lovejoy, University of Toronto, Canada

S5-1.
EVOLUTION OF SUPERFAMILIES FOR GNRH AND ITS RECEPTOR
Nancy M. Sherwood, Graeme J. Roch, and Ellen R. Busby
Department of Biology, University of Victoria, Victoria, BC, Canada.

Gonadotropin –releasing hormone (GnRH) and its receptor are well established in the vertebrates, but their identity and function in the invertebrates are just beginning to emerge. Genomics has made available DNA sequences for a number of key genomes. These sequences combined with phylogenetic analysis are used here to consider whether invertebrate and vertebrate GnRHs share a common ancestor. Structural homology of GnRH peptides from vertebrates, tunicates, echinoderms, molluscs and annelids supports grouping the GnRH peptides into one family provided that function need not be strictly related to reproduction. The once solitary GnRH peptide family is structurally related to the invertebrate peptides corazonin and adipokinetic hormone (AKH). We propose that these families form a superfamily of peptides with an ancient evolutionary origin. The corresponding receptors for GnRH, corazonin and AKH are seven-transmembrane G protein-coupled receptors belonging to Family A (Rhodopsin) that also form a superfamily, as demonstrated by phylogenetics. Phylogenetic analysis of invertebrate GnRH receptors show that the four tunicate and two out of four amphioxus GnRHRs cluster closer to the vertebrate receptors, but the other two amphioxus, hemichordate, sea urchin, annelid and mollusc GnRHRs cluster in a basal position. The two vertebrate-related amphioxus GnRHRs responded *in vitro* to vertebrate GnRH1 and 2, whereas the functional protostome-related amphioxus receptor responded preferentially to octopus GnRH and AKH. The vasopressin/oxytocin receptor superfamily is phylogenetically most homologous to the GnRH receptor superfamily, possibly sharing a common ancestral gene in pre-bilaterian animals. The peptide and receptor superfamilies are compared to the origin of pituitary hormones and kisspeptin. (Supported by Canadian NSERC grant to NMS).

S5-2.
COMPLEX EVOLUTION OF SOMATOSTATIN AND UROTENSIN II RECEPTORS: ELEVEN ANCESTRAL GNATHOSTOME GENES OF WHICH ONLY SIX REMAIN IN MAMMALS
Dan Larhammar, Christina Bergqvist, Görel Sundström and Daniel Ocampo Daza
Dept of Neuroscience, Uppsala University, Uppsala, Sweden.

Somatostatins, urotensin II and the urotensin II-related peptides (URPs) form a family of related short peptides. They are expressed in both endocrine cells and neurons and have numerous roles mediated by somatostatin and urotensin II receptors. Mammals have been reported to have five somatostatin receptor genes called SSTR1-5 and one gene for a urotensin II receptor (UTS2R) located close to the SSTR2 gene. We have investigated the evolution of these receptor genes by sequence comparisons for a broad range of species, followed by phylogenetic tree analyses using both Neighbor-Joining and Maximum Likelihood methods. We have also compared chromosomal positions and phylogenetic tree topologies for a large number of adjacent gene families to investigate conserved synteny. To our surprise, we discovered that the teleost fish somatostatin receptor gene that is annotated as either SSTR1-like or SSTR4 is in fact orthologous to neither of these in tetrapods. We therefore call this fish receptor SSTR6. Another surprise was that while mammals have only one UTS2R gene, the anole lizard has no less than five UTS2R genes and the western clawed frog and the investigated bird species have four genes. Reciprocal losses have occurred in the avian and amphibian lineages. We propose that the vertebrate ancestor had two SSTR genes; these were duplicated in the two basal vertebrate tetraploidizations (2R), followed by some losses. One of the ancestral genes generated SSTR2, 3 and 5. The other gave rise to SSTR1, 4 and 6, and subsequently SSTR6 was lost in tetrapods. A single ancestral UTS2R gene was quadrupled in 2R and one of these underwent a local duplication resulting in five genes. Thus, mammals have lost the SSTR6 gene and four of the five ancestral UTS2R genes. It will be interesting to investigate what roles the much more advanced urotensin II system has in non-mammalian vertebrates. (Supported by the Swedish Research Foundation and Carl Trygger's Foundation)

S5-3.
ALLATOSTATIN-LIKE RECEPTORS INFLUENCE BEHAVIOUR IN *DROSOPHILA MELANOGASTER* AND *CAENORHABIDITIS ELEGANS*
William G. Bendena, Christine Wang and Ian Chin-Sang
Dept of Biology, Queen's University, Kingston On, K7L 3N6, Canada.

Neuropeptides and their action on receptors are involved in regulating various behaviours of many organisms. With the arrival of sequenced invertebrate genomes many new invertebrate neuropeptides as well as their G-protein coupled receptors (GPCRs) have been identified. In *Drosophila melanogaster* GPCR expression studies, generally in mammalian cells, have matched neuropeptides to their cognate receptor(s). Apart from the matching of receptor with ligand, very little is known about signal transduction pathways or how activation of the receptor may regulate animals physiology or behaviour. The first allatostatin family of neuropeptides found in invertebrates (PheGlyLeu-ASTs) share a conserved C-terminal-sequence of Tyr/Phe*Xaa*PheGlyLeu-NH_2. In insects, depending on the insect PheGlyLeu-AST function varies. This neuropeptide family may contribute to several physiological mechanisms such as inhibition of juvenile hormone biosynthesis, inhibition of muscle contraction, myoendorine regulation, neuromodulation, and regulation of enzymatic activities. Two allatostatin receptors were first identified in *Drosophila* (Dar1 and Dar2) and orthologs have been identified in a variety of other invertebrates. The role of each receptor to a particular phyisological function or behaviour is unknown. As a comparative approach to understanding the contribution of allatostatin receptors to behaviour(s) we identified two allatostatin/galanin-like GPCRs in the nematode *C. elegans.*A deletion mutant of a *C.elegans* receptor that resembles *Drosophila* Dar-1 lost roaming behaviour with increased pivoting which impairs their ability to travel long distances on food. Animals with a mutant receptor gain fat. In the absence of food the mutant has normal foraging/roaming behaviour. With these observations we have assessed whether an RNAi reduction of Dar-1 in *Drosophila* leads to altered foraging/roaming behaviour in larvae on versus off food.

OR5-1.
STANNIOCALCIN: A CALCIUM REGULATOR WITH AN ANCIENT ORIGIN IN UNICELLULAR EUKARYOTES
Graeme J. Roch and Nancy M. Sherwood
Department of Biology, University of Victoria, Victoria, BC, Canada.

Stanniocalcin (STC) is a large glycoprotein hormone that was originally isolated in teleost fishes, where it is secreted by specialized glands known as the corpuscles of Stannius to inhibit calcium uptake at the gills. Orthologs of this hormone, STC1, were later isolated in mammals, where they were found to regulate calcium and phosphate homeostasis and influence a variety of physiological actions including bone formation, growth and neuroprotection. A paralog, STC2, was also identified in vertebrates, with a role in phosphate regulation. We identified and isolated novel forms of stanniocalcin in the protchordate models *Ciona intestinalis* (tunicate) and *Branchiostoma floridae* (amphioxus). The protochordate STCs shared structural features with their vertebrate homologs, such as the conserved core cysteine residues and gene organization. One of the amphioxus peptides also shared the C-terminal dimerization motif, although the other two protochordate STCs did not. Quantitative PCR analysis revealed the majority of tunicate STC expression was in the heart. More recently, we discovered a number of homologous STC gene models and ESTs from a variety of protostomes, cnidarians, sponges and unicellular eukaryotes. Although these putative STCs shared little sequence conservation with the vertebrate hormones, they retained critical structural characteristics including the conserved cysteine residues, and most had a signal peptide for secretion. Phylogenetic analysis demonstrated that these STCs have evolved as a distinct group from their chordate counterparts, with the exception of a third STC from amphioxus that was homologous with the non-chordate hormones. Although the receptor and signaling mechanism for stanniocalcin is not yet understood, we propose that this diverse family of hormones with an ancient origin may directly influence cation channels that have similar evolutionary roots, like the voltage-dependent calcium channels. (Supported by a Canadian NSERC grant).

OR5-2.
IDENTIFICATION OF ANCESTRAL VERTEBRATE GLYCOPROTEIN HORMONES FROM A BASAL VERTEBRATE, THE SEA LAMPREY
Takayoshi Kosugi, Michael T. Wilmot and Stacia A. Sower
Center for Molecular and Comparative Endocrinology, University of New Hampshire, Durham, NH, U.S.A.

One major family of vertebrate pituitary hormones is the glycoprotein hormones that regulate gonadal/thyroid functions. These glycoprotein hormones, FSH, LH and TSH belong to the cystine knot-forming glycoprotein hormone family that consists of the common alpha subunit and unique beta subunits. Recently, novel glycoprotein hormone alpha (GPA2) and beta (GPB5) subunits have been identified in both vertebrates and invertebrates, and named thyrostimulin since the heterodimeric hormone stimulates the TSH receptor. In the sea lamprey, an agnathan and ancestral lineage of the vertebrates, gonadotropin beta subunit has been identified and proposed as a common ancestral glycoprotein hormone beta subunit to gnathostome FSH, LH and TSH beta subunits (Sower et al., 2006). Despite extensive efforts, the alpha subunit had not been identified in lampreys. Therefore, in silico screening of the alpha gene was performed using the lamprey genome database resulting in identification of a partial GPA2-like sequence instead of the typical alpha sequence (GPA1). Further screening of the lamprey genome identified a partial GPB5-like sequence, suggesting a thyrostimulin homologue (GPA2 and GPB5) in the lamprey. We performed molecular cloning of lamprey GPA2 and GPB5 full-length cDNAs with RT-PCR and RACE techniques. The lamprey GPA2 full-length cDNA encodes 121 amino acid residues containing 20 amino acid residues of a signal peptide. The deduced amino acid sequence contains conserved 10 cysteine residues at the homologous positions of gnathostome GPA2 and a putative *N*-glycosylation site at the position of one of the two conserved sites of gnathostome GPA2. From our data, we propose that lampreys have a GPHbeta, GPA2 and GPB5. Phylogenetic data support that an ancestral GPA2 and GPB5 gave rise to GPHalpha and GPHbeta of the vertebrate glycoprotein hormone family and that GPA1 may be lost in the lampreys. (Supported by NSF 0849569 and NH AES Hatch 332 to SAS).

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Thursday, July 14th
9:20 – 11:10 a.m.
Vandenberg Room

NASCE 2011 Symposium 6:
Endocrine Control of Growth, Body Size, and Allometric Scaling

Chairpersons:
Russell Borski, North Carolina State University, USA
Alexander Shingleton, Michigan State University, USA

S6-1.
ECDYSONE REGULATES SIZE-DEPENDENT DEVELOPMENT
Takashi Koyama, Marisa Oliveira and Christen Mirth
Instituto Gulbenkian de Ciência, Fundação Calouste Gulbenkian, Oeiras, Portugal.

Environmental factors like nutrition carefully tune growth to produce organisms of a size appropriate for the conditions. Nutrition regulates body size by controlling larval growth rates and the duration of the larval growth period. Both the insulin and target of rapamycin (TOR) signalling pathways regulate the rate of growth in response to nutrition. In addition, insulin and TOR act to control the duration of the growth period by regulating a size-dependent developmental transition called critical weight. Critical weight represents a developmental switch in the response to starvation. Larvae starved before reaching critical weight significantly delay their development; after reaching critical weight, starvation induces premature metamorphosis. This switch is controlled by nutrition-dependent production of the steroid hormone ecdysone. Our current work examines the molecules responsible for crosstalk between the insulin/TOR and ecdysone signalling pathways, and works to elucidate how ecdysone switches the developmental response to starvation. Our studies suggest that the Forkhead Box O (FoxO) transcription factor, a negative regulator of insulin and TOR signalling, binds directly to Ultraspiracle (Usp). Usp and Ecdysone Receptor (EcR) heterodimerize to form the functional ecdysone receptor. Preliminary results show that FoxO and Usp may act in the PG to control the level of ecdysone synthesis in a nutrition-dependent manner. In turn, the production of ecdysone controls the growth and patterning of the presumptive adults tissues, the imaginal discs, through its action on EcR. These exciting results reveal how nutrition regulates body size by modulating the insulin/TOR pathways, ecdysone synthesis and signalling, and the pathways that regulate tissue patterning and growth.

S6-2.
KEEPING THINGS IN PROPORTION: THE COORDINATION OF ORGAN GROWTH IN DROSOPHILA
Nathan F. Parker and Alexander W. Shingleton
Department of Zoology, Michigan State University, East Lansing, MI, 48824, U.S.A.

The developmental mechanisms by which growth is coordinated among developing organs are largely unknown and yet are essential to generate a correctly proportioned adult. In particular, such coordinating mechanisms must be able to accommodate perturbations in the growth of individual organs caused by environmental or developmental stress. By autonomously slowing the growth of the developing wing discs within *Drosophila* larvae we show that growing organs are able to signal localized growth perturbation to the others organs in the body and slow their growth also. Growth rate is so tightly coordinated among organs that they all show approximately the same reduction in growth rate as the developing wings, thereby maintaining their correct size relationship relative to one another throughout development. Further, we show that the systemic growth effects of localized growth-perturbation are mediated by ecdysone. Application of ecdysone to larvae with growth-perturbed wing discs rescues the growth rate of other organs in the body, indicating that ecdysone is limiting for their growth. Collectively our data demonstrate the existence of a novel growth-coordinating mechanism in *Drosophila* that synchronizes growth among organs in response to localized growth perturbation. (This research was funded by grants from Michigan State University GEDD, and NSF Grant 0845847.)

S6-3.
LEPTIN STIMULATES HEPATIC GROWTH HORMONE RECEPTOR EXPRESSION: POSSIBLE ROLE IN ENHANCING GH-MEDIATED ANABOLIC PROCESSES IN FISH
Russell J. Borski, Eugene T. Won, and David A. Baltzegar
Department of Biology, North Carolina State University, Raleigh, NC, U.S.A.

Growth hormone (GH) exerts much of its anabolic actions through binding cytokine receptors (GHRs) and stimulating production of insulin-like growth factors (IGFs). Leptin is an anorexigenic hormone produced by adipocytes and that circulates in proportion to lipid deposition in mammals. Its regulation of the GH-IGF system, particularly GHRs, is poorly understood. Virtually nothing is known of leptin control of the GH-IGF axis in fishes. In hybrid striped bass (HSB, *Morone chrysops* x *M. saxatilis*) and many other vertebrates, fasting leads to a hepatic GH resistant state whereby elevated GH secretion is accompanied by declines in hepatic expression of GHR1, GHR2, IGF-1, and IGF-2 and low growth rate. Upon refeeding, HSB undergo a robust compensatory growth response, characterized by an alleviation of the GH resistant state, increase or overcompensation in expression of hepatic IGFs and GHRs, and growth that exceeds normal rates. We cloned and characterized the leptin gene in HSB and show there is likely only one form of leptin that is produced predominantly in the liver. Human recombinant leptin acutely inhibits appetite in HSB. HSB hepatic leptin mRNA levels decline during fasting and increase with refeeding, paralleling gene expression patterns observed with the GHRs and IGFs. This suggests the hormone may modulate GHRs and IGF expression under different metabolic states. To assess leptin regulation of hepatic GHRs, a primary cell culture system was established and validated for HSB hepatocytes. Leptin increased GHR1 and GHR2 gene expression by as much as 6-fold in HSB hepatocytes. The dose-dependent response occurred as early as 8 hours, and lasted for as long as 24 hours with the highest (50 nM) concentration. Additionally, leptin stimulated IGF-1 gene expression in hepatocytes over 24-hr incubation. The results suggest that leptin may promote anabolic processes in vertebrates through stimulation of hepatic GHRs and IGF-1 expression.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

OR6-1.
REGULATION OF ALLOMETRY BY MODULATING LOCAL IGF ACTIONS: ROLE OF IGF BINDING PROTEINS
H. Kamei and C. Duan
Department of Molecular, Cellular, & Developmental Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Growth in multi-cellular organisms is tightly controlled and coordinated such that different organs maintain their size in proportion to each other and to the body. This relationship is called static allometry. Understanding how allometry is controlled is a fundamental question. Recent progress suggests that the insulin-like growth factor (IGF) signaling pathway plays a central role in regulating body growth in vertebrates. The IGF system is composed of multiple IGF ligands, IGF receptors, and IGF binding proteins (IGFBPs). While the IGF ligands and receptors are expressed in all embryonic tissues, the expression of IGFBPs is highly tissue-specific and dynamically regulated. Because IGFBPs are high affinity binding partners for IGFs and can regulate IGF bioavailability and bioactivities, we hypothesized that local IGFBPs are crucial factors in determining the local growth rate and body allometry. We have been testing this hypothesis using the zebrafish model. We have shown that *igfbp-3* is expressed in the inner ear in zebrafish embryos and targeted knockdown of *igfbp-3* decreased inner ear size and disrupted hair cell differentiation. Likewise, knockdown of zebrafish *igfbp-2a* resulted in localized angiogenic defects in the retina and brain, where this gene is highly expressed. Targeted overexpression of Igfbp-2a in the lens resulted in disproportional reduction in eye size. Co-expression of IGF-I rescued Igfbp-caused reduction in eye size. Furthermore, overexpression of ligand-binding domain mutant forms of Igfbp (which have impaired IGF binding ability) had little effect, suggesting that Igfbps act by binding to and inhibiting IGF actions in local tissues. Likewise, when Igfbp-2a was targeted overexpressed in the developing heart or inner ear, it caused a disproportional reduction in their size. These results suggest that changes in local Igfbp levels can alter the net IGF activity and regulate the specific organ growth.

OR6-2.
THE ROLE OF TESTOSTERONE IN DEVELOPMENT OF SEXUAL SIZE DIMORPHISM IN THE TROPICAL LIZARD *PAROEDURA PICTA* (SQUAMATA: GEKKONIDAE)
Z. Starostova(1), L. Kubicka(2), A. Golinski(3), and L. Kratochvil(2)
(1) Department of Zoology, Charles University in Prague, Prague, Czech Republic; (2) Department of Ecology, Charles University in Prague, Prague, Czech Republic; (3) Graduate Program in Endocrinology and Animal Biosciences, Rutgers University, New Brunswick, NJ 08901, U.S.A.

Sexual size dimorphism (SSD) is a nearly ubiquitous phenomenon and has been extensively studied in animals, including lizards. However, the proximate mechanism responsible for development of SSD is still only poorly understood in this group. Currently the most pervasive candidates are (1) sex steroid effects, whereby elevated circulating levels of androgens in males (but not females) stimulates growth in male-larger species, or (2) sex differences in energy allocation, whereby females invest relatively greater amount of energy (compared to males) towards reproduction. We tested these alternative hypotheses in the tropical lizard *Paroedura picta*, in which hatchlings do not exhibit SSD and both sexes reach sexual maturity at a relatively small body size, but males attain much larger final body size than females. Individuals of *P. picta* were kept separately under the same conditions. When animals reached sexual maturity we established three groups of males (intact control, surgically castrated, castrated with testosterone replacement) and four groups of females (intact control, intact control allowed to mate regularly, testosterone supplemented, castrated). Body size was measured monthly until the lizards reached asymptotic body size. Neither of the hypotheses was supported by the results. Castrated males reached a final structural body size (i.e., snout-to-vent length) comparable to control males or castrated males with testosterone implants. Thus, testosterone had no significant effect on growth (i.e., final body size) in males. Control females maintained in social isolation to prevent reproduction reached the same final body size as mated females that produced eggs frequently. Interestingly, ovariectomized females and females with testosterone implants grew to larger body size than both groups of intact females. This finding suggests that not the cost of reproduction but gonadal hormones in females (estrogenes and/or gestagenes) may retard growth in *P. picta*. [The research was supported by the Czech Science Foundation, projects no. 206/09/0895 (LK and LK) and P505/10/P174 (ZS) and by the Fulbright Commission (AG).]

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Thursday, July 14th
9:20 – 11:10 a.m.
Hussey Room

NASCE 2011 Symposium 7 (Co-sponsored by ISAREN 2011):
Sex Determination and Differentiation

Chairpersons:
Lou Guillette, Medical University of South Carolina. USA
Taisen Iguchi, Okazaki National Research Institutes, Japan

S7-1.
SEX DETERMINATION / DIFFERENTIATION AND SEXUAL PLASTICITY IN FISH
Yoshitaka Nagahama
Institute for Collaborative Relations, Ehime University, Matsuyama 790-8577, Japan and Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan.

Fish exhibit a range of gonadal forms, thus providing an excellent animal model to study the molecular mechanisms of sex determination/differentiation and sexual plasticity in vertebrates. We identified *DMY* (DM-domain gene on the Y chromosome) (also called *dmrt1bY*)) as the sex-determining gene of the medaka, the first one in non-mammalian vertebrates. A search for the target genes of DMY led to the identification of *gonadal soma derived factor (GSDF)*, a member of the transforming growth factor-beta superfamily. Importantly, *GSDF* and *DMY* were found to be co-localized in the same cell type in XY gonads. These results suggest that GSDF plays an important role in testicular differentiation in medaka, probably downstream of DMY. We also show that *R-Spondin 1* and *estrogen receptor β2* are expressed specifically in XX gonads prior to ovarian differentiation. Loss- and gain-of function evidence indicates that these two factors are critical to initiate the ovary pathway in medaka. With the exception of certain hermaphroditic species, the sexual plasticity of the gonads is not retained after the completion of sexual differentiation. Recently, we show that the depletion of estradiol-17β (E_2) by aromatase inhibitors (AIs) for up to six months resulted in a functional female-to-male sex reversal in sexually-mature adults of two gonochoristic fish species, medaka and Nile tilapia. The sex-reversed XX fish showed a typical male pattern of E_2 and androgen levels, secondary sexual characteristics, and male-like sex behavior, producing fertile sperm. Cysts on the dorsal side of the adult ovaries are the origin of germ cells (germ-line stem cells) and Sertoli cells in the newly formed testicular tissue. Our studies further suggest that GSDF plays a role in the initiation of Sertoli cell differentiation during AI-induced female to male sex reversal. Gonochoristic fish maintain their sexual plasticity until adulthood and E_2 plays a critical role in maintaining the female phenotype. (Supported by JST and MEXT to YN).

S7-2.
WHICH ESTROGEN RECEPTOR (ESR1; ERα OR ESR2; ERβ) IS INVOLVED IN SEX DETERMINATION AND GONADAL DIFFERENTIATION?
S. Kohno(1)(2), Y. Katsu(3), Y. Ohta(4), T. Iguchi(5)(6), L. J. Guillette Jr(1)(2)
(1) Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, U.S.A.; (2) Marine Biomedicine and Environmental Science Center, Hollings Marine Laboratory, Charleston, SC, U.S.A.; (3) Graduate School of Life Science and Department of Biological Sciences, Hokkaido University, Sapporo, Japan; (4) Laboratory of Experimental Animals, Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan; (5) Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan; (6) Department of Basic Biology, The Graduate School for Advanced Studies (SOKENDAI), Okazaki, Japan.

All crocodilians and most turtles exhibit temperature-dependent sex determination (TSD) where the temperature of egg incubation determines sex during a thermo-sensitive period (TSP). Estrogens play a critical role in sex determination in crocodilians and turtles, as it likely does in most non-mammalian vertebrates. Indeed, administration of an estrogen during TSP overrides a male-producing temperature. Embryonic exposure to estrogenic compounds can alter sex, but does it also lead to other health consequences as it does in mammals? Sensitivity to temperature and exogenous chemicals during TSP could be a powerful model to investigate the impact of estrogenic EDCs as well as climate change. It isn't clear how estrogens or EDCs override TSD. Most vertebrates have two types of nuclear estrogen receptors, ESR1 (ERα) and ESR2 (ERβ). In turtles, localization and timing of ESR expression suggested that both might work during sex determination and gonadal differentiation, respectively. However, there is no direct evidence which receptor is involved or if both are. We identified specific pharmaceutical agonists of each ESR cloned from the American alligator by using an *in vitro* trans-activation assay employing each receptor. PPT (4,4',4"-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol) and WAY (7-Bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol) were highly specific to alligator ESR1 and ESR2, respectively. We "painted" E_2 (estradiol-17β), PPT or WAY onto alligator eggs at the TSP and examined sex-reversal at the male-producing temperature. E_2 and PPT induced sex-reversal, whereas WAY didn't sex-reverse embryos nor did it induce oviducal hyperplasia as seen with E_2 and PPT. PPT-exposed embryos exhibited hyperplasia and advanced differentiation of the Müllerian duct, not seen in E_2- or WAY-exposed embryos. These results indicate that ESR1 could be a principal estrogen receptor for the sex determination as well as Müllerian duct differentiation in alligators. [This work was supported by a grant from National Institutes of Health (R21 ES014053-01) and the Howard Hughes Professors Program (L.J.G.), Grants-in-Aid for Scientific Research 17052032 (Y.K.), 20570064 (Y.K.), 21510068 (Y.O.), and 19370027 (T.I.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.]

S7-3.
OPPOSITE ROLES OF DMRT1 AND ITS W-LINKED PARALOGUE, DM-W, IN THE ZZ/ZW-TYPE SEX DETERMINATION IN *XENOPUS LAEVIS*
Michihiko Ito, Shin Yoshimoto, Shuuji Mawaribuchi, Nozumi Ikeda, Kazuko Fujitani, Kei Tamura, and Nobuhiko Takamatsu
School of Science, Kitasato University, Sagamihara, Japan.

In the genetic sex-determining system of vertebrates, heterogametic sex chromosomes determine the male (XY) or female (ZW) fate. In the XX/XY sex-determining systems, the Y-linked *SRY* gene of most mammals and the *DMY/dmrt1bY* genes of the teleost fish medaka act as sex (male)-determining genes. In contrast, the molecular mechanisms for the ZZ/ZW systems remain largely unclear, although a recent work suggested the chicken Z-linked *DMRT1* gene as a sex (male) sex-determining gene. We previously isolated a W-linked gene *DM-W* as a paralogue of *DMRT1* in the clawed frog *Xenopus laevis* that has a

ZZ/ZW-type. Interestingly, DM-W has highly or no significant sequence similarity with the DNA-binding domain or transactivation domain of DMRT1, respectively. Transgenic and expression analyses indicated that DMRT1 and DM-W could function as a male- and female-determining gene, respectively. We also found that DM-W antagonized the transcriptional activity of DMRT1 on a luciferase reporter system in cultured cells. From these findings, we propose a novel model for the ZZ/ZW-type sex determination in which DM-W determines the development of bipotential gonads into ovaries as a sex (female)-determining gene by repressing the DMRT1-driven testis formation. Next, to obtain clues to understanding molecular mechanisms on sex differentiation following sex determination in *X. laevis*, we examined expression patterns of several sex-related genes. Real-time PCR analysis revealed that *Cyp19*, *Foxl2*, and *RspoI* showed ZW gonad-abundant expression during sexual development. We also found by in situ hybridization that *DMRT1* might be expressed in male germ stem cells (spermatogonia) in developing ZZ testes. Finally, we will discuss gene expression cascades during early stages of ovary/testis development, and a DMRT1-driven male sex-determining system as well as distinct roles of DMRT1 in gonadal masculinization during vertebrate evolution. (Supported by MEXT KAKENHI 22132003 to MI).

OR7-1.
ZWY SEX DETERMINATION IN *XENOPUS TROPICALIS*
Allen Olmstead and Sigmund Degitz
U.S. E.P.A., NHEERL, Mid-Continent Ecology Division, Duluth, MN, U.S.A.

Most vertebrate species with described genetic sex determination are either male (XY) or female (ZW) heterogametic. To date, studies with Xenopus species indicate that members of this genus operate under a ZW sex determination system. We used two different approaches and demonstrated a ZW sex determination system in lab raised spawns of X. tropicalis. Sex-reversed males were generated by larval exposure to the synthetic estrogen, ethynylestradiol and when mated to control females resulted in all-male offspring. Larval exposure to the aromatase inhibitor, fadrozole, resulted in sex-reversed females that when mated to control males produced offspring with a 1:3 male:female sex ratio. One out of three of these female offspring possessed a WW genotype that produced all female offspring when mated with normal males. These results were corroborated using sex-linked genetic markers that showed that the sex-determining factor was inherited maternally in these frogs. Most breeding pairs of this species outside of this study group, however, did not corroborate these results. Breeding trials and inheritance analysis of sex-linked markers show that this species uses a polyfactorial sex determination system with three possible alleles at the same locus. Males of this species can possess one of three genotypes; ZZ, ZY, or WY. Females possess either ZW or WW genotypes. While most spawns results in 1:1 sex ratios, there are occasionally male- or female-biased spawns. These results have implications for this species' usage in endocrine disruptor toxicity testing and provides a unique opportunity to study the evolution of sex determining systems especially with the molecular tools available for this species.

OR7-2.
THE DECLINE IN YOLK PROGESTERONE CONCENTRATIONS DURING DEVELOPMENT IS DEPENDENT ON THE PRESENCE OF A DEVELOPING EMBRYO IN THE EUROPEAN STARLING
Ryan T. Paitz and Joseph M. Casto
School of Biological Sciences, Illinois State University, Normal, IL, U.S.A.

Oviparous amniotes, particularly birds, have become model systems in which to study how mothers may utilize steroids to adaptively adjust offspring development. Although there is now ample evidence that maternally derived steroids in the egg at oviposition can influence offspring phenotype, very little is known about how these steroids elicit such effects. Of the major avian steroid hormones found in yolk, progesterone is by far the most abundant at oviposition, but has received little research attention to date. In this study, we examine the metabolism of [^{3}H] progesterone injected into freshly laid European starling eggs throughout the first five days of development by characterization of radioactivity within the egg homogenate. We also introduce a technique that utilizes a focal, freeze/thaw cycle to prevent embryonic development and allows us to assess the role of the embryo in metabolizing progesterone during early incubation. Two major findings result. First is that [^{3}H] progesterone is metabolized in eggs possessing a developing embryo, but not in eggs with disrupted embryonic development. Second is that the change in the distribution of radioactivity within eggs possessing an embryo is the result of metabolism of [^{3}H] progesterone to a more polar form that is subsequently conjugated. Together, these data suggest live embryos are necessary for metabolism of progesterone during early incubation, underscoring the potentially important contribution of embryos to functional modulation or mediation of maternal yolk steroid effects. Future research should investigate environmental and physiological conditions that might alter the metabolism of maternally derived steroids present in freshly laid eggs.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
9:20 – 11:10 a.m.
Vandenberg Room

NASCE 2011 Symposium 8:
Hormone Action in Neural Development and Plasticity

Chairpersons:
Susan Fahrbach, Wake Forest University, USA
Steve Harvey, University of Alberta, Edmonton, Canada

S8-1.
DOES THE MOLTING HORMONE SHAPE THE ADULT BEE BRAIN?
Susan Fahrbach and Rodrigo A. Velarde
Department of Biology, Wake Forest University, Winston-Salem, NC, U.S.A.

Studies in vertebrates have demonstrated key roles for nuclear receptors (NRs) in development, reproduction, and regulation of metabolism. A similarly broad range of roles is attributed to insect NRs. An unusual aspect of NR function in insects is a conserved cascade of NR gene expression triggered by the liganded EcR-USP receptor complex. This cascade represents the initial cellular response to ecdysteroids, and includes other insect NRs expressed in a sequence first described for larval-pupal transition in *Drosophila melanogaster*. *EcR*, *USP*, *HR3*, *HR4*, *HR78*, *E75*, *E78*, and *FTZ-F1* function as so-called early genes in the metamorphosis cascade. The same NRs assigned roles at metamorphosis also regulate the vitellogenesis that follows a blood meal in female mosquitos. Prior to the *Apis mellifera* genome project, partial cDNA sequences for *USP*, *Hr3*, *ERR*, *SVP*, *Ftz-f1* and *Hnf4* were identified in an expressed sequence tag library developed from adult bee brain. Possible relevance of the metamorphosis transcriptional cascade to patterns of gene expression in the adult honey bee brain was suggested by localized expression of molting hormone responsive genes (*E93*, E75, *E74*, *Hr38 and EcR*) in the mushroom bodies of the worker. It was subsequently shown that expression of the *EcR*, *USP*, *E75*, *Ftz-f1*, and *Hr3* genes in adult honey bee mushroom bodies is modulated by endogenous ecdysteroid pulses, and that treatment with a high dose of 20E induces a cascade of gene expression similar to the canonical cascade defined for *D. melanogaster* metamorphosis and *A. aegypti* vitellogenesis. Current investigations use neuronal cytoarchitecture (studied *in vivo* with the Golgi technique and *in vitro* in primary neuron culture) coupled with quantitative studies of gene expression to define a role for ecdysteroids in the regulation of adult brain plasticity. (Supported by NSF grant IOS 0949728 to SF and RAV).

S8-2.
HORMONE ACTIONS IN NEURAL DEVELOPMENT AND PLASTICITY OF TELEOST FISH
Olivier Kah(1), Nicolas Diotel(1), Jean-Luc Do Rego(2), Isabelle Anglade(1), Colette Vaillant(1), Elisabeth Pellegrini(1), and Hubert Vaudry(2)
(1) Neurogenesis And Œstrogens, UMR CNRS 6026, Campus de Beaulieu, Université de Rennes 1, 35042 Rennes cedex, France.; (2) Différenciation et Communication Neuronale et Neuroendocrine, INSERM U982, PRIMACEN, IFRMP 23, Université de Rouen, Mont-Saint-Aignan, France.

The brain of adult fish has several unique properties compared to that of other vertebrates. 1- It exhibits an intense adult neurogenesis supported by the persistence of radial glial progenitors in the whole brain. 2- It shows a very high aromatase activity due to the strong central expression of the estrogen-synthesizing enzyme, aromatase B (AroB), the product of the *cyp19a1b* gene. 3- The brain of fish shows high sexual plasticity as shown by the variety of reproductive strategies, including sex change that is never observed in other vertebrates. We have previously documented the fact that aromatase B is only expressed in radial glial progenitors and this observation raises several important questions notably regarding the roles of locally produced estrogens on neurogenesis and the origin, central or peripheral, of androgens available for aromatization. Recently, we reported that the brain of adult zebrafish also exhibits 3α- and 3β-HSD, CYP17, 17 β-HSD and 5α-reductase activities. *In situ* hybridization studies showed that *cyp11a1*, *cyp17* and *3 β-hsd* mRNAs have an expression pattern very similar to that of *cyp19a1b* mRNAs. Consequently, it is possible that radial glial cells express a large set of steroidogenic enzymes and could be true steroidogenic cells. Furthermore, we have documented the fact that radial progenitors are also targets for steroids or neurosteroids. They express nuclear and membrane estrogen receptors, and nuclear progesterone receptors. Accumulating data now show that estrogens modulate neurogenesis in adult fish. These data provide strong indication that estrogens, and possibly progestins, produced by radial glial progenitors, can act in an autocrine manner to modulate their proliferative activity. It will be of great interest to explore whether similar mechanisms could occur in mammals during embryonic development. (This work is supported by the CNRS, INSERM, MRT, the ANR NEED and the EU programme LIFECYCLE [European project n° 222719])

S8-3.
GROWTH HORMONE AND RETINAL NEUROGENESIS DURING CHICK EMBRYO DEVELOPMENT
Steve Harvey and Esmond J. Sanders
Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The growth hormone (GH) gene, identical to that in the pituitary gland, is expressed in the neural retina of chick embryos. GH immunoreactivity is also present in the optic cup of chick embryos from day 2 of the 21 day incubation period and in the retina from ED 4 (embryonic day 4). This immunoreactivity is largely associated with the retinal ganglion cells (RGCs) and their axons that form the optic fibre layer (OFL). These fibres form the optic nerve (ON), which synapses within visual centers (the optic tectum, OT) in the brain. GH immunostaining is abundant in the RGCs and OFL between ED 5 and ED 12 but declines at ED 14 and is absent by ED 18. As the ON synapses within the OT by ED 12, this temporal pattern of GH immunoreactivity suggests roles for GH in retinal neurogenesis. This possibility is supported by the presence of GH receptor (GHR) mRNA and protein in the chick retina and by the colocalizaiton of GH and GHR immunoreactivities. A role for retinal GH in neurogenesis is also supported by the sprouting of RGC axons in response to exogenous GH and by impaired RGC axonal growth following the siRNA knockdown of endogenous GH mRNA. Retinal GH may also be involved in RGC survival during neurogenesis, since it is abundant during periods of development characterized by sequential waves of apoptosis. A role for retinal GH in retinal cell survival is indicated by antiapoptotic actions of exogenous GH and by the increased cell death following the immunoneutralization of endogenous GH or the siRNA knockdown of endogenous GH mRNA *in vivo* and *in vitro*. This neuroprotective action involves caspase-dependent are caspase-independent mechanisms and may be direct or indirectly mediated by insulin-like growth factor (IGF) -1, since GH induces retinal IGF-1 and the immunoneutralization of retinal IGF-1 similarly induces RGC death. Retinal GH may therefore be an autocrine or paracrine neurotrophic factor during retinal neurogenesis in early chick embryo development. (Supported by NSERC of Canada.)

OR8-1.
CHARACTERIZATION OF MAMMALIAN RECOMBINANT PAQR6 AND PAQR9 (mPRδ AND mPRε) AND THEIR POTENTIAL INVOLVEMENT IN MEDIATING ANTIAPOPTOTIC EFFECTS OF NEUROSTEROIDS IN NEURONAL CELLS
Yefei Pang, Jing Dong and Peter Thomas
Marine Science Institute, University of Texas at Austin, Port Aransas, TX, U.S.A.

Three members of the progestin and adipoQ receptor family (PAQR7, 8 and 5) have been previously characterized and classified as plasma membrane progesterone receptors mPRα, β and γ, in both mammalian and non-mammalian model systems. Two other PAQRs, PAQR6 and PAQR9, heterologously expressed in yeast were shown to be capable of responding to progesterone in the absence of G proteins and were designated as mPRδ and mPRε, respectively. In the present study, we stably expressed PAQR6 and PAQR9 in nuclear progesterone receptor negative MDA-MB-231 human breast cancer cells and examined their steroid binding characteristics; G protein coupling and second messenger signaling. Specific, saturable, and high capacity [^{3}H]progesterone (P4) binding was detected on the plasma membranes of PAQR6- and PAQR9- transfected cells with Kds of 7.97 nM and 2.85 nM, respectively. Competition studies showed that P4 had the highest binding affinities for both of the receptors, whereas R5020, the nuclear PR specific binding agonist, displayed negligible binding. Treatment with 100nM P4 increased GTPγS binding to the cell membranes of transfected cells and immunoprecipitation studies suggested that both receptors activate stimulatory G proteins (Gs). The finding that cAMP was significantly elevated in PAQR6- and PAQR9- transfected cells treated with 5, 20 and 100 nM of P4, further supports their activation of a stimulatory G protein. Treatment with P4 and the neurosteroid allopregnanolone increased ERK phosphorylation and demonstrated a protective effect against serum starvation-induced cell death of PAQR6 transfected cells. It is concluded that PAQR6 and PAQR9 have the characteristics of membrane progestin receptors and activate stimulatory G proteins. The two receptors may mediate important neurosteroid actions in the human central nervous system since the mRNAs of both of them were detected in several brain regions in a tissue array.

OR8-2.
C-TERMINAL REGION OF THE EVOLUTIONARY CONSERVED TENEURIN-1 IN MOUSE HIPPOCAMPUS INTERACTS WITH THE DYSTROGLYCAN COMPLEX AND REGULATES ERK-DEPENDENT STATHMIN MODULATION OF THE CYTOSKELETON
Dhan Chand(1), Lifang Song(1), Dalia Barsyte-Lovejoy(2) and David A. Lovejoy(1)
(1) Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada; (2) Structural Genomics Consortium, University of Toronto, Toronto ON, Canada.

Teneurin C-terminal associated peptides (TCAP) are a new family of four bioactive peptides located on the final exon of the teneurin gene. The teneurin-TCAP gene is phylogenetically conserved among the metazoans and has been described in *Caenorhabditis elegans*, *Drosophila*, *Danio rerio*, *Gallus gallus* and *Mus musculus. While the teneurins have been implicated as signaling molecules and receptors during vertebrate development, previous in vitro* studies indicate that TCAP-1 can regulate cell proliferation, axon fasciculation and spine density in the rodent hippocampus. Thus, new studies were aimed at understanding the molecular mechanisms of TCAP-1 and how it relates to neuronal morphology and plasticity. Cellular localization in mouse hippocampal cultures indicate that immunoreactive (ir) teneurin is found in the plasma membrane, whereas ir-TCAP is predominantly found in the cytosol. Moreover, FITC-labeled TCAP-1 binds to the same hippocampal regions in the mouse brain that label ir-TCAP indicating a paracrine action. *In vitro* studies in cultured hippocampal cells indicate that TCAP-1 co-localizes with the dystroglycan complex upon binding to the plasma membrane and stimulates extracellular signal-regulated kinases (ERK1/2)-dependent phosphorylation of the cytoskeletal regulatory proteins, stathmin at serine 25 and ribosomal S6 kinase (p90RSK) at serine 380. Furthermore, hippocampal cells treated with TCAP-1 for 1 hour, showed a reorganization of actin- and tubulin-based cytoskeletal elements, and a corresponding increase in neurite outgrowth. We postulate that this cytoskeletal reorganization is a prelude to, and is associated with changes in neuronal morphology that ultimately modulates neuronal plasticity within the hippocampus. In addition, the TCAP-dystroglycan interaction may represent a novel mechanism that may be part of the constellation of mechanisms associated with the regulation of hippocampal-function. (Supported by NSERC grant and funding from Protagenic Therapeutics to DAL).

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
9:20 – 11:10 a.m.
Mendelssohn Theater

NASCE 2011 Symposium 9:
Extreme Endocrinology: Physiological and Behavioral Adaptation to Extreme Environments

Chairpersons:
John Wingfield, University of California–Davis
Brian Barnes, University of Alaska

S9-1.
SHUTTING DOWN FOR THE WINTER: A ROLE FOR INSULIN SIGNALING IN INSECT DIAPAUSES
David L. Denlinger(1) and Cheolho Sim(1,2)
(1) Department of Evolution, Ecology and Organismal Biology; Department of Entomology, Ohio State University, Columbus, OH, U.S.A.; (2) Department of Biology, Baylor University, Waco, TX, U.S.A.

The low temperatures of winter pose a major and recurring seasonal obstacle for insect development and survival. In response, most insects living in temperate zones enter a dormant state (diapause) that is programmed by the short daylengths of late summer. One regulatory pathway that is emerging as a possible common theme in diapause is the involvement of insulin signaling. Females of the mosquito *Culex pipiens* overwinter in a reproductive diapause, characterized by a switch from blood feeding to sugar feeding, accumulation of fat reserves, suppression of metabolism, enhancement of stress responses, and a shut-down in reproduction. One key endocrine regulator of diapause is juvenile hormone, but we now propose that insulin signaling contributes to diapause by acting upstream of the juvenile hormone response. When the insulin receptor is blocked using RNA interference, mosquitoes programmed by long daylength for immediate reproduction enter a diapause-like state. Insects have numerous insulin-like peptides (ILPs), but we propose that ILP-1 is the most important ILP involved in the diapause response. In the absence of insulin signaling, the transcription factor FOXO is activated leading to the fat hypertrophy and enhanced stress resistance that characterize the diapause state. A role for insulin signaling is also evident in the regulation of diapause and other forms of dormancy, as noted in flesh flies, fruit flies, and nematodes, thus suggesting that this may be a theme common to diverse forms of developmental arrest. (Supported by NIH grant 1 R01 AI 1058279 and NSF grant IOS-0840772 to DLD).

S9-2.
WIDESPREAD REPRODUCTIVE DISRUPTION, MASCULINIZATION AND ENDOCRINE IMBALANCE IN CROAKER EXPOSED TO ENVIRONMENTAL HYPOXIA
Peter Thomas and Md. Saydur Rahman
University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX78373, U.S.A.

One of the most dramatic global changes due to human activities over the last half century has been the marked increase in the extent of seasonal coastal hypoxia (dissolved oxygen, DO < 2 mg O_2/L) which now affects over 400 regions and covers a total area of approximately 250,000 sq. km. The long-term impacts on marine ecosystems of this dramatic worldwide increase in coastal hypoxia are unknown. Here we show widespread reproductive disruption in Atlantic croaker collected from hypoxic sites 120 km apart in the extensive hypoxic region in the northern Gulf of Mexico, the second largest hypoxic region in the world. Gonadal growth and gamete production were impaired in croaker from hypoxic sites compared to fish from reference normoxic sites. Male germ cells were detected in 16-24% of croaker ovaries collected in the hypoxic region, but were absent in croaker ovaries from normoxic sites. In addition, the sex ratio was skewed towards males at the hypoxic sites. The masculinization and other reproductive disruptions were associated with declines in neuroendocrine function as well as ovarian and brain expression of aromatase, the enzyme that is critical for ovarian differentiation in fish. A similar incidence of intersex and decline in ovarian aromatase expression was observed in croaker after chronic laboratory exposure to hypoxia, indicating that masculinization of ovaries is a specific response to hypoxia and is due to decreased aromatase activity. The results suggest that severe reproductive impairment can occur over large coastal regions in marine fish populations exposed to seasonal hypoxia, with potential long-term impacts on population abundance. (Supported by NOAA Coastal Ocean Program Gulf of Mexico GOMEX grant NA09NOS478079 to P.T.)

S9-3.
WHAT ARE EXTREME ENVIRONMENTAL CONDITIONS AND HOW DO ORGANISMS COPE WITH THEM?
John C. Wingfield
Department of Neurobiology, Physiology and Behavior, University of California, One Shields Avenue, Davis, CA 95616, U.S.A.

Long-term weather data suggest that extreme meteorological events are increasing in frequency and intensity. Severe environmental conditions affect organisms in two major ways. The environment may be predictably severe such as in deserts, polar and alpine regions, or unpredictable with temporarily extreme conditions. Existence in an extreme environment may be possible, but then to breed or molt in addition can present major bottlenecks that have resulted in the evolution of hormone-behavior adaptations both to cope with unpredictable events. Examples of hormone-behavior adaptations in extreme conditions include attenuated testosterone secretion because territoriality and excess courtship may be too costly when there is one opportunity to reproduce. Insensitivity to testosterone and target areas of the brain regulating reproductive behavior may also develop. Similarly, reduced sensitivity to glucocorticoids occurs following acute stress during the breeding season or molt. This may allow successful reproduction and/or a vital renewal of the integument to endure extreme conditions during the rest of the year. Reduced sensitivity of the adrenocortical response to acute stress could involve: a) modulated response of the hypothalamo-pituitary-adrenal axis, b) reduced sensitivity to high glucocorticoid levels or c) a combination of a and b. Moreover, corticosteroid binding proteins (CBP) buffer responses to stress by reducing the movement of glucocorticoids into target cells. Finally, intracellular enzymes (11β-hydroxysteroid dehydrogenase and variants) can deactivate glucocorticoids entering cells thus reducing interaction with receptors. These mechanisms have important implications for climate change and increasing extremes of weather.

OR9-1.

PREPARING TO HIBERNATE IN A DEEP FREEZE: ADRENAL ANDROGEN PRODUCTION IN SUMMER LINKED TO ENVIRONMENTAL SEVERITY IN WINTER IN ARCTIC GROUND SQUIRRELS

Rudy Boonstra (1,2), Adrian Bradley (3) and Brendan Delehanty (1,2)
(1) Department of Biological Sciences, University of Toronto Scarborough, Toronto, Ontario, Canada; (2) Centre for the Neurobiology of Stress Scarborough, University of Toronto Scarborough, Toronto, Ontario, Canada; (3) School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia.

At high latitudes, evolutionary adaptations for winter focus on those that maximize survival, with hibernation being a major one used by many smaller mammals. Typically, mammalian hibernators overwinter in sites that are $^{3}0$ °C. In the arctic, such sites do not exist. Lipid, the normal fuel of most hibernators, may not provide sufficient glucose needed by certain tissues to permit survival, with muscle breakdown being required. Critical to enhancing muscle stores are high concentrations of anabolic androgens at a time when the gonads are inactive. We compare arctic ground squirrels (AGS) from the Arctic and Columbian ground squirrel (CGS) from Alberta. In males, changes in testes mass over the active season were similar in AGS and CGS, being at a maximum prior to breeding, declining rapidly during breeding and, by late summer, being <10% of that at emergence. In contrast, during the breeding and pre-hibernation periods, androgen levels in AGS were 6-10 and 20-25 times, respectively, those of CGS. From the breeding to the pre-hibernation periods, levels declined 41% in AGS, but 86% in CGS. In female AGS, androgen levels were high throughout the activ e season and, prior to hibernation, were 24 times those in female CGS. Levels in both female and male AGS, decreased≥10% in response to dexamethasone and increased ≥18% in response ACTH. These results implicate the adrenals as the source of the androgens. In male AGS, GnRH had no effect on androgen levels, whereas ACTH stimulated them by > 40%, both before and after gonadectomy. Adrenalectomy caused levels to fall by 80%. Thus, the adrenals, not the testes, are the source of the AGS androgens. We conclude that pre-hibernating AGS have evolved a solution seen in no other known mammal: exploiting the benefits of high adrenal androgen levels in nonbreeding animals to build muscle that is then catabolized overwinter. (Supported grants from NSERC to RB, U. Queensland to AJB)

OR9-2.

DO STATE-MEDIATED HORMONES PREDICT REPRODUCTIVE DECISIONS IN ARCTIC-NESTING COMMON EIDERS?

Holly L. Hennin(1), Joël Bêty(2), H. Grant Gilchrist(3) and Oliver P. Love(1)
(1) Department of Biological Sciences, University of Windsor, Windsor, ON, Canada; (2) Département de Biologie et Centre d'études nordiques, Université du Québec à Rimouski, Rimouski, QC, Canada; (3) National Wildlife Research Centre, Environment Canada, Ottawa, ON, Canada.

Variation in individual state and the abiotic environment is predicted to influence the reproductive decisions and hence success of Arctic migratory species. Previous studies have indicated that body mass and arrival date on breeding grounds can explain some of the variation observed in the reproductive decisions individuals make; however we still observe a substantial amount of unexplained variation among individuals. Employing a state-dependent framework we are using arrival physiology to enhance our ability to explain individual variation in reproductive decisions in a model migratory Arctic species. Our objective is to determine whether variation in state-dependent physiological traits combined with environmental context enhances our predictive ability to explain variation in reproductive decisions and how this variation influences reproductive success. We are studying Canada's largest colony of Arctic-breeding common eiders (*Somateria mollissima*; 4000 – 6000 pairs annually) at East Bay Island, Nunavut. From 2006-2009 we captured over 1000 pre-breeding females, collected baseline hormone levels (corticosterone and leptin), and recorded both the reproductive decisions (e.g. whether to defer reproduction, when to reproduce) and reproductive success (e.g. ability to hatch ducklings) of these individuals. Since 2005 avian cholera (*Pasteurella multocida*) has spread through the colony, potentially creating strong selection pressure on individual physiological phenotypes. Furthermore, an increasingly variable spring climate in the Eastern Arctic is potentially selecting for individuals with physiologically plastic responses. We hope to use state-dependent physiological measures to i) predict variation in reproductive decisions, ii) relate individual physiological plasticity to reproductive success, and iii) better understand how individual state and the external environment interact to shape variation in life-history traits in this Arctic-breeding species of concern. (Research supported by Environment Canada, Arctic Net, Northern Studies Training Program, NSERC and the University of Windsor.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
9:20 – 11:10 a.m.
Hussey Room

NASCE 2011 Symposium 10:
Iodothyronine Actions Throughout the Life Cycle

Chairpersons:
Andreas Heyland, University of Guelph
Carlos Valverde Rodríguez, UNAM

S10-1.
MOLECULAR AND GENETIC STUDIES OF HISTONE-MODIFYING COMPLEXES IN THYROID HORMONE ACTION DURING XENOPUS DEVELOPMENT
Yun-Bo Shi
Laboratory of Gene Regulation and Development, NICHD, NIH, Bethesda, MD 20892, U.S.A.

Thyroid hormone (T3) plays a causative role in frog metamorphosis. T3 is believed to function mainly to alter gene expression through T3 receptor (TR), although non-genomic effects of T3 also exist. Transgenic studies by us and others have shown that TR is both necessary and sufficient to mediate the metamorphic effects of T3. More recently, we have analyzed the role of TR-binding cofactor complexes during frog development. We will show that the histone acetylase/methylase-containing coactivator complexes SCR/p300//PRMT1 are recruited by liganded TR to endogenous target genes during natural and T3-induced metamorphosis. More importantly, these complexes are necessary for gene regulation by TR and frog metamorphosis and their levels play a role in regulating the rate of metamorphosis in different organs/tissues, thus ensuring coordinated transformations of different organs/tissues. On the other hand, during premetamorphic development, unliganded TR recruits histone deacetylase complexes containing TR-binding corepressors N-CoR/SMRT. Disrupting the recruitment of corepressor complexes with a dominant negative form of N-CoR leads to derepression of T3-response genes in transgenic animals. Interestingly, transgenic tadpoles develop faster than wild type siblings, initiating metamorphosis by as much as 7 days earlier out of the 30-day experiment. These data thus demonstrate that deacetylase complexes participate in gene repression to control metamorphic timing while acetylase/methylase complexes are involved in gene activation to control the rate of metamorphosis. The similarities between metamorphosis and postembryonic development in mammals suggest that such mechanisms are conserved during evolution.

S10-2.
REGULATION OF THYROID HORMONE BIOACTIVITY BY DEIODINASES; FROM INVERTEBRATES TO HUMANS
Theo J. Visser
Dept Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands

In most species, the thyroid largely secretes the prohormone T4 and a small amount of the bioactive hormone T3. Most T3 is generated by outer ring deiodination (ORD) of T4. T4 is inactivated by inner ring deiodination (IRD) to rT3, and T3 by IRD to 3,3'-T2. In mammals, 3 deiodinases are involved in these processes. D1 is highly expressed in liver and kidneys and has both ORD and IRD activities. It is important for production of serum T3. D2 has only ORD activity; it is highly expressed in brain, pituitary and brown adipose tissue, where it plays an important role in local T3 production. D3 has only IRD activity and is highly expressed in fetal tissues (e.g. brain), placenta and pregnant uterus, and in adult brain and skin. With D2, D3 is crucial for local regulation of thyroid hormone (TH) bioactivity in the brain, and thus for brain development. In all vertebrates, the deiodinases are homologous selenoproteins, containing a selenocysteine (Sec) residue in the active center. Most fish have 2 closely related D3 genes, D3a and D3b, the purpose of which is unknown. In addition to deiodination, TH is metabolized by conjugation with glucuronic acid or sulfate and by side chain modification. The latter produces, among others, the acetic acid metabolites Tetrac (TA4) and Triac (TA3). Interestingly, TA3 has equal or even higher affinity for the T3 receptors TRα and TRβ, respectively. TH is essential for metamorphosis in different species, including fish, amphibians and invertebrates such as ascidians and amphioxus. We have recently characterized a deiodinase from *Branchiostoma floridae*, with remarkable properties. Firstly, this deiodinase, termed bfDy, is not a selenoprotein but features a Cys residue instead of Sec. Secondly, this enzyme does not act on iodothyronines but specifically catalyzes the IRD of TA3 and TA4. This is interesting as the TH receptor of *B. floridae* is activated by TA3 but not by T3. This suggests that TA3 may be the primordial bioactive TH.

S10-3.
IODINE METABOLISM AND THYROID-LIKE FUNCTION IN SEA URCHINS
Andreas Heyland, Berta Lautens, and Ashley E. M. Miller
Integrative Biology, University of Guelph, ON, Canada.

Thyroid hormones (THs) accelerate larval development in several echinoids (sea urchins and sand dollars) and preliminary evidence indicates that these species can synthesize THs endogenously from iodine, the essential component of THs. In contrast to vertebrates, nothing is known about the iodine uptake mechanisms in echinoderms or any other invertebrate species. New experiment on iodine uptake mechanisms, employing I_{125} radioisotope and pharmacology experiments show that inhibitors of vertebrate sodium iodine symporters (NIS) have no effect on iodine uptake in sea urchin larvae. However, peroxidase inhibitors as well as reducing agents drastically lower the iodine content of larvae after exposure. Moreover, iodine uptake is significantly increased when larvae are exposed to hydrogen peroxide H_2O_2. Therefore, our data suggest that sea urchins do not utilize a vertebrate-type NIS system for iodine uptake but instead rely largely on peroxide facilitated diffusion. This mechanism has also been confirmed in several plant and bacterial species and therefore appears to be evolutionary ancient. Finally, preliminary data indicate that iodine may act as a strong inorganic antioxidant due to its ability to scavenge endogenous H_2O_2. Future experiments examining iodine's effects on development may provide further insight into its function.

OR10-1.

TRANSCRIPTION OF THYROID HORMONE CO-ACTIVATOR PRMT1 IS REGULATED BY A THYROID HORMONE-INDUCED TRANSCRIPTION FACTOR C-MYC DURING *XENOPUS* INTESTINAL REMODELING

Kenta Fujimoto and Yun-Bo Shi
Section on Molecular Morphogenesis, Laboratory of Gene Regulation and Development, Program in Cellular Regulation and Metabolism (PCRM), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD 20892, U.S.A.

The thyroid hormone (T3)-dependent *Xenopus* metamorphosis resembles the mammalian postembryonic development and offers a unique opportunity to study the adult stem cell development. During metamorphosis, the intestine is remodeled from the larval to adult form and the adult epithelial stem/progenitor cells are developed *de novo* from the larval epithelium through yet known mechanism. T3 exerts its metamorphic effects through T3 receptor (TR)-mediated transcriptional modulation of downstream genes. We have shown that the TR-coactivator protein arginine methyltransferase 1 (PRMT1) is upregulated in a small number of larval epithelial cells that appear to give rise to the adult stem cells and that PRMT1 upregulation is the earliest molecular marker known to date. Interestingly, stem cell specific expression of PRMT1 is conserved during zebrafish and mouse intestinal development, suggesting that PRMT1 function is conserved. To analyze how PRMT1 is specifically upregulated in the stem cells, we first characterized the *X. laevis* PRMT1 promoter in CaCo-2 cells, a human intestinal epithelial cell line with intestinal stem cell characters. We identified evolutionally conserved elements in the promoter and in the first intron involved in the transcriptional regulation of PRMT1 gene. Our findings suggest the involvement of the c-MYC transcription factor, which is known to be important for stem cells and cell proliferation. We hypothesized that T3 induces c-MYC to regulate PRMT1 transcription. We showed that c-MYC regulates the promoter via the conserved binding site in PMRT1 gene through promoter mutational and DNA binding studies. Furthermore, we found that c-MYC is upregulated during natural and T3-induced metamorphosis prior to PRMT1 and that TR binds to a putative T3 response element of *X. tropicalis* c-MYC gene *in vivo*. These results suggest that T3 induces c-MYC directly in the intestine, which in turn enhances PRMT1 expression through the cis-element in the first intron.

OR10-2.

THYROID HORMONE METABOLISM IS ACTIVE IN THE DEVELOPING XENOPUS BRAIN

Ghislaine Morvan-Dubois, Jean-Baptiste Fini, Karine Le Blay, Sébastien Le Mével, Marine Perret-Jeanneret and Barbara Demeneix
UMR CNRS 7122, Evolution des Régulations Endocriniennes, Département Régulations, Développement et Diversité Moléculaire, Muséum National d'Histoire Naturelle, 75231 Paris, France.

In most vertebrates, the main thyroid hormone (TH) secreted by the thyroid gland is thyroxine or T_4. However, the more biologically active form of TH is tri-iodothyronine or T_3. T_3 is produced locally in the periphery from T_4 by specific enzymes. T_4 conversion to T_3 is carried out by activating deiodinases (D1 and D2) whereas an inactivating deiodinase (D3) can convert both T_4 and T_3 into less active forms, unable to bind TRs. In both cases, the balance between deiodinase activation and inactivation determine TH availability and action. We previously demonstrated that D2 is expressed and active in pre-metarmorphic embryo Xenopus and that T4 is converted into T_3 within the embryo. Here we investigated the role of TH metabolism through deiodination during early Xenopus development with a special emphasis on brain. Expression of activating and inactivating deiodinases in Xenopus embyos was followed by ISH and its regulation by TH was studied by quantitative PCR. All three deiodinases are present as maternal mRNAs and display dynamic profiles from neural stages onwards. ISH showed periventricular expression in the brain. To determine the localisation and temporal changes in expression we generated tadpole reporters for deiodinases expression. In parallel, we used a pharmaceutical approach in order to identify T_3 target genes during Xenopus brain development. The combination of these two approaches showed several genes implied in steminality to be regulated by TH signalling during Xenopus neurogenesis. In order to determine in which cell types TH is metabolised during neurogenesis we generated double transgenic tadpoles that express one fluorescent protein under control of the D2 promoter and a second fluorescent protein under control of a TH response element. The analysis of these tadpoles will enhance knowledge of how deiodinases control thyroid signalling during early brain development.(Supported by EU project ‘Crescendo’ and ANR ‘Signator.’)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
1:30 – 3:20 p.m.
Vandenberg Room

NASCE 2011 Symposium 11:
Nuclear Hormone Receptors: Evolution and Roles in Development and Physiology

Chairpersons:
Penny Hopkins, University of Oklahoma, USA
Yun-Bo Shi, National Institute of Child Health and Human Development, NIH, USA

S11-1.
ECDYSTEROIDS AND THEIR RECEPTORS IN THE CRUSTACEAN, *UCA PUGILATOR*
Penny M. Hopkins
Department of Zoology, University of Oklahoma, U.S.A.

The ecdysteroids are a family of steroid hormones unique to the arthropods. The various forms of these steroids varies from group to group with most insects having 20-hydroxyecdysone as the primary molting hormone and some crustaceans having more than one active form. Crustacean ecdysteroids and ecdysteroid nuclear receptors aresimilar to those of insects, but differ in the number of hormones and in the number and structure of the receptor isoforms. Moreover, the control(s) of ecdysteroid synthesis by crustacean Y-organs is primarily inhibitory - through molt-inhibiting hormone (MIH) - whereas in insects ecdysteroid synthesis is positively stimulated by a very different neurosecretory hormone. The *in vivo* effects of ecdysteroids are less understood in crustaceans than in insects but appear to have some concordance. Ecdysteroid-responsive genes in crustaceans are just beginning to be uncovered and may have some identities to insect genes. The differences in ecdysteroid control between insects and crustaceans are thought to have evolved to accommodate the differences in life-histories seen in these diverse arthropod groups.

S11-2.
ROLE OF JUVENILE HORMONE AND ITS RECEPTOR IN *DROSOPHILA* METAMORPHOSIS AND REPRODUCTION
Lynn M. Riddiford, Julide Bilen and James W. Truman
Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, U.S.A.

Juvenile hormone (JH) has two roles: it prevents the switching actions of ecdysone that are necessary for metamorphosis and it regulates reproductive maturation in the adult. In *Drosophila melanogaster* the *Methoprene-tolerant* (*Met*) gene encodes a JH receptor (a basic helix-loop-helix, Pas domain protein), yet the Met^{27} null mutant becomes an adult that shows delayed reproduction and reduced fecundity. We found that genetic ablation of the corpora allata (allatectomy) that produce JH in *Drosophila* larvae causes the premature maturation of the optic lobe during the prepupal period in response to the pupariation peak of ecdysone and death at the pupal molt (head eversion). These effects can be rescued by feeding JH to final stage larvae. Loss of Met in the Met^{27} mutant mimics the effect of allatectomy on development of the optic lobe. Expression of Met RNAi in selected neurons shows that JH is acting on one of the photoreceptors itself to prevent premature differentiation caused by ecdysone. The cause of death of the allatectomized prepupae is unknown; it is mediated not by Met but apparently by a highly similar protein, Germ Cells Expressed (GCE). Genetic allatectomy during the molt to the adult results in a delay in the onset of female receptivity for mating that can be restored by application of JH at the time of eclosion. The loss of Met causes a similar delay in onset of female receptivity. Expression of Met RNAi in brain neurons known to be involved in female receptivity also delays its onset, suggesting that JH acts via Met to promote the maturation of these neurons. Thus, Met functions as the JH receptor in some of JH's roles in both development and reproduction, and manipulation of its expression allows the identification of cellular targets for these quite different actions of JH.

S11-3.
WHOLE GENOME MAPPING OF THYROID HORMONE RECEPTOR IN *XENOPUS TROPICALIS*
Nicolas Buisine(1), Patrice Bilesimo(1), Gladys Alfama(1), Alexis Grimaldi(1), Xiaoan Ruan(2), Ed Liu(2), Barbara A. Demeneix(1), Yijun Ruan(2) and Laurent M. Sachs(1)
(1) CNRS UMR 7221, Muséum National d'Histoire Naturelle, 7 rue Cuvier F75231 Paris cedex 05, France; (2) Genome Institute of Singapore, 60 Biopolis street, 138672, Singapore.

One striking example of how thyroid hormones (THs) regulate vertebrate developmental processes is amphibian metamorphosis. Tadpoles transformation is marked by dramatic TH-induced changes including de novo morphogenesis, tissues remodeling and organ resorption. These changes involve cascades of gene regulation initiated by THs and their receptors (TR). Metamorphosis is thus an attractive model to decipher the molecular mechanisms controlling the cascades of transcriptional programs dependent on TR/THs signaling. Our aims is to build a genome wide profile of TR binding sites and to map the physical interactions network between DNA bound TR complexes. The latter should provide insight into how the TH responsive genome is organized into high-level three-dimensional structures according to functional input. To this end, the recently developed chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) was used in the context of *Xenopus tropicalis* metamorphosis. ChIA-PET libraries, corresponding to tail fin tissues with treatment with THs, have been constructed and sequenced using high throughput sequencing technologies. Strong TR binding sites have been found and analyzed for THs response elements. The linkage of a binding site to its target gene is not trivial and require genes annotation and RNA expression levels information to take the full advantages of ChIA-PET data. As DNA array results reveal the limitation of this technology (low number of gene represented on the array) and the annotation of the genome is partial, shotgun sequencing of transcripts (RNA-Seq) and the paired-end tag approach to sequence RNA (RNA-PET) were used. ChIA-PET analysis strongly benefits from the acquisition of such transcriptome studies. The results highlight enrichment of key gene categories and open new perspectives for functional studies on control of cell fate. (Research supported by European FP6 Crescendo, European FP7 Ideal, ANR JCJC Trigger, Partenariat Hubert Curien Merlion.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

OR11-1.

DISCOVERING THE REGULATORY LOGIC OF AN ANCIENT, EVOLUTIONARILY CONSERVED NUCLEAR RECEPTOR ENHANCER MODULE

Pia Bagamasbad(1), Ronald M. Bonett(2) and Robert J. Denver(1)(3)
(1) Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, U.S.A; (2) Department of Biological Science, The University of Tulsa, Tulsa, OK; (3) Neuroscience Program, University of Michigan, Ann Arbor, MI, U.S.A.

Different hormones may cooperate to regulate physiology and development. A striking example is the acceleration of tadpole metamorphosis by the synergistic actions of thyroid hormone (T_3) and glucocorticoid (GC). Krüppel like factor 9 (KLF9) is a transcription factor that mediates hormone action during development; the *Klf9* gene is directly regulated by T_3 receptor (TR) and GC receptor (GR). We investigated whether *Klf9* is synergistically transactivated by T_3 plus GC. Gene expression analysis in frog and mouse cell lines, and in tadpole brain showed that treatment with T_3 plus GC synergistically transactivated *Klf9*. Kinetic analysis of mRNA and heteronuclear RNA accumulation supported that synergy occurred at the transcriptional level. Transfection assays with reporter constructs containing 1 kb fragments of the 5' flanking region of the frog *Klf9* gene identified a region between -5 and -6 kb that supported synergistic transactivation. Comparative sequence analysis showed an evolutionarily conserved region of ~180 bp within the -5 to -6 kb fragment. The frog and mouse ~180 bp sequences supported synergistic transactivation, supporting that this region comprises an enhancer module. *In silico* analysis identified several putative hormone response elements (HREs) for TR and GR. Using gel shift, DNAseI footprinting and chromatin immunoprecipitation (ChIP) assays we found that TR and GR associate with specific sequences within the enhancer module. Site-directed mutagenesis of any of the HRE half sites abolished the synergistic response, supporting that direct DNA binding by TR and GR are necessary for synergistic transactivation. ChIP assays spanning the *Klf9* locus for acetylated histones 3 and 4 showed elevated histone acetylation at the NR synergy module, but no augmentation of acetylation by combined hormone treatment. We are now investigating differential recruitment of RNA polymerase II as a possible molecular basis for hormone synergy. (Supported by NIH grant R01 NS046690 and NSF grant IOS 0922583 to RJD).

OR11-2.

3,5-T_2 AND T3 ACTIVATE DIFFERENT ISOFORMS OF THE THYROID HORMONE RECEPTOR B1 IN *FUNDULUS HETEROCLITUS*

A. Mendoza-Cisneros, P. Villalobos, C. Valverde-R, and A. Orozco
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla Querétaro, Querétaro México.

Besides the non-genomic mitochondrial effects documented by others, we demonstrated that 3,5-T_2 regulates the transcription of T_3-responsive genes, at least in some teleosts. Furthermore, EMSAs of hepatic nuclear proteins of hypothyroid killifish replaced with 3,5-T_2 or T_3 showed two TRE-TR (T_3 responsive element-thyroid receptor) complexes of different weight, suggesting that 3,5-T_2 and T_3 bind to transcriptional protein complexes that could involve one or more isoforms of the classic TR. We addressed this hypothesis by cloning the TR expressed in killifish liver and found two different TRβ1 mRNAs. Both fish TR lack 75 amino acids at the N-terminus, as compared to the human TRβ1. Most importantly, one of them, designated as long or L-TRβ1, contains a 9 amino acid insert in the ligand-binding domain, whereas the other one, named short or S-TRβ1 lacks it. To test the putative influence of this insert in the selectivity and binding proprieties of the TR to a different iodothyronine, we evaluated the transactivation capacity of both TRβ1 isoforms in the presence of T_3 or 3,5-T_2 using a reporter gene coupled to luciferase and containing a TRE. We observed different patterns of response depending on the iodothyronine tested and the nature of the transfected cell line. In GH3 cells, a higher transactivating activity was observed in cells transfected with L-TRβ1 and treated with 3,5-T_2, while those cells transfected with S-TRβ1 showed activity only when treated with T_3. In contrast, in the CV1 cell line, the highest activity was observed when cells were transfected with S-TRβ1and treated with T_3. These results suggest that the effects of 3,5-T_2 could be mediated by a different TR isoform. Furthermore, the lack of transactivation activity in CV1 cells transfected with L-TRβ1 and treated with T_3 or 3,5-T_2 suggests that this TR isoform might interact with transcription factors that are not expressed in all cell types.

Acknowledgements: We thank Miguel Angel Maqueda. This work was partially supported by: CONACYT 080420 and PAPIIT IN203409

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
1:30 – 3:20 p.m.
Mendelssohn Theater

NASCE 2011 Symposium 12:
Neuroendocrine Control of Reproduction

Chairpersons:
Stacia Sower, University of New Hampshire, USA
Pei-San Tsai, University of Colorado, Boulder, USA

S12-1.
DEVELOPMENT AND HORMONAL REGULATION OF REPRODUCTIVE NEURAL CIRCUITS IN RODENTS
Alexander S. Kauffman
Department of Reproductive Medicine, University of California San Diego, La Jolla, CA, U.S.A.

The mammalian neuroendocrine reproductive axis is controlled by various hormonal and neural pathways that converge upon and regulate forebrain gonadotropin-releasing hormone (GnRH) neurons. The status of the reproductive axis and GnRH secretion differs between various life stages, including perinatal development, puberty, and adulthood. However, until recently, many of the key neural circuits underlying the control of GnRH neurons at different life stages were only poorly understood. The newly-identified neuropeptide kisspeptin, encoded by the *Kiss1* gene, has now been implicated as an important regulator of GnRH neurons in numerous species. In rodents, *Kiss1* neuronal populations are located in several discrete brain regions, including two hypothalamic nuclei and the amygdala. Accumulating evidence indicates that *Kiss1* neurons are potent stimulators of the reproductive axis at different life stages, including puberty and adulthood. Studies have now begun to examine how *Kiss1* circuits are themselves regulated, both in adulthood and development. Findings from multiple species indicate that *Kiss1* neurons are robustly regulated by sex steroids in both sexes. Intriguingly, the manner in which sex steroids regulate *Kiss1* neurons (stimulatory or inhibitory) is region-specific within the brain, a finding which may illuminate the cellular mechanism of steroid-mediated positive and negative feedback regulation of GnRH secretion. Recent findings also indicate that the various *Kiss1* neural populations have different developmental patterns and ontogeny, culminating in different phenotypic identities, and perhaps functions, in adulthood. Intriguingly, certain *Kiss1* populations in rodents undergo sexual differentiation, a developmental process shown to be driven by sex steroids during critical postnatal periods. The development and regulation of *Kiss1* reproductive circuits at different life stages will be summarized and discussed for several species, primarily rodents.

S12-2.
APLYSIA GNRH: A ROLE IN REPRODUCTION?
Pei-San Tsai, Scott I. Kavanaugh and Biao Sun
Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO 80301-0354, U.S.A.

Gonadotropin-releasing hormone (GnRH) is a neuropeptide critical for the activation of vertebrate reproduction. The recent discoveries of GnRH-like molecules in protostomes shed light on the structure and expression pattern of these molecules, but the functional data remain scarce. To close these knowledge gaps, we characterized the physiological roles of an endogenous GnRH, named ap-GnRH, in a gastropod mollusk (*Aplysia californica*). Treatment of sexually mature and immature animals with synthetic ap-GnRH had no effects on a number of reproductive parameters such as gonadal mass, reproductive tract mass, egg laying, and penile eversion. ap-GnRH also failed to alter oocyte growth and the accumulation and secretion of egg-laying hormone. Interestingly, ap-GnRH triggered acute behavioral and locomotive changes unrelated to reproduction, and was found at high levels in central neurons and efferents projecting to the foot and parapodia, suggesting a role in motor regulation. Electrophysiological recordings revealed that ap-GnRH had diverse effects on central neurons that ranged from excitatory, inhibitory, to alteration of input resistance. Unexpectedly, ap-GnRH suppressed the activation of reproductive bag cell neurons, suggesting an inhibitory effect on female reproduction. The central ganglia of *A. californica* expressed a putative ap-GnRH receptor (ap-GnRHR) that was >60% identical to GnRHR in the common octopus and similar to the Type 2 vertebrate GnRHR, suggesting ap-GnRH does in fact bind to a receptor homologous to vertebrate GnRHR. Overall, we performed extensive functional tests and found little evidence that ap-GnRH was stimulatory to reproduction. In fact, ap-GnRH was more likely to have central effects on behavioral and motor regulation. As such, we propose that the observed role of vertebrate GnRH as a reproductive activator is not universally applicable to protostomes. (Supported by NSF grant IOS-0743818 to PST).

S12-3.
AGNATHA AND AVIAN: EVOLUTIONARY RECRUITMENT OF GNRH SYSTEMS
Nerine Joseph(1)(2)(3)(4), Kevin Morgan(2), Robert Millar(2), Ian Dunn(3), Gregoy Bedecarrats(4), and Stacia Sower(1)
(1) Center for Molecular and Comparative Endocrinology, 46 College Road, University of New Hampshire, Durham NH 03824, U.S.A.; (2) MRC Human Reproductive Sciences Unit, The Queens Medical Research Institute, Edinburgh, EH16 4TJ, U.K.; (3) The Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, Edinburgh, EH25 9RG, U.K.; (4) Animal and Poultry Science, University of Guelph, 50 Stone Road East, Guelph, N1G 2W1, Canada.

Gonadotropin releasing hormone (GnRH) plays a pivotal role in the regulation of reproductive function through activation of its corresponding receptor (GnRH-R). Loss of a GnRH ligand and receptor isoforms may be correlated with the evolution of complexity of reproductive physiology. With the identification of two novel type III GnRH-Rs in the lamprey and the previously identified lGnRH-R-1, the total number of identified GnRH-Rs in the lamprey is three, which coincides with three endogenous lamprey GnRH ligands. In the chicken, two GnRH-R subtypes (type I and III) coincide with two endogenous chicken GnRH ligands. A comparison of the GnRH systems in a basal vertebrate and an avian species provides a unique insight into the recruitment of GnRH systems in these two divergent species and the evident plasticity in utilization of specific GnRH receptors in particular tissues. We have examined expression profiles, ligand specificity and activation of intracellular signaling in lamprey and chicken GnRH-Rs. Our latest findings describe which GnRH-R is the probable major mediator of pituitary gonadotrope function in each species and demonstrate that evolutionary recruitment of specific ligand-receptor pairing for particular physiological processes does not necessarily correlate with ligand–binding affinity or potency of second messenger activation. We propose that type I and III GnRH-Rs are more prevalent than type II GnRH-Rs and the gene duplication event from which type II and III

GnRH-Rs emerged may have occurred before the split of the tetrapods and teleosts lineages. The retention of type II and III GnRH ligands may correlate with diverse endocrine functions for GnRH, including intra-gonadal functions and coincides with a diffusional hypothalamic-pituitary system in lampreys, whereas the loss of the type III ligand coincides with a developed hypothalamic portal system in chickens. (Supported by NSF IOS-0849569, NH AES Hatch 332 to SAS and BBSRC scholarship to NJ.)

OR12-1.

DIFFERENTIAL INVOLVEMENT OF PKC AND PKA IN GHRELIN-INDUCED GROWTH HORMONE AND GONADOTROPIN RELEASE FROM PRIMARY CULTURES OF DISPERSED GOLDFISH PITUITARY CELLS

Caleb L. Grey and John P. Chang*
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Ghrelin (GRLN) stimulates feeding and the release of pituitary growth hormone (GH) and maturational gonadotropin (LH) in goldfish, thus providing a possible link between energy balance and reproduction. Previous work shows that mRNAs for GRLN and its receptors are expressed in the goldfish pituitary. Extracellular Ca^{2+} entry through L-type voltage-sensitive Ca^{2+} channels and increases in intracellular Ca^{2+} are important signaling components leading to goldfish (g)$GRLN_{19}$-elicited GH and LH release. In this study, we examined the role of protein kinase C (PKC) and A (PKA) in $gGRLN_{19}$-induced hormone secretion via column perifusion of dispersed goldfish pituitary cells, and single cell Ca^{2+}-imaging of identified goldfish somatotropes and gonadotropes. PKC inhibitors (bisindolylmaleimide-II and Gö 6976) attenuated $gGRLN_{19}$-stimulated hormone secretion and associated Ca^{2+} signals. $gGRLN_{19}$-induced hormone release and Ca^{2+} signals were not observed when cells were already stimulated by a PKC agonist (DiC8). PKA inhibitors (H-89 and KT5720), however, only inhibited $gGRLN_{19}$-induced hormone release and Ca^{2+} responses in LH cells. On the other hand, an adenylate cyclase activator (forskolin) enhanced $gGRLN_{19}$-induced GH, but not LH, release. Ca^{2+} signals elicited by combined forskolin and $gGRLN_{19}$ treatment were not different from those to either treatment alone in either cell type. These results suggest that while PKC and PKA mediate GRLN actions on LH release by actions upstream of Ca^{2+} signals, similar responses in GH cells only involve PKC. In addition, activation of PKA may enhance GRLN-stimulated GH secretion by actions downstream of Ca^{2+} changes. (Supported by NSERC.)

OR12-2.

THE DUPLICATED ZEBRAFISH KISSPEPTIN RECEPTOR (KISSR) GENES EXHIBIT NON-OVERLAPPING EXPRESSION PATTERNS AND FUNCTIONS: THE IDENTIFICATION OF A NUCLEAR KISSR VARIANT THAT HAS TRANSACTIVATING ACTIVITY

Takeshi A. Onuma and Cunming Duan
Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Kisspeptin and its receptor KiSSR (GPR54) are key players in the neuroendocrine control of reproduction. In zebrafish, there are two kisspeptin genes, *kiss1* and *kiss2*, and two KiSSR genes, *kissr1* and *kissr2*. The functional relationship of these KiSS ligands and receptors is largely unclear. In this study, we have performed molecular and functional analyses of the two KiSSR genes and tested the hypothesis that they have evolved non-overlapping expression patterns and distinct functions. We show that there are at least five *kissr1*transcripts resulted from alternative splicing. In addition to the previously reported transcript encoding the full-length KiSSR (referred as *kissr1-1)*, there are 4 novel *kissr1* transcripts (referred as *kissr1-2*, *1-3*, *1-4* and *1-5*) that encode 4 truncated forms of KiSSR-1. These *kissr1* transcripts are expressed in the brain and in many peripheral tissues. In comparison, *kissr2* has only a single transcript and is only expressed in the brain. *Kissr1* and *kissr2* exhibits different temporal expression patterns in early development. Functional assays showed that KiSSR1 and KiSSR2 have different ligand selectivity. While KiSSR1 can be selectively activated by KiSS1, KiSSR2 can be activated by both KiSS1 and KiSS2. None of the truncated KiSSR1 isoforms can be activated by KiSS1 or KiSS2. To determine whether these *kissr1* variants encode membrane proteins, they were tagged with EGFP and transfected into COS-7 and GT1-7 cells for subcellular localization study. Surprisingly, one of them, KiSSR1-4, is localized to the nucleus. We next performed one-hybrid assay to test its possible nuclear activity. When fused to GAL-4 DNA dinging domain, the KiSSR1-4 has significant transactivation activity (TA). Mutation analysis revealed that the TA domain is located in the extracellular N-terminus region of KiSSR1. These results indicate that the two KiSSR genes have evolved non-overlapping spatial and temporal expression patterns and distinct functions. The discovery of a nuclear form of KiSSR-1 that has TA activity has raised the possibility of new function of KiSSR in the nucleus.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
1:30 – 3:20 p.m.
Hussey Room

NASCE 2011 Symposium 13:
Ion and Water Balance

Chairpersons:
Ian Orchard, University of Toronto. Canada
Steve McCormick, USGS and University of Massachusetts, USA

S13-1.
NEUROENDOCRINE REGULATION OF DIURESIS AND ANTI-DIURESIS IN THE CHAGAS' DISEASE VECTOR, RHODNIUS PROLIXUS
Jean-Paul Paluzzi and Michael J. O'Donnell
Department of Biology, McMaster University, Hamilton, Ontario, Canada.

Hormones such as neuropeptides and biogenic amines regulate an assortment of biological functions in vertebrates and invertebrates. For insects, one particular biological role regulated by hormones and, of great interest in our research, is the maintenance of salt and water homeostasis. Such regulation is necessary when insects increase food intake, change their metabolism, or are presented with a challenging habitat in which they must either retain or eliminate excess water and/or salts. In the blood-feeder, *Rhodnius prolixus*, a blood meal which often weighs more than ten times the unfed body weight of the insect is imbibed during each post-embryonic developmental stage. A hormone-regulated rapid diuresis ensues soon after the onset of feeding to remove the excess water and salts associated with the non-nutritive plasma portion of the blood meal. The endogenous hormones leading to the rapid diuresis have been identified and recently a native anti-diuretic factor was also elucidated. We discuss the interplay between these various neuroendocrine-derived factors and their regulation on tissues associated with salt and water balance. The anti-diuretic hormone, RhoprCAPA-2, inhibits serotonin-stimulated diuresis, however, it does not inhibit RhoprCRF-stimulated diuresis. An unexpected finding is that, at concentrations below 100nM, RhoprCAPA-2 augments the diuretic effects of RhoprCRF but attenuates natriuresis. (This work was supported by NSERC.)

S13-2.
MOLECULAR DIVERSITY OF AQUAPORIN FOR WATER ADAPTATION STRATEGY IN ANURAN AMPHIBIANS
Shigeyau Tanaka(1,2) and Masakazu Suzuki(2)
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To maintain water homeostasis in the body, most adult anuran amphibians except for the aquatic species absorb water across the ventral pelvic (VP) skin and reabsorb it from urine in the urinary bladder (UB). Aquaporin (AQP), a water channel protein, plays a fundamental role in these water absorption/reabsorption processes, which are regulated by antidiuretic hormone. Anuran AQP family consists of at least ten AQPs and two anuran-specific types, designated as AQPa1 and AQPa2 (The letter "a" represents anuran). In Hyla japonica, AQP2 (AQP-h2K) and two forms of AQPa2 (AQP-h2 and AQP-h3) reside in tight-junctioned epithelial cells of three major osmoregulatory organs, i.e. AQP-h2K in the kidney, AQP-h2 in the UB, and both AQP-h2 and AQP-h3 in the VP skin. They show translocation from the cytoplsmic pool to the apical plasma membrane in response to arginine vasotocin (AVT). Tissue distribution of AQPa2 in five anuran species, from aquatic to arboreal habitats, suggests that AQP-h2 is an UB-type AQP, while AQP-h3 is a VP skin-type AQP. Further, AQP-h2K seems to be specific to the kidney. These findings suggest that anuran AVT-dependent osmoregulatory organs utilize AQP3 (AQP-h3BL), located in the basolateral membrane, at the exit site of the epithelial water transport, whereas at entry site they basically adopt different AQPs as translocable water channel: h2-like AQPa2 in the UB, h3-like AQPa2 in the VP skin, and AQP2 in the kidney. In terrestrial and arboreal species, the UB-type AQP is further expressed in the VP skin, together with the VP skin-type AQP. In contrast, the VP skin-type AQP (AQP-x3) of the aquatic Xenopus has lost the ability of efficient protein production. The positive transcriptional regulation of UB-type AQP in the VP skin and negative post-transcriptional regulation of VP skin-type AQP provide flexibility in the water regulation mechanisms, which might have contributed to the evolutionary adaptation of anurans to a wide variety of water environments.

S13-3.
OSMORECEPTION AND ENDOCRINE RESPONSES IN THE EURYHALINE TILAPIA, *OREOCHROMIS MOSSAMBICUS*
A. P. Seale(1), J. P. Breves(2), S. Watanabe(3), T. Kaneko (3), D. T. Lerner(1)(4), T. Hirano (1), E. G. Grau(1)
(1) Hawai'i Institute of Marine Biology, University of Hawaii, Kaneohe, HI, U.S.A.; (2) Department of Biology & Center for Neuroendocrine Studies, University of Massachusetts, Amherst, MA, U.S.A.; (3) Department of Aquatic Bioscience, University of Tokyo, Tokyo, Japan; (4) Sea Grant College Program, University of Hawai'i, Honolulu, HI, U.S.A.

Osmoregulation is essential to life in vertebrates. Prolactin (PRL) is well established as a central regulator of hydromineral balance in teleosts inhabiting fresh water (FW), regulating both the active and passive movements of ions across epithelial surfaces such as gill, kidney, gut and the integument. Tilapia PRL cells of the rostral pars distalis (RPD) have special attributes, including responsiveness to physiologically relevant alterations in extracellular osmolality and a homogenous arrangement within the RPD, that make them a valuable model for studying the fundamental aspects of osmoreception. These characteristics facilitate cell culture and the simultaneous assay of PRL release and gene expression. Consistent with its role in FW osmoregulation, PRL release is inversely related to extracellular osmolality, both *in vivo* and *in vitro*. Osmotically driven increases in cell volume, mediated by the water channel aquaporin 3 (AQP3), are coupled with a rapid influx of Ca^{2+} through stretch-activated channels, which in turn leads to an increase in intracellular Ca^{2+} that initiates a rise in PRL release. *In vitro* PRL release from the cells of FW tilapia is more robust after a decrease in osmolality than that from PRL cells of seawater (SW) fish. Interestingly, a hyposmotically-induced increase in PRL gene expression was observed only in cells from SW fish; possibly because expression is already maximal in FW PRL cells. RPD expression of the stretch-activated Ca^{2+} channel, transient receptor potential cation channel, subfamily V, member 4 (TRPV4), is directly proportional to extracellular osmolality *in vivo* and *in vitro*. Together, these studies indicate that hyposmotically-induced PRL release is dependent on extracellular Ca^{2+} and that differences in the responsiveness of PRL cells in FW and SW tilapia are correlated with changes in the expression of AQP3, TRPV4, and PRL. (This work was supported by NSF grant IOB05-17769 and by the Pauley Foundation.)

OR13-1.
HORMONAL AND DEVELOPMENTAL REGULATION OF SALINITY-DEPENDENT ISOFORMS OF THE BRANCHIAL SODIUM PUMP IN ATLANTIC SALMON

S. D. McCormick(1)(2), A. Regish(1), J. G. Chadwick, Jr.(2), A. K. Christensen(2), T. O. Nilsen(3), S. O. Stefansson(3), and B. T. Björnsson(4)
(1) USGS, Conte Anadromous Fish Research Center, Turners Falls, MA, U.S.A.; (2) Department of Biology, University of Massachusetts, Amherst, MA, U.S.A.; (3) Department of Biology, University of Bergen, High Technology Centre, Bergen N-5000, Norway; (4) Fish Endocrinology Laboratory, Department of Zoology/Zoophysiology, Göteborg University, Sweden.

The sodium pump, Na+,K+-ATPase (NKA), in the gills of teleost fish is involved in ion regulation in both freshwater and seawater. Recent molecular evidence indicates that two isoforms of the main catalytic alpha (α) subunit may be differentially regulated by environmental salinity. We have developed and validated antibodies specific for the NKA α1a and NKA α1b isoforms of Atlantic salmon, and used western blots and immunocytochemistry to examine their abundance and localization. Branchial NKA α1a decreases and NKA α1b increases after Atlantic salmon are acclimated to seawater. The abundance of branchial NKA α1a is higher in parr than in the downstream migratory smolt, and decreases in parr and smolt from winter through spring. NKA α1b increases dramatically in smolts in spring coincident with their increased salinity tolerance. There is a further increase in NKA α1b abundance in smolts after seawater exposure. Plasma growth hormone (GH) and cortisol increase during smolt development. Gill NKA α1a and α1b abundance and chloride cell size were increased by treatment with exogenous cortisol. Exogenous GH by itself did not affect the abundance of either isoform. However, when co-injected with cortisol, GH caused a decrease in NKA α1a and an increase in NKA α1b abundance, indicating that GH is acting as a switch to alter the effects of cortisol. Exogenous prolactin was shown to increase the abundance of NKA α1a and decrease the abundance of NKA α1b. These results indicate that prolactin and cortisol act to promote ion uptake in freshwater, whereas growth hormone and cortisol interact to promote salt secretion in seawater. (This project was supported by USGS and National Research Initiative Competitive Grant no. 2008-35206-18782 from the USDA National Institute of Food and Agriculture.)

OR13-2.
CHARACTERIZATION OF LEPTIN AND ITS PUTATIVE RECEPTOR (LEPR) IN EURYHALINE TILAPIA: A NOVEL LINK BETWEEN ENERGY STATUS AND OSMOREGULATORY FUNCTION?

David A. Baltzegar, Emily S. Brune, William M. Johnstone III, and Russell J. Borski
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Leptin is secreted by adipocytes and acts via its cytokine receptor (LepR) to reduce appetite. Additionally, leptin has multiple peripheral actions including stimulation of reproductive maturity, immunity, and epithelial tissue development. In teleost fishes, the actions of leptin remain poorly described, however similar effects upon appetite have been reported. Leptin stimulates pituitary prolactin secretion in tilapia (*Oreochromis mossambicus*), and in mammals may itself be responsive to elevated cortisol. As prolactin and cortisol are major hormones controlling osmoregulation, leptin may be a critical link in the integrated endocrine network controlling growth, reproduction, and osmotic homeostasis. Here, we describe the molecular sequence of leptin and its putative receptor (LepR). Tilapia leptin and LepR share 46-56% sequence identity with Fugu, but only 24-28% identity with human orthologs, suggesting a significant evolutionary divergence in vertebrates. In basal teleosts, two leptins have been described likely arising from genome duplication. Gene synteny and Northern blotting suggests the presence of only a single leptin homolog in Perciformes, the largest order of vertebrates. In tilapia, leptin is expressed in both liver and adipose tissue, but at lower levels in gill, kidney, skin and hypothalamus/brain. LepR expression was detected in all tissues examined, supporting pleiotropic actions for leptin in teleosts. To ascertain potential osmoregulatory functions gill leptin gene expression was measured in tilapia during 24-hr salinity challenge. An 8-fold higher abundance of gill leptin mRNA was found in freshwater (FW) compared with seawater (SW) tilapia ($p<0.01$). No significant change in gill leptin mRNA was observed after FW or SW transfer, albeit levels increased over the first 3 hr of FW challenge. Elevated production of leptin in the gill of FW tilapia may be associated with paracrine-mediated gill remodulation to a FW phenotype.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
3:40 – 5:30 p.m.
Mendelssohn Theater

NASCE 2011 Symposium 14:
Coping with Environmental Change: Adaptive Roles for Neuroendocrine Stress Pathways

Chairpersons:
L. Michael Romero, Tufts University, USA
Matt Vijayan, University of Waterloo, Canada

S14-1.
STRESS AND INVASION: ELEVATED LEVELS OF CORTICOSTERONE MAY FACILITATE SURVIVAL-ENHANCING BEHAVIOR OF NATIVE LIZARDS
Tracy Langkilde, Nicole A. Freidenfelds and Travis Robbins
Department of Biology, Pennsylvania State University, State College, PA 16802, U.S.A.

Non-native species introductions are becoming increasingly common, and can impose novel threats to the native communities they invade. Populations exposed to such environmental perturbations often exhibit elevated physiological stress levels including increased levels of circulating glucocorticoids, such as corticosterone (CORT). Red imported fire ants, *Solenopsis invicta*, are a globally important invader. Native fence lizards, *Sceloporus undulatus*, have developed behavioral strategies to mitigate potentially lethal impacts of frequent attack by these predatory ants. We conducted staged encounters between fence lizards and fire ants in the field to assess the role that physiological stress levels and prior exposure to this invader plays in driving lizard escape behavior. This study reveals that both population-level exposure to fire ants, and the physiological stress response to ant attack, affect the behavioral response of lizards. Lizards from fire ant invaded areas are more likely to adaptively respond to fire ant attack than lizards from uninvaded sites. Lizards from both sites exhibit elevated levels of CORT following attack by fire ants, and by experimentally elevating lizard CORT levels we were able to trigger a survival-inducing behavioral response to fire ant attack. Elevated CORT levels can have important consequences for populations, including suppressing immune function, growth and reproduction. However, our study suggests that this physiological stress response to invaders may permit native species to survive initial invasion by promoting an adaptive behavior response to this novel threat. (Supported by NSF grant DEB-0949483 to TL).

S14-2.
USING REACTIVE SCOPE TO UNDERSTAND PHYSIOLOGICAL RESPONSES DURING STRESS
L. Michael Romero
Dept. of Biology, Tufts University, Medford, MA, U.S.A.

Even though the term "stress" is widely used, a precise definition is notoriously difficult. Notwithstanding this difficulty, stress continues to be an important concept in biology because it attempts to describe how animals cope with environmental change under emergency conditions. Without a precise definition, however, it becomes nearly impossible to make testable a priori predictions about how physiological and hormonal systems will respond to emergency conditions and what the ultimate impact on the animal will be. We recently proposed the Reactive Scope Model as an attempt to formulate testable predictions. Reactive Scope presumes that hormones and other physiological mediators exist in four distinct ranges. The predictive homeostasis range encompasses concentrations during normal daily and yearly activities and the reactive homeostasis range encompasses concentrations during acute emergency conditions. Together, these two ranges comprise the normal reactive scope of the animal. Concentrations below the reactive scope (homeostatic failure) are insufficient to sustain life and concentrations above the reactive scope (homeostatic overload) begin to create pathological problems typified by chronic stress. Using this model, it is possible to formulate predictions of both acute and chronic hormonal responses to stress.

S14-3.
THE ROLE OF CORTISOL IN TELEOSTS: STRESS ADAPTATION AND EARLY DEVELOPMENT
Dinushan Nesan and Matt M. Vijayan
Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada.

In teleosts, cortisol is the primary circulating corticosteroid and this hormone is produced by the steroidogenic cells (interrenal tissue) distributed mainly in the head kidney region. The release of corticosteroid in response to stress is well conserved across the vertebrate lineage and includes the activation of the hypothalamus-pituitary interrenal axis in fish. The plasma cortisol response to stressor exposures has been extensively studied in teleosts, and the magnitude and duration of this hormonal response is influenced by various factors, including type and intensity of the stressor, and it also appears to be species-specific. A major role for cortisol in stress adaptation is the mobilization of energy substrates to cope with the increased energy demand associated with stress in fish. However, relatively less is known about the mechanism of action of cortisol in the stress adaptation process in fish. Teleosts have multiple copies of corticosteroid receptors, including two glucocorticoid receptors (GRs), but the functional significance of these multiple receptors are currently unknown. Zebrafish (*Danio rerio*) is the only teleost to date with only a single GR gene in the genome. The temporal changes in zebrafish embryo cortisol content and GR mRNA levels during early development suggested a critical role for cortisol in developmental programming. Recently we showed that a functional cortisol stress axis was evident only after hatch in zebrafish suggesting that the early developmental events are driven by maternal cortisol. Using GR knockdown studies we have confirmed that maternal cortisol signaling is critical for early development and survival in zebrafish. Specifically, our results suggest that GR activation is essential for developmental regulation of the bone morphogenetic proteins in zebrafish. (Supported by Natural Sciences and Engineering Research Council of Canada Discovery grant to MMV).

OR14-1.

ADAPTIVE HORMONE-MEDIATED MATERNAL EFFECTS IN FREE-RANGING RED SQUIRRELS

B. Dantzer(1), R. Boonstra(2), S. Boutin(3), R. Palme(4), M. M. Humphries(5), A. G. McAdam(1,6)
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How organisms adapt to changing environments is a question that pervades all biological disciplines. The neuroendocrine system is highly sensitive to changes in the ecological or social environment and often responds by increasing or decreasing circulating levels of androgens or glucocorticoids. In mammals, changes in maternal hormone levels that are induced by the environment can generate variation in prenatal hormone exposure, which can have profound consequences on offspring phenotype. Red squirrels (*Tamiasciurus hudsonicus*) in the Canadian Yukon live in a highly variable environment. Fluctuations in population density driven by resource pulses generate density-dependent selection on offspring phenotype. Population density during pregnancy reflects the competitive environment offspring will encounter and is positively associated with the strength of directional selection on offspring growth rates. We conducted a multiyear study (2007-2010) to investigate whether the hormonal responses of breeding female squirrels to variation in population density can adaptively manipulate offspring phenotype. We examined relationships between maternal hormones and offspring growth rates across a gradient of population density and also during a large-scale experimental manipulation of perceived (acoustic) population density. We found that population density was positively correlated with fecal glucocorticoid (FCM) and androgen (FAM) metabolite concentrations in females during gestation and early lactation. As we had predicted, these changes in FCM and FAM significantly increased offspring growth rates. Finally, we found that experimentally increasing population density by adjusting the frequency of territorial vocalizations using long-term playbacks caused elevation of FCM and FAM in breeding females. This study suggests that the endocrine responses of female red squirrels to variation in population density influences offspring phenotype in a direction that may be adaptive. [This research was funded by the National Science Foundation (AGM) and Natural Sciences and Engineering Research Council of Canada (RB, SB, MMH, AGM).]

OR14-2.

***TAENIA CRASSICEPS* WFU CYSTICERCI SYNTHETIZE GLUCOCORTICOIDS IN VITRO: METIRAPONE REGULATES THE STEROID PRODUCTION**

Marta C. Romano(1), Ricardo A. Valdez(1), Lorena Hinojosa(1), Kaethe Willms(2).
(1) Dpto. Fisiología, Biofísica y Neurociencias, Cinvestav, Apdo. Postal 14-740, 07000, México D.F.; (2) Depto. de Microbiología y Parasitología, Facultad de Medicina, UNAM, México 04510, D.F.

We have demonstrated the ability of cysticerci to synthesize steroid hormones in vitro (Valdez et al, 2006; Jimenez, et al, 2006) and that *Taenia solium* (*T.s*) and *Taenia crassiceps* WFU (*T.c* WFU) tapeworms and cysticerci have a functional 3β-hydroxisteroid dehydrogenase (Fernández Presas et al, 2008), a key enzyme in the steroidogenic pathways in mammals and birds. We have recently shown that glucocorticoids (GCs) stimulate estrogen production in *T.c* WFU cysticerci. The aim of this work was to study the ability of *T. crassiceps* WFU cysticerci to synthesize glucocorticoids and to investigate if metirapone, an inhibitor of GC synthesis, regulates their production. *T. crassiceps* WFU cysticerci were obtained from the abdominal cavity of mice, washed and pre-incubated for 24 h in DMEM + antibiotics/antimycotics and tritiated progesterone (^{3}H-P4). Blanks containing the culture media plus ^{3}H-P4 were simultaneously incubated. After 24, 48 or 72h culture media were ether extracted and analyzed by thin layer chromatography (TLC) in two different solvent systems. Data were expressed as percent transformation of the tritiated precursor. Corticosterone production was also measured in the culture media by RIA. In some experiments Metirapone (0.1 mM to 0.5 mM) was added for 24 or 48h. Results showed that *T.c*. WFU cysticerci synthesized 3H-11-deoxy corticosterone (29.35±2.8 %) and small amounts of corticosterone (2.5± 0.9%) that was also found by RIA, as well as small amounts of 3H-11-deoxy cortisol (3.28±3.2 %). GCs synthesis was time dependent. The addition of metirapone significantly inhibited ^{3}H -11-deoxy corticosterone synthesis compared to controls. These results show for the first time that parasites synthesize GCs and that metirapone regulates the GC synthesis. Data suggest that 11-deoxy corticosterone is the main GC in cysticerci. (Partially supported by Conacyt grant # 69347.)
Acknowledgements: The authors thank IBT Viridiana Salvador and MVZ José A. Jiménez for technical assistance.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
3:40 – 5:30 p.m.
Hussey Room

NASCE 2011 Symposium 15:
Ectohormones, Environmental Chemicals, and their Perception

Chairpersons:
Nicholas Johnson, US Geological Survey
Jeremy McNeil, University of Western Ontario, Canada

S15-1.
PHEROMONE MEDIATED MATING IN MIGRATORY MOTHS
Jeremy N. McNeil
Department of Biology, The University of Western Ontario, London, ON, N6A 5B, Canada.

In response to predictable habitat decline (e.g. short days, low temperature) insects may enter diapause or undertake seasonable migration. We have been using the true armyworm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) as a model species to investigate the underlying physiology related to both sexual maturation (including pheromone production) and seasonal migration. In recent years we have been carrying out studies, comparing North American migratory populations with non-migratory ones from the Azores, a volcanic archipelago in the Atlantic Ocean. There are significant differences in morphology and reproductive output (females from the Azores being smaller but with a higher total fecundity). Furthermore, there are marked differences in the temporal patterns of sexual maturation, as well as the rates of JH biosynthesis and JH haemolymph titres, when reared under identical temperature and photoperiod conditions. The implications related to the costs associated with migration and reproductive success will be discussed.

S15-2.
IDENTITY, FUNCTION AND APPLICATION OF A MALE PHEROMONE IN SEA LAMPREY
N. S. Johnson(1), M. J. Siefkes (2), C. O. Brant(3) and W. Li(3)
(1) United States Geological Survey, Great Lakes Science Center, Hammond Bay Biological Station, Millersburg, MI, U.S.A.; (2) Great Lakes Fishery Commission, Ann Arbor, MI, U.S.A.; (3) Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, U.S.A.

Sexually mature male sea lampreys (*Petromyzon marinus*) release mating pheromones which coordinate reproduction with ovulated females during their terminal life stage. Male sea lampreys, after becoming spermiated, actively release a bile acid, 7α,12α,24-trihydroxy-5α-cholan-3-one-24-sulfate (3kPZS), across the gills through specialized glandular cells at a rate of 0.5 mg/fish/h. Ovulated female sea lampreys respond to synthesized 3kPZS at concentrations as low as 10^{-13}M by swimming directly upstream to the source. An analytical method to measure 3kPZS has been developed using ultra high performance LC/MS and can measure 3kPZS and other related bile acids in stream water at concentrations as low as 10^{-13} M. 3kPZS is being registered with U.S. Environmental Protection Agency as the first vertebrate pheromone for use in an invasive species control program. Recent research shows that the sea lamprey male mating pheromone has multiple functions and components and each component may function over different stream distances. Ongoing research objectives are to identify and characterize additional mating pheromone components and determine if 3kPZS is a conserved mating pheromone among *Petromyzonid* species.

S15-3.
CHEMICAL ECOLOGY OF SNAKES: FROM PHEROMONES TO RECEPTORS
Robert T. Mason(1) and Mimi Halpern(2)
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We study the evolution of chemical communication systems in vertebrates by examining both the diversity of chemical signals and the underlying physiological mechanisms mediating their production, expression, and reception. Reproduction in reptiles, snakes in particular, is dependent on the production and perception of sex pheromones. One of the few vertebrate pheromones isolated, characterized, and synthesized is the sex pheromone of the Red-sided Garter Snake, *Thamnophis sirtalis parietalis*. When males encounter a female expressing the pheromone, they exhibit stereotyped courtship behaviors including chin-rubbing and rapid tongue-flicks. The pheromone, a nonpolar, hydrophobic blend of 13 long-chain (C_{29}–C_{37}) saturated and monounsaturated methyl ketones, is insoluble in aqueous solutions. This pheromone is detected by the vomeronasal organ (VNO), which is specialized for the reception of nonvolatile chemical cues. Male garter snakes deprived of a functional vomeronasal (VN) system are unable to detect or respond appropriately to pheromones. But the mechanism by which the hydrophobic pheromone gains access to the aqueous environment of the VNO remained unknown. Results to date indicate that the Harderian glands' (HG) secretions, which duct exclusively into the VNO in snakes, contain pheromone-binding proteins. For over 300 years, the function of the cephalic HG of vertebrates has been the subject of speculation. Our studies in garter snakes demonstrate that the HG serves as a mediator in providing access for the female sex pheromone to the VNO of male garter snakes. In addition, feeding involves detection of prey chemoattractants by the VN system as well, and may require carrier molecules to deliver prey chemoattractant proteins to the VNO. (Supported by NSF grant 0620125 to RTM).

OR15-1.
CHANGES IN ODORANT RECEPTOR MESSENGER RNA EXPRESSION ASSOCIATED WITH MATURATION, REPRODUCTIVE HORMONES, AND HOMING IN SOCKEYE SALMON *(ONCORHYNCHUS NERKA)*
A. Dittman(1), D. May(2), L. Felli(2), and P. Swanson(1)
(1) Environmental Physiology Program, Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA 98112, U.S.A.; (2) School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105, U.S.A.

Seasonal migrations for reproduction are common in many mid-high latitude animals and for many species there are strong links between hormones, reproduction and migration. In addition, hormones associated with sexual maturation may modulate the sensory systems involved in migratory and reproductive behaviors. The homing migration of Pacific salmon from oceanic feeding grounds back to their river of origin to spawn provides an ideal model for studying the endocrine control of neuronal plasticity and sensory physiology involved in migration. The final stages of salmon homing migrations are governed by olfactory discrimination of homestream odors that juvenile salmon learn (imprint to) prior to their seaward migrations. One aspect of the imprinting and homing process involves long-term sensitization of the peripheral olfactory system to specific odorants associated with their natal stream. Concurrent with these homing migrations, salmon experience dramatic changes in hormones associated with gametogenesis and final maturation. In this study, we examined changes in odorant receptor (OR) mRNA expression in the olfactory epithelium of sockeye salmon exposed to specific odorants during sensitive periods for imprinting. We then examined the patterns of OR mRNA expression during final maturation, the period when salmon would be homing in the wild. Developmental increases in OR mRNA expression were correlated with surges in plasma luteinizing hormone (LH) and reproductive steroids that occurred just prior to final maturation. Differences in OR mRNA expression levels between imprinted and unimprinted fish were apparent prior to the surge in LH and final maturation and were consistent with increased sensitivity to imprinted odors during the period salmon would be homing to their natal streams. (Supported by the Bonneville Power Administration and the NWFSC).

OR15-2.
ODORANT RESPONSES DEPEND ON PHYSIOLOGICAL STATE IN AXOLOTLS *(AMBYSTOMA MEXICANUM)*
Nicholas W. Bellono and Heather L. Eisthen
Department of Zoology, Michigan State University, East Lansing, MI, U.S.A.

The terminal nerve is an anterior cranial nerve that contains gonadotropin releasing hormone (GnRH) and neuropeptide Y (NPY), peptides that play important roles in regulating reproduction and feeding. Our previous work suggests that responses to odorants in salamanders are modulated by both peptides in a context-dependent manner. We have now examined the effects of both peptides on responses evoked by odorants in 154 adult axolotls of both sexes in different reproductive and nutritional states. Specifically, we used electro-olfactogram (EOG) recordings to measure responses elicited by food odorants as well as those from male and female axolotls. As a control we used isoamyl acetate, an odorant with no biological significance to axolotls. For each recording we measured baseline odorant responses; then bathed the epithelium with 1 μM NPY, 10 μM GnRH, or Ringer's solution (control) for 20 min; then washed off the peptide and recorded responses for another 40 min. In Ringer's, we found that responses to different odorants change in diverse ways over the course of the recording, suggesting that endogenous modulation of odorant responses may be occurring in our preparation. In addition, the effects of GnRH and NPY vary with the animal's sex as well as its reproductive and nutritional state in a way that depends on the behavioral significance of the odorant stimulus. For example, the effects of GnRH on responses evoked by female odorants depend on the animal's sex, nutritional state, and reproductive state; in contrast, the effects of NPY depend only on the animal's nutritional state. Responses to food odorants are modulated very differently: GnRH has no effect, and the effects of NPY depend on the animal's sex and nutritional state. These studies contribute to understanding the ways in which the vertebrate brain regulates incoming sensory activity to emphasize stimuli that are most relevant to the animal's current physiological state and behavior. (Supported by funding from US National Science Foundation (IOS 0817785), Michigan State University College of Natural Science, and the Marine Biological Laboratory.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Friday, July 15th
3:40 – 5:30 p.m.
Vandenberg Room

NASCE 2011 Symposium 16:
Advances in Comparative Neuroendocrinology

Chairpersons:
Angela Lange, University of Toronto, Mississauga, Canada
Vance Trudeau, University of Ottawa, Canada

S16-1.
PEPTIDERGIC SIGNALING CASCADES IN THE REGULATION OF INSECT ECDYSIS
M. E. Adams(1), D. Kim(1), Y. J. Kim(1), and D. Zitnan(2)
(1) Department of Entomology, University of California, Riverside, CA, U.S.A.; (2) Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia.

Molting and ecdysis are among the most distinctive and characteristic features of arthropod physiology and are vital for growth and metamorphosis from juvenile to the winged, reproductive adult. These events are by precisely timed release of steroid and peptide hormones. We are interested in how hormones initiate and schedule the ecdysis sequence, a series of innate behaviors that allows escape from the old cuticle to terminate the molt. These behaviors are initiated and scheduled by ecdysis triggering hormones (ETHs) that regulate neuropeptide signaling cascades in the central nervous system (CNS). Ecdysteroids signal onset of the molt and program expression of genes encoding ecdysis signaling molecules. In moths (*Bombyx mori*, *Manduca sexta*) and flies (*Drosophila melanogaster*), we have identified primary neuronal targets of ETH in the CNS by detecting expression of ETH receptor (ETHR) transcripts. We monitor activities of ETHR neurons by electrophysiology and calcium imaging. ETHRs are expressed predominantly in "peptidergic ensembles", groups of central neurons that release FMRFamides, eclosion hormone, kinins, CCAP, MIPs, and bursicon into the CNS to schedule activation of central pattern generators that drive pre-ecdysis, ecdysis, and post-ecdysis. We are examining the roles played by these neurons in behavioral scheduling using genetics and physiological manipulations. Our findings offer insights into how neural substrates for behaviors are assembled and regulated. (Supported by NIH grant GM 067310)

S16-2.
TENEURIN C-TERMINAL ASSOCIATED PEPTIDES (TCAP): NEW PEPTIDES INVOLVED IN THE NEURAL REGULATION OF CORTICOTROPIN-RELEASING FACTOR (CRF)
D. A. Lovejoy(1), D. Chand(1), L. A. Tan(1), R. De Almeida(1), T. G. Nock(1), M. Xu(1), T. Ng(1), C. Yeung(1), L. Song(1), and D. Barsyte-Lovejoy(2)
(1) Dept. of Cell and Systems Biology, University of Toronto, Toronto ON, Canada; (2) Structural Genomics Consortium, University of Toronto, Toronto ON, Canada.

The existence of the teneurin C-terminal associated peptides (TCAP) was reported in 2004 after identification in a rainbow trout hypothalamic cDNA library. The peptide was named on the basis of a close structural association with the teneurin transmembrane protein gene. The teneurin-TCAP system is present in all major bilateral metazoan taxa, although in some lineages an independent TCAP system has been lost. In vertebrates, there are four teneurin genes, each of which possesses a unique, but highly conserved TCAP sequence. TCAPs 1 and 3 can be either transcribed independently or as part of the full-length teneurin sequence. TCAPs 2 and 4 appear are transcribed as part of the full- length teneurin where they may be cleaved from the teneurin proprotein or remain attached where they may act as a tethered peptide. Loss or knockdown of the teneurin-TCAP gene results in lethality or a profound decrease in cell viability. Exposed or liberated TCAP in the extracellular regions interacts with the dystroglycan complex which induces a MEK-ERK1/2-dependent pathway to regulate cytoskeletal reorganization leading to changes in process formation such as axonal growth, dendritic arborization and spine formation of the latter. In addition, a BAD-p90rsk pathway is activated which may confer neuroprotective elements upon the neurons. After TCAP stimulus, the neuron undergoes a set of dynamic metabolic changes impacting on a number of cell systems, including elements of the hypothalamic-pituitary-adrenal axis hormones. In vivo, these changes manifest as a selective inhibition of CRF-induced cfos labelling in regions of the limbic system and regulation of CRF-induced behaviors associated with anxiety, depression, exploration, social interaction and cocaine reinstatement in rats. We theorize that the teneurin-TCAP system evolved early in metazoan evolution and plays a fundamental role in the regulation and integration of sensory-motor processing in the central nervous system. (Supported by grants from NSERC and Protagenic Therapeutics Inc. to DAL).

S16-3.
QUANTITATIVE PROTEOMICS FOR FISH NEUROENDOCRINOLOGY AND NEUROTOXICOLOGY
C. J. Martyniuk(1), S. Alvarez(2), B. Chown(1), J. T. Popesku(3), V. L. Trudeau(4), and N. D. Denslow(5)
(1) Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, NB, Canada; (2) Donald Danforth Plant Science Center, St Louis, MO, U.S.A.; (3) Child & Family Research Institute, University of British Columbia, Vancouver, BC, Canada; (4) Department of Biology, University of Ottawa, Ottawa, ON, Canada; (5) Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, U.S.A.

Neuroendocrine transcriptomics has played a role in characterizing cell signaling cascades underlying fish physiology and toxicology. To complement neurogenomics, neuroproteomics approaches in teleost fishes promise to offer new insight into the study of molecular cascades that are mediated by neurotransmitter and nuclear receptor agonists/antagonists. The number of proteins quantified in recent studies using proteomics in fish neuroendocrine regions (hypothalamus, Hyp and telencephalon, TEL) ranged from 75 in fathead minnow (FHM) to 225 in goldfish. Gene ontology reveals that the majority of proteins identified in neuroendocrine regions have biological functions in transport, metabolism, and glycolysis. Proteins include those with a role in synaptic transmission and receptor activity. A significant challenge to proteomics in fish is the lack of well annotated fish databases. The NCBI nr database contains a limited number of protein sequences for teleost fish, ranging from a few hundred for FHM to tens of thousands for zebrafish. However, the number has increased approximately 2-20 times for different teleosts in the last three years, due to the completion of genomics projects for some species (i.e. zebrafish and salmon). The increase in information has resulted in an increase of 11% for proteins identified in the FHM TEL when the raw data was analyzed two years later. In addition to database improvements, the use of hybrid LTQ-FT and Orbitap mass spectrometers with high resolution are able to further increase protein identification rates in fish. Lastly, fish neuroendocrine studies using both high-throughput genomics and proteomics must consider

that gene and protein correlations are poor for single time point experiments, highlighting the need for time course analyses. This is important if both genes and proteins are to be studied for hormone signaling pathways or used as bioindicators of adverse effects in the CNS after exposures to neuroactive toxicants.

OR16-1.
NEW INSIGHTS INTO THE PITUITARY GH/IGF-I SYSTEM IN ONTOGENY AND PHYLOGENY
Elisabeth Eppler
Research Group Neuro-Endocrine-Immune Interactions, Institute of Anatomy, University of Zürich, Zürich, Switzerland.

IGF-I is a major hormonal regulator of differentiation, growth, proliferation and development. Liver is the principal source of endocrine IGF-I. The main stimulus for its synthesis and release is growth hormone (GH) from the anterior pituitary. IGF-I specifically inhibits GH gene transcription and secretion via a negative feedback loop. Throughout phylogeny, IGF-I is also produced in extrahepatic sites, such as in pituitary, the central endocrine organ involved in the regulation of virtually all physiological processes in mammals like in fish. Bony fish adenohypophysis preserves its embryonic compartmentalization lifelong, i.e. the endocrine cell types are located in distinct regions. Thus, fish pituitary is an excellent tool for morphological investigations. The distribution patterns of IGF-I mRNA and/or peptide in endocrine cell subtypes are similar in lower and higher vertebrates, suggesting that intrapituitary IGF-I is highly conserved and has important physiological roles. The major task of IGF-I is to prevent apoptosis and promote cell proliferation. Therefore, IGF-I released from endocrine adenohypophyseal cells may have protective and proliferative autocrine and/or paracrine effects. This idea is supported by the presence of type1 IGF receptors at all endocrine subpopulations of the rat and the pronounced presence of IGF-I in ACTH cells of bony fish and mammals. ACTH cells are probably stressed by pro-apoptotic cytokines and hormones which demands for IGF-I. The transiently increased expression of IGF-I in gonadotrophs during puberty and in subordinate tilapia males suggests an impact on sexual differentiation and maturation. Pronounced inter-individual differences of the IGF-I content in GH cells may indicate that synthesis and release of IGF-I from GH cells depend on the physiological status and the serum IGF-I level and constitute an additional intrapituitary feed-back loop. Further studies are required to verify these morphofunctional observations. [This study was supported by Swiss National Science Foundation (Project Nos. 4050-66580; 111028, 118165) and the Hartmann Müller-Foundation for Medical Research (Grants 972, 1115).]

OR16-2.
THE REGULATION OF INSECT CARDIAC ACTIVITY AND A FRANK-STARLING-LIKE MECHANISM
Rosa da Silva(1), Sara R. da Silva(2) and Angela B. Lange(1)
(1) Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada; (2) Department of Chemical and Physical Sciences, University of Toronto Mississauga, Mississauga, ON, Canada.

Vertebrate and invertebrate cardiovascular systems are under myogenic and neural control through the action of neurotransmitters, neuromodulators and neurohormones. The excitatory neuropeptide, crustacean cardioactive peptide (CCAP), first isolated from the pericardial organs of the shore crab, *Carcinus maenas*, is present in the innervation to the heart in many insects, and can be released from neurohemal sites in arthropods. CCAP triggers an increase in heart rate in the Vietnamese stick insect *Baculum extradentatum*, leading to an increase in cardiac output. However, in the African migratory locust, *Locusta migratoria*, CCAP significantly increases stroke volume and cardiac output without modifying heart rate or aortic contraction frequency. In the locust, CCAP increases the volume of hemolymph in the dorsal vessel by the synchronous closing of the excurrent ostia (small openings or valves that allow hemolymph to exit the dorsal vessel directly into the perivisceral sinus), resulting in more forceful heart contractions and increased stroke volume and cardiac output. This is achieved without modifying heart rate through a physiological mechanism analogous to the Frank–Starling mechanism in the vertebrates. The absence of CCAP-like immunoreactive staining on the segmental vessels (short tubes that connect the excurrent ostia to the dorsal vessel) and excurent ostia in *L. migratoria* indicates that CCAP may be acting as a neurohormonal regulator of cardiac activity. Therefore, CCAP differentially alters the contractile activity of the cardiac tissue in both *B. extradentatum* and *L. migratoria*, with each mechanism allowing for circulatory changes in hemolymph flow throughout the insect. (This work was supported by the Natural Sciences and Engineering Research Council of Canada.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Saturday, July 16th
10:20 – 12:10 p.m.
Mendelssohn Theater

NASCE 2011 Symposium 17:
Hormones and Behavior

Chairpersons:
Rosemary Knapp, University of Oklahoma, USA
Juli Wade, Michigan State University, USA

S17-1.
HORMONE-INDUCED ADULT NEUROPLASTICITY AND THE ACTIVATION OF BEHAVIOR IN BIRDS
Gregory F. Ball
Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, 21218, U.S.A.

Steroid hormones bind to their cognate receptors and act as transcription factors in the brain to facilitate changes in behavior. These hormonal effects on behavior are often associated marked changes in the brain, even in adult animals. I will review in this presentation work on steroid-dependent plasticity in birds based on two cases: the medial preoptic nucleus (POM) of Japanese quail in relation to the control of male sexual behavior and the song nucleus HVC in canaries that regulates aspects of song behavior. In male quail, POM volume changes in volume seasonally (larger under breeding conditions than non-breeding conditions. In castrated subjects testosterone can almost double POM volume within two weeks. Significant volume increases are, however, already observable after one day of treatment. The Steroid Receptor Coactivator-1 is part of the mechanism mediating these effects. Increases in POM volume reflect changes in cell size or spacing and dendritic branching but are not associated with an increase in neuron number. In contrast, seasonal changes in HVC volume reflect incorporation of newborn neurons in addition to changes in cell size and spacing in neurons and glia. HVC volume is larger when birds are sampled in pre-breeding or breeding conditions than in those who are not breeding. These types of changes can be induced by treatments with exogenous testosterone or its estrogenic and androgenic metabolites. Expression of doublecortin, a microtubule-associated protein that is a marker of new neurons, is increased by testosterone in HVC but not in the adjacent nidopallium suggesting that neuron production in the subventricular zone, the birthplace of newborn neurons, is not affected. Testosterone acting through both androgenic and estrogenic metabolites promotes the recruitment of new neurons to HVC. Together these data illustrate that hormone effects on behavior can be mediated by distinct action of steroids at the cellular level. (Supported by NIH/NINDS R01 NS35467 and NIH/NIMH R01 MH50388.)

S17-2.
EFFECTS OF THE ENVIRONMENT ON ESTROGEN-DEPENDENT MECHANISMS OF AGGRESSIVE BEHAVIOR
Brian C. Trainor
Department of Psychology, University of California, Davis, CA 95616, U.S.A.

The effects of hormones on behavior are often context specific. For example, a surge in progesterone will only stimulate female reproductive behavior in many rodents if it is preceded by surge in estradiol. More rare, are cases in which effects of hormones on behavior can be reversed by context. We have observed that differences in photoperiod, which predicts seasonal variation in environmental conditions in nature, can reverse the effects of estrogens on male aggressive behavior. In two species of *Peromyscus*, inhibition of estrogen synthesis (with the aromatase inhibitor fadrozole) was found to increase male aggressive behavior under long days. In contrast, under short days estrogens act rapidly to increase aggressive behavior. In the course of following up on these observations in California mice (*P. californicus*) we inadvertently discovered that husbandry conditions (the bedding in the cages) can have a similar effect on estrogen-dependent aggression. Experiments using two common forms of bedding (carefresh and corncob) show that fadrozole has essentially opposite effects on aggression depending on which bedding is used. These data show that the effects of estrogens on complex social behavior are flexible and are affected by many environmental variables. (Supported by NIH R01 MH085069.)

S17-3.
CROSS-TALK BETWEEN ENDOCRINE STRESS AND REPRODUCTIVE AXES IN A LIVEBEARING FISH
Rosemary Knapp(1) and Edie Marsh-Matthews(1)(2)
(1) Department of Zoology, University of Oklahoma, Norman, OK, U.S.A.; (2) Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK, U.S.A.

Across vertebrates, glucocorticoids and sex steroids influence behavior and responses to environmental stimuli and stressors, especially with respect to reproductive behavior and function. Although glucocorticoids often inhibit the production of sex steroids and reproductive behavior, the exact nature of the interplay between these hormones can be quite complex and can differ between the sexes. As an example, we will discuss our recent, unexpected finding that exposure of sexually mature female mosquitofish (*Gambusia affinis*) to the stress hormone cortisol induced morphological and behavioral masculinization (Knapp, Marsh-Matthews, Vo and Rosencrans. 2011. *Biology Letters* 7:150-152). Cortisol masculinized the sexually dimorphic anal fin, a known androgen target tissue, in a dose-dependent manner. Some cortisol-treated females attempted copulations with stimulus females with behaviors similar to those exhibited by normal males. Males also responded differentially to cortisol-treated vs. control females, and some masculinized females even attempted copulations with males. In addition to the effects on copulatory behavior and morphology, females exposed to the higher cortisol doses also had lower somatic lipid content and more late stage embryos showing arrested development and decomposition. Numerous females also showed evidence of early stage brood reduction via the presence of yolkless embryo remnants, which suggest that females are able to reclaim, and presumably re-distribute, nutrients to the remaining brood under stress or other conditions that affect resources available for post-fertilization provisioning. Our findings thus provide support for a key assumption of the Trexler-DeAngelis model for the evolution of matrotrophy (post-fertilization nutrient transfer). As time permits, we will discuss potential mechanisms by which cortisol might masculinize behavior and morphology and implications of our findings for studies of endocrine disruptors. (Financial support for the research described here came from various University of Oklahoma funding sources.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

OR17-1.
ENDOCANNABINOIDS REGULATE VASOTOCIN SIGNALING USING A NOVEL NEUROMODULATORY MECHANISM
Erin McEvoy(1), Sarah Sonnenfeld(1), Kurt Dolence(2), and Emma J. Coddington(1)(3)
(1) Department of Biology, Willamette University, 900 State St., Salem, OR, 97301, U.S.A.; (2) Department of Pharmacology, University of Wyoming, Laramie, WY, 83071, U.S.A.; (3) Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA, 02543, U.S.A.

Cannabinoids play many different roles in the brain and can affect a wide range of behaviors. The connection between the behavioral effects and the cellular mechanisms used by cannabinoids remains a mystery in most cases. This study used *Taricha granulosa* (roughskin newt) to investigate the cellular mechanism by which cannabinoids suppress the effects of a behavior-enhancing hormone, vasotocin (VT). Previous studies using *Taricha* demonstrate that the typical clasp-enhancing effects of VT are blocked by cannabinoid agonists. We hypothesized that cannabinoids suppress behavioral effects of VT by blocking VT signaling in the clasp-controlling neurons of the hindbrain. Other researchers using cell culture have shown that complete signaling by the mammalian homologue vasopressin requires endocytosis and internalization of vesicles containing vasopressin and its V1a receptors. Thus, we predicted that by imaging VT tagged to a fluorescent probe, Oregon green (OG), we could visualize the effects of cannabinoids on VT internalization. Animals were cannulated and received a high (100ng/µl), medium (5ng/µl), low (100pg/µl), or control dose of the cannabinoid agonist CP 55,940, followed by VT-OG (40ng/µl). Brains were removed 30 min post-VT-OG, then fixed, frozen, sliced, and imaged on a confocal microscope. Each image was analyzed for sum intensity and area percent to quantify the amount of VT internalized in the experimental (rostral reticular formation, Rf) and control (inferior reticular formation, Ri) brain regions. Our results showed that the cannabinoid agonist suppressed VT internalization in a dose dependent manner in the Rf and Ri regions. This is the first evidence showing that cannabinoids can block hormone action by interfering with hormone-receptor signaling. (Supported by NSF IOS-0817785 grant to EJC; Rodgers Family Student Collaborative Research Program, Willamette University to EM and SS; Marine Biological Laboratory to EJC).

OR17-2.
ANDROGEN RESPONSIVENESS TO PARENTAL BEHAVIOR: NURTURANT CONTEXTS MODULATE EFFECTS OF INFANT CUES ON HUMAN MALE TESTOSTERONE
Sari M. van Anders(1)(2), Richard M. Tolman(3), and Brenda L. Volling(1)
(1) Department of Psychology, University of Michigan, Ann Arbor, MI, U.S.A; (2) Department of Women's Studies, University of Michigan, Ann Arbor, MI, U.S.A.; (3) School of Social Work, University of Michigan, Ann Arbor, MI, U.S.A.

Testosterone (T) is theorized to be one of the proximate mechanisms driving life history trade-offs, with parenting/low T on one side, and challenges/high T on the other. Paradoxically, some infant-related behavioral contexts have been tied to higher T, e.g. in humans and fish. We hypothesized that nurturant responses to infants would decrease T, whereas cues signaling the need for infant defense - and not accompanied by a nurturant response - would increase T. Similar to non-human animal studies using fake conspecifics, we tested the effects of three conditions on T responses in men: an interactive infant doll that cried and was calmed via nurturant responses, the same infant cries presented via sound, and a control condition. We showed that infant cues do decrease T in men, but only when coupled with nurturant behavioral responses; whereas cues presented with no possibility of nurturant response increase T. These findings underscore that 'parental' or 'infant' behavioral contexts are not phenomenologically whole, and instead encompass behaviors linked to both low T (via nurturance) or high T (via infant defense). Moreover, this research highlights the importance of considering context and the potential for behavioral response, in lab studies of androgen responsivity.

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Saturday, July 16th
10:20 – 12:10 p.m.
Vandenberg Room

NASCE 2011 Symposium 18:
Feeding and Metabolism

Chairpersons:
J. Sook Chung, University of Maryland, USA
Suraj Unniappan, York University, Canada

S18-1.
NEUROPEPTIDES AND AMINO ACID SENSING COORDINATELY REGULATE BLOOD DIGESTION AND REPRODUCTION IN THE MOSQUITO *AEDES AEGYPTI*
Mark R. Brown, Monika Gulia-Nuss, Anne E Robertson, and Michael R Strand
Department of Entomology, University of Georgia, Athens, GA, 30602, U.S.A.

Reproduction in mosquitoes encompasses a highly regulated sequence of behavioral, metabolic, and synthetic processes that result in the production of eggs. Prior studies implicate both hormonal and nutritional cues in regulation of reproduction. Ingestion of a blood meal triggers the release of insulin-like peptides (ILPs) and ovary ecdysteroidogenic hormone (OEH) from the brain neurosecretory cells, which in turn stimulate ovaries to produce ecdysteroid hormones (ECD). Amino acids released from the blood meal by the action of gut proteases activate the target of rapamycin (TOR) pathway in the fat body, and together with ECD drive vitellogenin (Vg) expression and ultimately egg production. Knockdown of the insulin receptor by RNA interference delayed gut serine protease expression thus egg maturation in blood-fed females, as did knockdown of TOR or treatment with rapamycin. Decapitation of females immediately after a blood meal also reduces gut protease activity and prevents egg maturation, but both ILP3 and OEH directly stimulate the expression of gut proteases and Vg in these females. Thus both neuropeptides synchronize digestion and amino acid availability with ovarian ECD production to maximize Vg expression by the fat body and ultimately egg maturation. The question of whether these neuropeptides activate redundant or specific signaling pathways has yet to be answered. (Research supported by NIH AI33108 to MRB and MRS).

S18-2.
COMPARATIVE ASPECTS OF THE ENDOCRINE REGULATION OF FEEDING IN FISH MODELS
Hélène Volkoff
Department of Biology, Memorial University of Newfoundland, St. John's, NL, Canada.

Feeding is regulated by several key appetite- stimulating (orexigenic) and appetite-inhibiting (anorexigenic) factors produced by both brain and peripheral tissues. Most fish homologs of mammalian appetite-regulating hormones appear to have similar appetite-regulating effects in fish as in mammals. Among these are the orexigenic factors neuropeptide Y (NPY) and orexin and the anorexigenic factors cocaine- and amphetamine-regulated transcript (CART), cholecystokinin (CCK) and amylin. The appetite-regulating role of hormones in fish can be assessed directly by examining feeding behavior of individual hormone-injected fish. Also, changes in appetite usually occur through modulations of the gene expression and the action of feeding-regulating hormones. Consequently, their appetite-regulating role can also be assessed indirectly by quantifying their gene expression in fish submitted to challenges that are known to modulate feeding in fish, such as nutritional (e.g. fasting vs. feeding) and reproductive (e.g. spawning vs. non-spawning) status as well as exogenous factors, such as environmental factors (e.g. temperature and photoperiod). Our current understanding of the regulation of feeding in fish is improving but is still based on studies involving only a few fish species. Fishes display a high level of diversity with regards to ecology and habitat (e.g. marine vs. freshwater and from tropical vs. cold-water) as well as feeding habits (omnivore vs. carnivore), which translate into differences in gastrointestinal tract morphology and gut hormone profiles. This diversity suggests that the endocrine control of feeding in fish might occur through molecules and mechanisms that are species-specific. This presentation will give a brief overview of the endocrine regulation of feeding in some fish with special focus on temperate freshwater (goldfish) as well as cold water marine (cod, flounder, skate) species (Supported by NSERC and CFI grants to HV).

S18-3.
RESOLVING THE GROWTH-PROMOTING AND LIPID-CATABOLIC ACTIONS OF GROWTH HORMONE
Mark A. Sheridan
Department of Biological Sciences, North Dakota State University, Fargo, ND, U.S.A.

Despite knowledge that growth hormone (GH) regulates numerous growth, metabolic, and other processes in vertebrates, the mechanistic basis of these manifold actions remain. We used teleost fish to elucidate the mechanisms by which GH regulates the divergent processes of growth promotion (anabolic) and mobilization of stored lipids (catabolic). Fish are advantageous for this study as their growth-promoting (e.g., IGF-1 synthesis and secretion) and lipid storage/lipolysis processes occur within the same cell type (e.g., hepatocytes). Moreover, fish have multiple genes that encode isoforms of GH receptor (GHR), providing an unique opportunity to examine subtype-specific linkages. Our findings to date indicate that a specific response to GH is determined by GHR characteristics and GHR-effector pathway linkages. The two GHRs of rainbow trout (GHR1 and GHR2) display overlapping and distinct ligand features. Although GH activates several signaling elements, GHR1 and GHR2 differentially activate signaling pathways. Reprogramming of GHR binding/ localization and/or effector pathways to which GHRs link can alter GH responsiveness. Ultimately, the action of GH depends on the distribution and abundance of GHR subtypes as well as on the signal pathways expressed in target cells under given developmental/physiological conditions to which the receptors link. (Supported by NSF grant 0920116).

OR18-1.
ONTOGENY OF LEPTIN SIGNALING IN HYPOTHALAMIC FEEDING CONTROL CENTERS IN THE FROG *XENOPUS LAEVIS*
Melissa Cui, Caroline Hu, Chris Pelletier and Robert J. Denver
Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, U.S.A.

The hormone leptin is well known to act on hypothalamic feeding control centers to reduce food intake in adults, but little is known about leptin's anorectic action during postembryonic development in any species. Using tadpoles of the frog *Xenopus laevis* we investigated the ontogeny of leptin mRNA expression and leptin actions on feeding, and correlated this with the expression of leptin receptor (LepR) and key hypothalamic feeding control genes. Analysis of gut contents throughout metamorphosis showed that tadpoles ceased feeding at metamorphic climax. Leptin mRNA levels peaked at this stage in the body region containing the fat pads. Intracerebroventricular (i.c.v.) injection of recombinant frog leptin (rxLeptin; 20 ng/g body weight) potently inhibited feeding in late prometamorphic, but not premetamorphic, tadpoles. LepR mRNA was expressed at a low level in premetamorphic tadpole preoptic area/hypothalamus; expression increased throughout metamorphosis and peaked in the metamorphic frog. Similar ontogenetic increases were seen for feeding control genes: proopiomelanocortin, corticotropin-releasing factor, neuropeptide Y, adenosine monophosphate activated protein kinase, and suppressor of cytokine signaling 3 (SOCS3). The increase in LepR mRNA correlated with functional LepR signaling as analyzed by immunohistochemistry for phosphorylated signal transducer and activator of transcription 3 (pSTAT3) and RTqPCR for SOCS3, a leptin-induced STAT3 target gene. Injection of rxLeptin i.c.v. increased pSTAT3 immunoreactivity (ir) in the parvocellular anterior preoptic area, ventral hypothalamic area, and anterior pituitary, with maximal pSTAT3-ir induced at metamorphic climax. rxLeptin increased SOCS3 mRNA in prometamorphic, but not premetamorphic tadpoles. Our findings show that the anorectic action of leptin develops in the tadpole during metamorphosis, and this correlates with upregulation of LepR and key hypothalamic feeding control genes. (Supported by NSF grant IOS 0641587 to RJD).

OR18-2.
NESFATIN-1: A NOVEL METABOLIC HORMONE IN FISH AND RODENTS
R. Gonzalez(1), R. L. S. Perry(2), B. K. Reingold(1), X. Gao(1), M.P. Gaidhu(2), B. Kerbel(1), R. G. Tsushima(1), R. B. Ceddia(2), and S. Unniappan(1)
Laboratory of Integrative Neuroendocrinology, (1) Department of Biology and (2)School of Kinesiology and Health Sciences, York University, Toronto, Ontario, Canada.

Nesfatin-1, a novel hormone, has recently generated great interest as a potential central and peripheral regulator of energy homeostasis in mammals. Nesfatin-1 or NEFA/nucleobindin 2-Encoded Satiety- and FAT-Influencing proteiN-1, is the N-terminal fragment of nucleobindin 2 (NUCB2). To date, multiple studies have demonstrated the inhibitory effect of nesfatin-1 on food intake and body weight providing clear evidence to support an anorectic role for nesfatin-1 in mammals. We recently reported, for the first time in a non-mammalian vertebrate, NUCB2 and nesfatin-1 biology in goldfish. NUCB2 is highly conserved among tetrapods and teleost and abundant expression of nesfatin-1-like immunoreactivity was found in the hypothalamus and gut. In addition, intraperitoneal and intracerebroventricular injections of synthetic goldfish nesfatin-1 inhibit food intake in goldfish. In rats, nesfatin-1 alters whole-body energy homeostasis by inhibiting feeding and stimulating fat oxidation. Nesfatin-1-like immunoreactivity is found in the β-cells of the rat and mouse pancreatic islets and nesfatin-1 has a glucose-dependent insulinotropic role *in vivo*. In agreement with our *in vivo* results, nesfatin-1 enhanced glucose-stimulated insulin secretion from isolated rat and mouse islets at high (16.7 mM), but not low (2 mM) glucose concentrations. Furthermore, we determined that nesfatin-1 stimulates insulin-mediated glucose uptake in isolated primary adipocytes of rats. Collectively, our novel data indicate that nesfatin-1 is a new hormone with multiple neuroendocrine and metabolic effects in vertebrates. (This work was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada to SU. SU is a Canadian Institutes of Health Research (CIHR) New Investigator and recipient of the Early Researcher Award from the Ontario Ministry of Research and Innovation.)

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Saturday, July 16th
10:20 – 12:10 p.m.
Hussey Room

NASCE 2011 Symposium 19:
Regulatory Pathways Controlling Gonadal Development and Gamete Maturation

Chairpersons:
Alex Raikhel, University of California, Riverside, USA
Glen Van Der Kraak, University of Guelph, Canada

S19-1.
INTRAFOLLICULAR COMMUNICATION NETWORK IN THE ZEBRAFISH OVARY – WHAT HAVE WE LEARNED FROM THE ZEBRAFISH MODEL?
Wei Ge
School of Life Sciences and Centre for Cell and Developmental Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

In the ovary there exists an intrinsic communication network within the follicle consisting of various growth factors. In the zebrafish, we have characterized several important growth factor families within the follicle, including epidermal growth factor (EGF), activin-inhibin-follistatin, Kit/Kit ligand, and GDF9/BMP families. These factors are likely involved in bidirectional communication between the oocyte and follicle cells. As a potential mediator of gonadotropin actions, activin subunits (*inhbaa* and *inhbb*) are exclusively expressed in the follicle cells whereas its receptors are abundantly expressed in the oocyte. In contrast, EGF and BMP families are mostly expressed in the oocyte, but their receptors (EGFR and BMPRII) are exclusively located in the follicle cells. The Kit/Kit ligand system is unique in that the system consists of two ligands (*kitlga* and *kitlgb*) and two receptors (*kita* and *kitb*). The distribution of Kits/Kit ligands in the follicle and the evidence for receptor specificity suggest that Kitlga-Kita and Kitlgb-Kitb may represent two paracrine regulatory pathways within the follicle that mediate the reciprocal communications between the two compartments. Our functional studies have demonstrated extensive interactions among these signaling pathways in the follicle. We have recently characterized inhibin, a natural activin antagonist, in the zebrafish ovary. Similar to activin subunits, inhibin alpha subunit (*inha*) is exclusively expressed in the follicle cells and its expression level surges at the full-grown stage prior to oocyte maturation, which has led us to hypothesize that the preovulatory surge of inhibin production may serve as an ovarian messenger to signal the pituitary for final maturation, and inhibin may act by antagonizing the effects of activin on FSH and LH biosynthesis in the pituitary. The expression of *inha* is likely subject to the regulation by oocyte-derived factors such as BMPs (supported by RGC grants to WG).

S19-2.
REGULATORY PATHWAYS CONTROLLING MOSQUITO REPRODUCTION
Alexander S. Raikhel
Department of Entomology and Institute of Integrative Genome Biology, University of California Riverside, Riverside California, U.S.A.

Hematophagous arthropods, such as mosquitoes, require vertebrate blood for their egg development with each gonadotrophic cycle being tightly coupled to a separate blood meal. As a consequence, mosquitoes are vectors of numerous disease pathogens of human and domestic animals, most importantly malaria, Dengue fever and West Nile virus. Thus, understanding of how cyclic egg development is controlled is of paramount importance for devising novel approaches for mosquito control. My research has been focusing on deciphering the molecular basis of regulatory pathways controlling mosquito egg development. The mosquito *Aedes aegypti* is being used for these studies due to its exceptional features as a vector model organism. I will discuss our research of four key aspects of cyclic egg production in this mosquito: our recent advances in understanding how juvenile hormone controls the pre-blood meal, preparatory phase; how the nutritional Target-of-Rapamycin kinase cascade mediates amino acid signaling after blood ingestion, permitting egg development to proceed; and how a master regulator of mosquito egg development, a steroid hormone 20-hydroxyecdysone, orchestrates vitellogenic and postvitellogenic events. Finally, we have recently discovered that small noncoding RNAs, called miRNAs, are involved in regulation of blood digestion and egg maturation in the mosquito. In particular, miR-275 is indispensible for these processes, and its specific depletion results in dramatic phenotypes disrupting major functions associated with utilization of blood digestion and egg maturation. (Support by NIH grants R37 AI244716 and RO1 AI59492.)

S19-3.
SEX, SURVIVAL, AND HEDGEHOG: A STORY OF HOW MOUSE EMBRYOS MAKE THEIR TESTES AND ADRENAL GLANDS
Humphrey Yao(1), Ivraym Barsoum(2), and Jeff Chen-Che Huang(3)
(1) Reproductive Developmental Biology Group, Laboratory of Reproduction and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC, U.S.A.; (2) Department of Cell & Developmental Biology, University of Illinois, Urbana, U.S.A.; (3) Department of Comparative Biosciences, University of Illinois, Urbana, U.S.A.

Adrenals and gonads are steroidogenic organs derived from a common primordium that consists of Steroidogenic factor 1 (SF1)-positive precursor cells. SF1 not only defines the steroidogenic lineages in these organs, but also controls their differentiation. We have found that the Hedgehog (Hh) signaling pathway serves as a downstream regulator of SF1 in the appearance of steroidogenic cells in these organs. In the fetal testis, activation of the Hh pathway is necessary and sufficient for the specification of fetal Leydig cell lineage. On the other hand in the fetal adrenal, the Hh pathway plays key roles in controlling the organ size by stimulating the expansion of progenitor cells in the adrenal capsule. This evidence demonstrates that the Hh pathway serves as a common crosstalk component and yet evolves diverse functions in the expansion and differentiation of the steroidogenic cells in a tissue-specific manner. (Supported by NIH-HD059961 and the Intramural Research Program at NIEHS).

OR19-1.
REGULATORY MECHANISM IN STARFISH REPRODUCTION BY A RELAXIN-LIKE GONAD-STIMULATING SUBSTANCE (GSS)
Masatoshi Mita(1), Kazutoshi Yamamoto(2), Masaru Nakamura(3) and Yoshitaka Nagahama(4)
(1) Department of Biology, Faculty of Education, Tokyo Gakugei University, Tokyo, Japan; (2) Department of Biology, School of Education, Waseda University, Tokyo, Japan; (3) Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan; (4) Laboratory of Reproductive Biology, National Institute for Basic Biology, Okazaki, Japan.

Gonad-stimulating substance (GSS) of starfish is the only known invertebrate peptide hormone responsible for final gamete maturation, rendering it functionally analogous to gonadotropins in the vertebrates. GSS stimulates ovary to induce oocyte maturation by producing maturation-inducing hormone, 1-methyladenine (1-MeAde) in the ovarian follicle cells. Recently, we purified GSS of starfish, *Asterina pectinifera*, from radial nerves and identified the chemical structure as a heterodimer composed of two different peptides (A- and B-chain) with disulfide cross-linkages. According to phylogenetic analyses, starfish GSS belongs to the insulin/IGF/relaxin superfamily and, more precisely, to the subclass of a relaxin-like peptide. In this study, we examined hormonal actions of GSS on follicle cells in starfish reproduction. The chemically synthesized GSS could stimulate follicle cells in the breeding season to produce 1-MeAde through an increase in cyclic AMP (cAMP). In the presence of GSS, 1-MeAde was synthesized from ATP as a substrate *via* methylation using S-adenosylmethionine. This suggests that a unique methyltransferase stimulated by cAMP dependent protein kinase is involved in 1-MeAde biosynthesis. In contrast, GSS failed to induce 1-MeAde and cAMP production in follicle cells of young ovaries during oogenesis. According to competitive experiments using radioiodinated and radioinert GSS, however, highly specific bindings were observed in the membrane fraction of follicle cells from ovaries in the growing and fully grown states, suggesting that GSS receptors are distributed on both follicle cell membranes. Additionally, Gsα was immunologically detected in these membrane fractions. At final maturation stage, upon secretion from nervous tissues, GSS interacts with its receptor on the follicle cell surface to activate G-proteins and adenylyl cyclase and induces 1-MeAde production. It may be possible that GSS has another hormonal action on follicle cells during oogenesis. (Supported by JSPS grant No. 21570063 to MM and by NIBB Cooperative Research Program No. 10-307 to MM)

OR19-2.
GHRELIN REGULATES REPRODUCTIVE PHYSIOLOGY IN FISH
Erin Shepperd and Suraj Unniappan
Laboratory of Integrative Neuroendocrinology, Department of Biology, York University, Toronto, ON, Canada.

Ghrelin, the only known orexigenic gut hormone has been proposed to integrate energy balance and reproduction. There is a large set of data available on the orexigenic and LH stimulatory roles of ghrelin in fish. However, the direct roles of ghrelin on fish gonads remain unclear. Our objective was to characterize the reproductive functions, especially the direct effects of ghrelin on oocyte maturation. We found ghrelin receptor expression in the ovaries and testes of goldfish and zebrafish. Ghrelin receptor mRNA expression in the ovary and testes was relatively lower during the sexually mature stages of goldfish. Further, incubation with native ghrelin at 10ng/mL, 50ng/mL and 100ng/mL concentrations inhibited zebrafish follicle maturation. Oocyte maturation assays also showed that ghrelin inhibited maturation inducing hormone triggered oocyte development. Ghrelin's role within oocyte maturation is further supported by IHC studies that indicate that ghrelin is localized to the theca and granulosa cell layers that surround the oocyte. Overall, our results indicate that the endogenous ghrelin system in the gonads changes during reproductive stages in goldfish. Ghrelin has an inhibitory role on zebrafish oocyte maturation *in vitro* and is localized in the cell layers that surround the oocyte. Collectively, our results for the first time indicate a direct role for ghrelin in the ovarian physiology of fish. [This research was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada through a Discovery Grant, and two Research Tools and Instruments Grants to SU. SU is a Canadian Institutes of Health Research (CIHR) New Investigator and is a recipient of the Early Researcher Award from the Ontario Ministry of Research and Innovation.]

NASCE 2011 INVITED SYMPOSIUM AND ORAL PRESENATATION ABSTRACTS

Saturday, July 16th
2:30 – 4:00 p.m.
Mendelssohn Theater

NASCE 2011 Special Symposium (Sponsored by Elsevier):
General and Comparative Endocrinology 50th Anniversary Symposium
Chairperson: Robert Dores, University of Denver, USA

S20-1.
THYROID HORMONE CONTROL OF REPRODUCTION IN GOLDFISH
H. R. Habibi, E. R. Nelson, E. R. O. Allan, and F. Y. Pang
Institute of Environmental Toxicology and Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

There is evidence that thyroid hormones influence reproduction in vertebrates, but the mechanisms are not clear. In the present study we investigated the role of of Triiodothyronine (T3) in the control of pituitary and gonadal hormone production in goldfish. At the pituitary level, T3 treatment decreased expression of LHß levels in both male and female goldfish. The effect of T3 on LHß expression was somewhat different *in vitro* and *in vivo*, indicating indirect action of T3 via other hormones. In addition we observed significant seasonal variations in T3-induced response. T3 treatment reduced LHβ mRNA levels in cultured pituitary cells obtained from goldfish at early stage of recrudescence, but not other stages of reproduction. Injection with T3, significantly reduced circulating estradiol (E2) level in male goldfish at early and mid stages of gonadal recrudescence, but was without effect in female fish. Treatment with T3 moderately reduced circulating testosterone level in both male and female goldfish in a seasonally dependent fashion. We also tested the hypothesis that the estrogen receptor subtypes (ER, ERß-1, andERß-2) are regulated by the thyroid hormone, (T3), in the gonads of goldfish. All three subtypes were down regulated by T3 in the testis and ovary. We also found evidence that T3 decreased the level of transcript for gonadal aromatase. Collectively, it appears that T3 acts to diminish reproduction by (1) decreasing pituitary LH expression and steroidogenesis, (2) down-regulating gonadal aromatase expression which also decrease estrogen synthesis and (3) decreasing sensitivity to estrogen by down-regulating the ER subtypes. Goldfish are seasonal breeders, spawning once a year, and thus have two distinct periods of growth: somatic and reproductive. Circulating thyroid hormone levels have been found to increase just after spawning. Therefore, we propose that this may be an endocrine mechanism to direct more energy to growth after reproduction. (Funded by Grants from NSERC of Canada.)

S20-2.
PEPTIDE IDENTITY CRISIS: IS SECRETONEURIN A NEW HORMONE?
Vance L. Trudeau(1), E. Zhao(1), Ajoy Basak(2), Gabriela C. López(3), Luis F. Canosa(3), Gustavo M. Somoza(3), Paula Pouso(4), and Ana Silva(4)
(1) Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, Canada; (2) Diseases Program, Ottawa Health Research Institute, Ottawa, ON, Canada; (3) Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico Chascomús, Argentina; (4) Laboratorio de Neurociencias, Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay.

Secretogranin II (SgII) is a 600 amino acid, tyrosine-sulfated protein localized to secretory granules of vertebrate neuroendocrine cells. Numerous small potentially bioactive peptides are derived from SgII precursor processing, but only the 33-34 amino acid segment termed secretoneurin (SN) is conserved from fish to mammals. SN has effects on angiogenesis, neuroinflammation and neurotransmitter release in rodents. The wide distribution of SN in neuroendocrine neurons and pituitary cells suggests important endocrine roles. We have shown that SN stimulates LH release in goldfish (GF) both in vivo and in vitro. The main SN-IR neurons in GF are the parvo- and magoncellular isotocin-positive preoptic cells. In GF, lactotrophs are SN-immunoreactive (SN-IR) and SN-IR neuronal fibres are located in the intermediate and distal pituitary lobes. Co-incubation of dispersed GF pituitary cells with anti-SN antiserum reduces the stimulatory effect of salmon GnRH on LH release, supporting a paracrine role for SN. SN-IR is found in mouse LβT2 cells, and SN stimulates LH release in vitro, suggesting an autocrine mechanism. Our data indicate that SN rapidly stimulates calcium entry into GF gonadotrophs, and SN activates cAMP production and ERK-dependent pathways in LbetaT2 cells. Neuromodulatory roles for SN have also been postulated. Similar to the GF, SN-IR neurons are found in the preotic area of the electric fish, *Brachyhypopomus gauderio*. SN-IR fibres also reach the hindbrain where the electromotor pacemaker nucleus (PN) lies, and SN modulates the firing rate of *B. gauderio* PN neurons in vitro. Critical evidence for the identity of the SN receptor in any species is still lacking; our data indicate that it is most likely a G-protein coupled protein. SN is multifunctional, has important neurohormonal activities in fish and mammalian cells, and exhibits all the characteristics of a new hormone.

S20-3.
PLASMA TESTOSTERONE AND BEHAVIOR IN FREE-RANGING VERTEBRATES: EVOLVING VIEWS ON TEMPORAL DYNAMICS, CONTROL MECHANISMS, AND SIGNIFICANCE
Pierre Deviche
School of Life Sciences, Arizona State University, Tempe, AZ U.S.A.

The behavioral role of the gonadal androgen testosterone (T) in free-ranging male vertebrates has been studied extensively at the population level through (1) correlative studies on relationships between seasonal plasma T (pT) changes and behavior, and (2) experimental work examining behavioral effects of pT manipulations. Relationships between pT and behavior at the individual level are less well understood. For example, social interactions between conspecifics can increase pT and this increase has been postulated to induce short-term adaptive behavioral changes. However, demonstrating the latter has been difficult because pT in free-ranging males is labile and individually variable, and this variation rarely correlates with differences in behavior. Progress in this area depends on a better understanding of the factors that determine individual differences in pT and are responsible for the frequently observed dissociation between individual pT and behavior. A better understanding of the individual variation of pT within a homogeneous group of animals will benefit from investigations on constitutive and/or regulated physiological differences in gonad sensitivity to gonadotropins, androgen secretory capacity, and the dynamics of plasma steroid-binding globulins. Additional research also is warranted on external factors, such as social interactions and acute stress that acutely influence pT. For example, we found in free-ranging songbirds that stress resulting from capture and mild restraint decrease pT by 30-40% within 15-30 minutes. Plasma T in acutely stressed territorial birds was depressed relative to pT in control birds for at least 1.5 hour after release, indicating that acute inhibitory effects of stress can persist. New work aimed at clarifying the time course and amplitude of T behavioral effects will enhance our understanding of the relationship between pT and behavior at the individual level. For example if, as proposed, short-term natural changes in pT has behavioral consequences, then acute experimental manipulation of pT should likewise result in rapid behavioral changes. Whether this is the case is largely unknown because many studies have investigated effects of chronic pT manipulations and acute effects, if any, of such manipulations have rarely been considered.

NASCE 2011 WORKSHOP ABSTRACTS

Wednesday July 13th
6:30pm – 8:00pm
Michigan Room

Chairpersons:
Dan Villeneuve, US EPA
Markus Hecker, University of Saskatchewan, Canada
Nil Basu, University of Michigan, USA

W1-1.
REPLICATION OF THE HYPOTHALAMUS-PITUITARY-GONADAL AXIS OF FATHEAD MINNOW (*PIMEPHALES PROMELAS)* ON A CHIP
T. Kissane(1), R. Morgan(2), D. Cropek(2)
(1) Department of Comparative Biosciences, University of Illinois, Urbana, IL U.S.A.; (2) Construction Engineering Research Lab, US Army Corps of Engineers, Champaign, IL U.S.A.

Endocrine disrupting chemicals (EDCs) are prevalent in waterways from industrial, municipal, and agricultural runoff. Traditional studies have focused on detection and monitoring techniques for EDCs that typically involve extensive whole-fish studies or *in vitro* cell cultures. Whole fish studies can require long time periods before results are detected. On the other hand, cell cultures provide faster results but often use only one cell type from a system composed of numerous cell types and organs. Our goal is to replicate the HPG-axis of the fathead minnow on a microfluidic platform using hypothalamus, pituitary, gonad (HPG), and liver tissues so that it detects EDCs rapidly with real system relevance. We dissected HPG and liver tissue from adult male and female fathead minnows and cultured them *ex vivo* for seven days. Our goal was to maintain viability and function of all tissues in co-culture. Co-cultures allowed hormonal communication among endocrine tissues. Our results support that the co-culture of endocrine tissues maintains tissue viability. Continued work will advance this lab-on-a-chip technique as a replacement model for live fish for in-depth studies of EDC modes of interaction.
Acknowledgements: We would like to thank the other members of the Cropek lab at CERL for input and assistance with this study, as well as Dave Johnson and Ed Perkins for their input. Thank you to Gary Ankley and Dan Villeneuve at the USEPA for teaching us fathead minnow dissection techniques.

W1-2.
SOCIAL STATUS MODULATES GENE EXPRESSION AND METABOLITE PROFILES IN THE FATHEAD MINNOW MALES
D. Martinović-Weigelt(1), D. L. Villeneuve(2), D. R. Ekman(3), M. Henderson(3), Q. Teng(3), T. W. Collette(3), N. Garcia-Reyero(4), C. James(1), E. Perkins(5), G. T. Ankley(2)
(1) Dept. of Biology, University of St. Thomas, St. Paul, MN, U.S.A.; (2) U.S. EPA, Mid-Continent Ecology Division, Duluth, MN, U.S.A.; (3) U.S. EPA, Ecosystems Research Division, Athens, GA, U.S.A.; (4) Dept. of Chemistry, Jackson State University, Jackson, MS, U.S.A.; (5) Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, MS, U.S.A.

Recent studies have successfully used genomic and metabolomic analyses to evaluate responses to endocrine disrupting compounds (EDCs) in urine of the FHM, but these results indicated an occurrence of substantial individual variation. To improve use of FHM for genomic and metabolomic aspects of EDC research, we investigated whether one source of this variability could be a social status. We hypothesized that social status may modulate expression of genes involved in regulation of behavior and reproduction, as well as urine abundance and metabolite composition. To test this, we paired two males with two females and one spawning substrate and examined their behavior, secondary sex characteristics (SSCs), and stored urine volume prior to and after territory acquisition. We also measured plasma androgen concentrations, gene expression patterns in the brains, and conducted NMR-based metabolomic analyses of urine once the social hierarchies were established. Results show that circulating androgens, expression of SSCs, and urine abundance increase with territory acquisition and either remain unchanged or decrease in non-territorial, subordinate individuals. Metabolomic analysis of the urine samples clearly distinguished the metabolite profiles of territorial and non-territorial males. Some of the affected metabolites included taurine, creatine, trimethylamine n-oxide, lactate and choline. Social status affected expression of multiple genes (*circa* 400) involved in immune and stress responses, steroid synthesis and signaling, reproduction and behavior. We demonstrated that social status significantly contributes to variability in gene expression and metabolite composition in male FHM thereby providing important baseline data for interpretation of the effects of EDCs on fish.

W1-3.
EXPRESSION OF MICRORNAS IN *CHIRONOMUS DILUTUS* EXPOSED TO OIL SANDS PROCESS AFFECTED WATER: INSIGHT INTO MECHANISMS OF TOXICITY
Steve Wiseman(1), Karsten Liber(1)(2), Markus Hecker(1)(2), and John P. Giesy(1)(3)(4)(5)
(1) Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada; (2) School of Environment and Sustainability, University of Saskatchewan, Saskatoon, SK, Canada; (3) Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada; (4) State Key Laboratory in Marine Pollution, Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR, Peoples Republic of China; (5) Department of Zoology and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, U.S.A.

Mining companies operating at the Alberta Oil Sands store oil sands process affected water (OSPW), generated during conventional extraction processes, in on-site tailing ponds. OSPW is a mixture of silt, clay, salts, metals, hydrocarbons, and naphthenic acids (NAs). Although a comprehensive understanding of the mechanism(s) of OSPW toxicity is unavailable it is generally accepted that NAs are the primary toxicant constituent. We are using the benthic invertebrate, *Chironomus dilutus*, to explore the toxicity of OSPW. For example, exposure to OSPW significantly impairs growth and development of *C. dilutus.* The growth and development of fly species is regulated, in part, by small non-coding microRNAs (miRNAs). In *Drosophila*, the steroid hormone ecdysone is an important regulator of growth and development, and regulation of ecydsone signaling is controlled by the miRNA, miR-14. Ecdysone also regulates the expression of other miRNAs. To explore the molecular basis of effects of OSPW on *C. dilutus* larval development we explored expression profiles of miRNAs. Sequencing of the miRNA transcriptome identified 74 miRNAs annotated in other species. Several miRNAs were differentially expressed in *C. dilutus* exposed to OSPW. After 4-days of exposure the expression of let-7 and miR-236 were 4.6- and 7.8-fold less, respectively, in OSPW exposed animals compared to controls. In contrast, expression of miR-1889 was 7.1-fold greater in OSPW exposed animals. After 9-days of exposure the expression of let-7 was not significantly different from the freshwater control. However, expression of miR-236 was 15.3-fold greater, and expression of miR-1889 was 2.3-fold less, in OSPW exposed animals. Expression of miR-14 was not significantly different between control and OSPW exposed animals after 4 days or 9 days of exposure. The analysis of miRNAs represents a powerful new tool in the identification of molecular mechanisms of toxicity and in deciphering organism responses to toxicant exposure.

Wednesday July 13th
6:30pm – 8:00pm
Michigan Room

W1-4.
DEVELOPMENT OF A CELL-FREE NEUROCHEMICAL SCREENING BATTERY TO PREDICT ADVERSE OUTCOMES IN MAMMALS, FISH, AND BIRDS

Nil Basu(1), Dong-Ha Nam(1), and Cheryl Murphy(2)
(1) Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI, U.S.A.; (2) Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, U.S.A.

Cell free, in vitro bioassays have been developed in biomedicine to screen, for example, neurotoxic agents and novel pharmaceuticals. Adaptation of such bioassays for predicting adverse outcomes is particularly attractive in ecological risk assessment owing to the lack of high-throughput in vitro screening methods that span several taxa. Here we describe a series of in vitro assays that may be used to assess whether contaminants interact with, and possibly disrupt the function of, various neurotransmitters receptors and enzymes that mediate fish reproduction. Specifically, in this presentation it was hypothesized that metals (Hg^{2+}, CH_3Hg^{+}, Pb^{2+}, total Se, Sn^{2+}, As^{3+}, Cd^{2+}, Cr^{6+}, Mn^{2+}) will emerge to inhibit binding to the muscarinic acetylcholnine receptor (mAChR) and N-methyl-D-aspartate receptor (NMDAR) in brain cortical tissues collected from several fish (goldfish, perch, lemon shark, mako shark), birds (chicken, bald eagle), and mammals (mouse, polar bear, mink, common dolphin, Atlantic white-sided dolphin). First, saturation binding curves were developed from each species to calculate mean receptor density (B_{max}) and ligand affinity (K_d). Next, samples were exposed to the aforementioned metals at several concentrations to derive IC50 (inhibition concentration 50%) values. Based on IC50 values and resulting inhibition constants (*K*i), generalized rank-order potencies for metals and species were developed and will be presented. These comparative results concerning interspecies and inter-metals differences potentially provide a high throughput, in vitro framework for evaluating neuroendocrine risk to ecological organisms. Further, we have developed (and continue to develop) cell-free in vitro methods for several other neuroendocrine receptors and enzymes (e.g., estrogen receptor, androgen receptor, GABA receptor) of concern to vertebrate reproduction, with the ultimate goal of modeling the in vitro results to individuals and populations.

W1-5.
LINKING SUBLETHAL STRESSORS TO POPULATION IMPACTS VIA COMPUTATIONAL MODELING

Cheryl Murphy(1), Sara Smith(1), Rick Goetz(2), Michael Carvan(2) Nil Basu(3), Shawn Sitar(4) and Jessica Head(5)
(1) Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, U.S.A.; (2) Great Lakes WATER Institute, University of Wisconsin – Milwaukee, Milwaukee, WI, U.S.A.; (3) Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI, U.S.A.; (4) Marquette Fisheries Research Station, Michigan Department of Natural Resources, Marquette, MI, U.S.A.; (5) Cooperative Institute for Limnology and Ecosystems Research (CILER), Ann Arbor, MI, U.S.A.

The obvious adverse effect of stressors on Great Lakes fish is the death of large, commercially valuable resident species, however, little is known about the population implications of subtle or sublethal effects. Several modeling techniques are available to predict effects of increased mortality due to stressors on populations. However, often stressors that do not cause direct mortality, can exert sublethal effects that can have repercussions at the population level. For example, stressors can interfere with bioenergetics, endocrine and neuroendocrine function and immunity; such factors can be linked to population relevant endpoints such as fecundity, growth and survival. Here we present two "in progress" case studies on species of relevance to the Great Lakes, lake trout (*Salvelinus namaycush*) and yellow perch (*Perca flavescens*). We compare and contrast the sublethal effects of two different stressors (sea lamprey parasitism and mercury contamination) and illustrate different modeling approaches to convert the effects of these stressors that often manifest at the suborganism level, to population relevant endpoints such as fecundity and growth. Such endpoints can then be input into population models to project population effects. [This work was funded by the Great Lakes Fishery Commission and the Environmental Protection Agency, Great Lakes Restoration Initiative (GLRI).]

TOPIC: *BRAIN AND BEHAVIOR*

P1.

MELATONIN REGULATES DIURNAL CHANGES IN LOCOMOTOR ACTIVITY BY REGULATING 7A -HYDROXYPREGNENOLONE SYNTHESIS IN NEWTS

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Steroids can be synthesized *de novo* in the central and peripheral nervous systems and such steroids are called "neurosteroids". Seasonally breeding wild animals, such as amphibians, have served as excellent animal models to investigate the biosynthesis and biological actions of neurosteroids. Our previous studies have demonstrated that the brain of amphibians possesses the key steroidogenic enzymes and produces pregnenolone, a precursor of steroid hormones, and other various neurosteroids. We recently found that the brain of seasonally breeding newts actively produces 7α-hydroxypregnenolone, a previously undescribed amphibian neurosteroid. Interestingly, this novel neurosteroid acts as a neuronal modulator to stimulate locomotor activity in male newts. Because male newts show marked diurnal changes in locomotor activity, we hypothesized that 7α-hydroxypregnenolone may be a key factor for the induction of diurnal changes in locomotor activity in male newts. In this study, we first found diurnal changes in 7α-hydroxypregnenolone synthesis in the brain of male newts. The production of 7α-hydroxypregnenolone in the male brain increased during the dark phase when locomotor activity of males was high. Thus, diurnal changes in 7α -hydroxypregnenolone synthesis in the brain paralleled with locomotor activity in male newts. We then identified melatonin as a key component of the mechanism regulating 7α -hydroxypregnenolone synthesis. Decreased synthesis of 7α -hydroxypregnenolone occurred in males *in vivo* after melatonin removal via pinealectomy and orbital enucleation (Px plus Ex). Conversely, increased synthesis of this neurosteroid occurred after melatonin administration to Px plus Ex males. This study demonstrates that melatonin regulates synthesis of 7α -hydroxypregnenolone, a key factor for induction of locomotor activity, thus inducing diurnal locomotor changes in male newts. (Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan to KT and a Japan Society for the Promotion of Science (JSPS) – Institut National de la Santé de la Recherche Médicale (INSERM) exchange program to KT and HV.)

P2.

DEVELOPMENTAL PROGRAMMING BY GLUCOCORTICOIDS: MODIFICATION OF POST-METAMORPHIC BEHAVIOR AND NEURAL GENE EXPRESSION AFTER EARLY-LIFE EXPOSURE TO CORTICOSTERONE IN THE FROG *XENOPUS LAEVIS*

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Exposure to stressors early in life can have profound effects on later life phenotypic expression. The effects of stressors may be mediated by glucocorticoids acting to 'program' gene expression, leading to long term, stable changes in physiology and behavior. We established an experimental paradigm using the frog *Xenopus laevis* to investigate long term phenotypic consequences of early life exposure to elevated glucocorticoids. We treated early prometamorphic tadpoles with corticosterone (CORT; 100 nM) or vehicle (ethanol; 0.00025%) for 5 days by addition to the aquarium water, and then reared animals to 2 months post-metamorphosis. This dose of CORT elevated whole body CORT content within the physiological range, which mimicked changes in endogenous CORT following ecologically relevant stressors. CORT-treated animals were smaller than vehicle-treated controls at metamorphosis, but showed catch-up growth, reaching similar body size to controls by 2 months post-metamorphosis. Behavioral assays conducted on juvenile frogs showed that CORT-treated animals displayed significantly greater anxiogenic-like behavior than controls (quantified as time spent escaping from a negative stimulus vs. time at rest). Immunohistochemical analysis showed that early life CORT exposure decreased glucocorticoid receptor immunoreactivity (ir) in the anterior preoptic area, medial pallium and anterior pituitary. By contrast, CORT exposure increased corticotropin-releasing factor-ir in the medial amygdala and bed nucleus of the stria terminalis. Microarray analysis conducted on RNA extracted from the preoptic area/hypothalamus of juvenile frogs identified 47 genes that were differentially expressed between control and CORT-treated animals; 18 upregulated and 28 downregulated. Our findings show distinct physiological and behavioral changes following early life exposure to elevated CORT, and our molecular analyses support that these changes may reflect altered neural gene expression. (Supported by NSF grant IOS 0922583 to RJD)

P3.

PLASMA ANDROGENS AND BRAIN AR-IR CELL COUNTS CORRELATE WITH SEX AND SPECIES DIFFERENCES IN AGGRESSION IN TWO *SCELOPORUS* LIZARDS

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In the studied *Sceloporus* lizards, species and sexes with blue belly patches exhibit more aggression than those without. We studied correlates of territorial aggression in adults of two species: *S. undulatus* (males blue, high aggression; females white, low aggression) and *S. virgatus* (both sexes white, low aggression). We measured plasma testosterone (T) concentrations and counts of cells with androgen receptor-like immunoreactivity (AR-ir) to an affinity-purified polyclonal AR antibody (PG-21), in three brain regions of breeding season adults. Males of the blue species had the highest mean plasma testosterone (T), and differed significantly from conspecific females. Mean plasma T did not differ between sexes in the white species. In the hypothalamic preoptic area, *S. undulatus* (blue) males had the highest mean AR-ir cell counts, and the sexes differed in *S. undulatus* but not in *S virgatus* (white); females of the two species did not differ in mean counts. In the ventral medial hypothalamus, *S. undulatus* (blue) males had higher mean AR-ir counts than females, but there was no sex difference in *S. virgatus*; for each sex, the species did not differ. The habenula did not exhibit significant sex or species differences in AR- ir cell counts. Thus, hypothalamic AR cell counts paralleled sex and species differences in aggression, as did breeding-season plasma T levels. (NIH-MH61788).

P4.

SOCIAL MODULATION OF JUVENILE HORMONE TITERS IN POLISTES WASP QUEENS AND THE WINNER LOSER EFFECT

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The 'challenge hypothesis' provides a conceptual framework for studying the interplay between social factors and endocrine responses and has framed much of the research on social modulation of androgens. Less is known about whether socially modulation of endocrine titers plays a role in the behavioral plasticity of invertebrates. Here, we test the causes and consequences of social modulation of endocrine titers in *Polistes dominulus* wasp queens. *P. dominulus* queens are a good system to study social modulation of endocrine titers because their social behavior combines intense conflict and extreme cooperation. When nest founding queens emerge from overwintering, they aggressively compete over dominance rank with numerous other queens before settling down to start a nest. Many queens nest in cooperative groups where the dominant queen reproduces, while subordinate queens do not. We explore the effect of aggressive conflict on juvenile hormone (JH) titers by staging aggressive contests among queens during the period of competition prior to nest foundation. Social conflict had strong effects on JH titers. JH titers increased in queens that won contests and decreased in queens that lost contests relative to non-fighting controls. Therefore, social interactions quickly and dramatically influenced the JH titers of nest founding wasp queens. We followed-up this result with experimental manipulation of JH titers and detailed behavioral observation to test the consequences of the endocrine change on subsequent social interactions. Our results suggest that there are surprising similarities in the causes and consequences of social modulation across vertebrates and invertebrate endocrine systems. The convergent evolution of endocrine responses suggests that social modulation is an adaptive feature of endocrine systems across diverse taxa. (Funding was provided by the University of Michigan.)

TOPIC: *ENVIRONMENTAL ENDOCRINOLOGY*

P5.

DOES TESTOSTERONE MEDIATE THE LINK BETWEEN ACOUSTIC AND VISUAL SIGNALS AND REPRODUCTIVE SUCCESS IN AN ARCTIC PASSERINE?

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Theory predicts that an individual's quality and fitness are closely linked; high-quality individuals are expected to signal this quality to enhance reproductive success. However, considerable intra-specific variation in quality-related traits suggests the presence of quality-mediated trade-offs. We are using an integrative approach to examine how variation in quality-mediated phenotypes relate to reproductive success in an Arctic passerine, the Snow Bunting (*Plectrophenax nivalis*). Specifically, we are examining whether variation in the underlying physiological traits linking male song and plumage quality drive variation in reproductive success in a high breeding density population at East Bay Island, Nunavut. By combining ecological (timing of arrival, territory size) and physiological (arrival condition, testosterone and sperm quality) traits within an evolutionary framework, we hope to elucidate the mechanisms by which quality-signaling drives reproductive success. Specifically, we are: 1) Assessing song quality via measures of song performance and complexity. 2) Quantifying male plumage quality using feather reflectance. 3) Measuring plasma testosterone as a driver of reproductive success and stress-mediated traits (immunoglobulins/oxidative stress) as costs to high reproductive effort.4) Determining reproductive success via offspring survival.We will present results on (1) how song and plumage may act together to signal individual quality, (2) the physiological mechanisms that link signal quality and reproductive success, and (3) potential trade-offs in breeding effort and immune function. Our overall goal is to understand how physiological mechanisms drive and constrain the evolution of quality-mediated phenotypic signals in free-living systems. (Research was supported by Environment Canada, Arctic Net, Northern Studies Training Program, NSERC and the University of Windsor.)

P6.

PUTTING NEUROENDOCRINOLOGY ON THE MAP: BIOGEOGRAPHICAL INTEGRATION OF ECOLOGICAL NICHE MODELING, CONSERVATION GENETICS, ENVIRONMENTAL ENDOCRINOLOGY IN THE WOOD FROG (*LITHOBATES SYLVATICUS*)

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While positive correlations among risk of extinction, genetic variability, and physiological stress are widely assumed, few studies have directly measured the relationships among these indices of population fitness. Working within a theoretical framework of species range dynamics, we aim to test whether independent assessments of habitat quality, generated from spatially-explicit ecological niche models (ENM), correlate with neuroendocrine and genetic indicators of population-level health within the eastern range of the wood frog (*Lithobates sylvaticus*). During the 2011 breeding season, we sampled males (n=20-30) from 18 populations, which spanned the latitudinal range of the eastern clade of wood frogs and were located within a range of habitat qualities as predicted by a climate-based ENM. We also sampled males from roadside and woodland breeding sites within select climatic regions to resolve the impact of local habitat conditions on genetic variance and stress responsiveness of populations. For each population, we measured baseline plasma corticosterone concentrations and the response to a standard dose of adrenocorticotropic hormone as our stress responsiveness assay, which has been shown in multiple vertebrates to be reduced in individuals that have experienced higher levels of stress during their lifetime. We also recorded body measurements, reproductive deformities, and assayed for chytrid, ranavirus, and trematode infections. At the genetic level, we harvested tissue from each frog to 1) measure individual and population-level genetic variability using microsatellite markers, and 2) assess environmental associations of genomic and epigenetic gene regulation. Ultimately, we will integrate these broad and fine-scaled measures of population fitness to understand the geography of population health, how species are distributed in space, and how these distributions will be altered by environmental change. (Supported by NSF grant BCS-1026700 to EJC and LJR).

P7.
THE SUBLETHAL EFFECTS OF SEA LAMPREY PARASITISM ON LAKE TROUT
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Sea lamprey (*Petromyzon marinus*) invasion of the Great Lakes has been linked directly to lake trout (*Salvelinus namaycush*) population decline through the effect of parasitism mortality. Less well understood are the sublethal effects of sea lamprey parasitism on lake trout. This study aims to identify and quantify sublethal effects such as changes in sex steroids and gonadotropin. To determine if sublethal effects differ between morphotypes, both siscowet and lean lake trout were used. Siscowets are found in Lake Superior at depths greater than 100m and usually have higher weights and lipid levels than leans. Leans are found in shallow water throughout the Great Lakes. Siscowet and lean lake trout (n=64) were subjected to sea lamprey parasitism for one to five days and analyzed for changes in testosterone, estradiol, gonadotropin, HSI, and GSI. Wild Lake Superior lake trout (n=58) were also sampled to establish a range of responses. Number of days parasitized and HSI reduction appear correlated, with lean morphotypes reacting more severely. Plasma sex steroid and gonadotropin levels show possible differences between parasitized and non-parasitized leans and siscowets. Results will be incorporated into a physiological model to link sex steroid and gonadotropin levels to changes in vitellogenin, an egg yolk precursor and good biomarker of reproductive investment. Cumulative vitellogenin production can then be scaled to population level impacts. This is important because current models that estimate spawning stock biomass per recruit do not take into account the likely decrease in reproductive ability due to sublethal sea lamprey wounding. (This research was funded through the Great Lakes Fishery Commission. Lab space was provided by the Hammond Bay Biological Station.)

TOPIC: *STRESS HORMONES*

P8.
LINKS BETWEEN BASELINE STRESS PHYSIOLOGY, HABITAT QUALITY, AND FITNESS
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Across taxa, measures of baseline stress hormones (eg. corticosterone) are increasingly being utilized as conservation tools for the assessment of individual and/or population condition. To be an effective biomarker in this regard, baseline corticosterone (cort) levels must show a predictable relationship with fitness, the general assumption being that high levels of baseline cort are indicative of individuals with low relative fitness (recently coined the Cort-Fitness Hypothesis). However, empirical evidence for this relationship has been largely inconclusive, showing variation both within populations and within individuals across different life history stages. Through the study of breeding Tree Swallows (*Tachycineta bicolor*), we investigated the relationship between baseline cort levels and reproductive success across two reproductive stages and within the context of habitat quality. We find that individual cort levels changed from the incubation stage to the nestling provisioning stage, with the direction of change being highly dependent on age. In addition, baseline cort levels predict fitness only for experienced individuals (those in their second breeding season or later). Moreover, the direction of this relationship differs based on habitat quality, showing a positive relationship in high quality habitats and a negative relationship in low quality habitats. Our results indicate that information on life history stage, age, and habitat metrics may be necessary to effectively apply stress hormones as relevant physiological indices for conservation. (Research support provided by NSERC, University of Windsor, Ruthven Park National Historic Site, and Haldimand Bird Observatory.)

P9.
ACUTE STRESS RAPIDLY INHIBITS PLASMA TESTOSTERONE IN HOUSE SPARROWS (*PASSER DOMESTICUS*)
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Organisms often perceive environmental changes, such as those associated with climate changes and human-driven habitat degradation, as physiologically stressful. In birds, stress may inhibit reproduction by reducing plasma testosterone (T). The time course of this reduction and its mechanistic basis have not been well investigated. We found capture and mild restraint (confinement in cloth bag) to decrease plasma T within 15-30 min in adult free-ranging males of several passerine species. In at least one of these species, the Rufous-winged Sparrow, *Peucaea carpalis*, the stress-induced decrease in plasma T was not associated with lowered plasma LH, suggesting that it is does not result from an inhibition of the hypothalamo-pituitary gland axis. To further investigate this issue, we used adult male House Sparrows, *Passer domesticus*, as the experimental model. Sparrows in breeding condition were mist-netted passively in Tempe, Arizona. Blood was collected from a jugular vein within 3 minutes of capture (baseline) and again after 5, 15, or 30 minutes of mild restraint (stress-induced). Blood was also collected from an alar vein within 3 minutes of capture and again 15 minutes later to test if plasma T reduction occurs at the central or peripheral level. Stress-induced plasma T was lower than plasma baseline plasma T after 30 minutes, but not after 5 or 15 minutes of stress. Furthermore, plasma baseline and stress-induced (15 min) T did not differ in samples collected from a jugular vs. an alar vein. Current research aims at investigating the mechanisms of action of acute stress on T secretion and in particular a potential direct impairment of the testicular endocrine function.

P10.
CORTICOSTEROID-BINDING GLOBULIN IN URSIDS: BINDING AFFINITIES AND NUTRITIONAL REGULATION
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The majority of corticosteroid in circulation in mammals is bound reversibly and with high affinity to a transport protein, corticosteroid-binding globulin (CBG). Cortisol bound to CBG is biologically unavailable to tissues, and consequently, CBG plays a key role in reducing free cortisol concentration and regulating the cellular actions of this hormone, including protecting tissues from potentially deleterious actions. However, little is known about either serum levels of CBG or its role in serum cortisol regulation in bears. Our lab previously reported differential expression of CBG between sexes in grizzly and polar bears, and between feeding and fasting polar bears by western immunoblotting. In this study, we report the development of a direct ELISA specific for grizzly bear CBG that accurately and reliably quantifies concentrations of this protein in grizzly, polar, and black bear sera. We have also calculated the equilibrium dissociation constants (K_D) of CBG in these species by Scatchard analysis. The black bear CBG showed the highest affinity for cortisol, followed by grizzly and polar bear CBG. The differences in K_D between these species may reflect differences in hormonal action in these species. With these

data and total cortisol concentrations, which were measured with a commercial RIA, we calculated the free cortisol concentrations in the serum of these ursid species. We compared free serum cortisol concentrations between feeding and fasting animals to help establish the physiological role of cortisol in the seasonal metabolic adjustments of these animals. (Funding provided by Alberta Innovation and Science, and the National Science and Engineering Research Council of Canada.)

P11.
FIVE WAYS TO SKIN A CAT: AN UNEXPECTED DIVERSITY OF STRESS PROFILES IN FIVE SPECIES OF GROUND SQUIRRELS
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Comparative physiologists often hypothesize that animals facing similar problems will have similar solutions; that is, we expect general patterns to emerge. The alternative hypothesis is that few, if any, patterns will emerge because each species is able to so finely manipulate its physiology that there are any number of ways to solve the same problem. We studied stress axis (hypothalamic-pituitary-adrenal axis) function over the course of the breeding season in a group of closely related ground squirrel species. Because the stress axis is highly conserved among vertebrates in terms of its basic functioning, and because all of the ground squirrel species we studied face similar reproductive challenges (all are hibernators that compete for mating opportunities shortly upon emerging from hibernation), we expected that species sharing similar life history strategies would have similar stress profiles. For each species we obtained pre- and post-breeding stress profiles of males that included stress-induced glucocorticoid (GC) levels, GC levels in response to a hormone challenge protocol (dexamethasone suppression and ACTH stimulation), blood glucose levels, free fatty acids, and complete blood counts. We found that life history did not predict stress profile: each species had a unique stress profile, with no two species sharing even basic trends in GC levels and binding capacity. Trends in total GC concentrations, GC binding capacity, and free GC concentrations over the course of the breeding season varied between all 5 species studied. Moreover, the downstream measures of stress (glucose, free fatty acids, hematocrit, complete blood counts) showed similar variability and could not be predicted from simple measures of GC concentrations. We conclude that many of our simplifying assumptions about how the stress axis supports or constrains reproduction need to be revisited. (Supported by NSERC grant to R.B. and NSTP Grant to B.D.)

P12.
ELEVATION OF PLASMA CORTICOSTERONE TO PHYSIOLOGICALLY RELEVANT LEVELS INCREASED METABOLIC RATE IN A TERRESTRIAL SALAMANDER
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Plasma glucocorticoid hormones (GCs) become elevated when individuals are exposed to stressors. GCs alter intermediary metabolism to increase blood glucose concentrations. In the absence of compensatory mechanisms, this increase in intermediary metabolism may be reflected in whole-animal metabolic rate. Studies in fish, birds, and reptiles have shown that GCs may alter whole animal metabolic rates, but results are conflicting and often involve GCs levels that are not physiologically relevant. A previous study in red-legged salamanders (*Plethodon shermani*) found that male courtship pheromone increased plasma corticosterone (CORT; primary GC in amphibians) concentrations in males but not females. To understand the possible metabolic effect of elevated plasma CORT, we measured the effects of male courtship pheromone and exogenous application of CORT on oxygen consumption in male red-legged salamanders. Exogenous application of CORT elevated plasma CORT to physiologically relevant levels. Compared to treatment with male courtship pheromone and vehicle, treatment with CORT increased oxygen consumption rates within two hours after treatment resulting in 12% more oxygen consumed over a 2 hr period. Contrary to our previous work, treatment with pheromone did not increase plasma CORT, perhaps because subjects used in this study were not in breeding condition. Our study is one of the few to evaluate the influence of physiologically relevant elevations in CORT on whole animal metabolism in wildlife, and the first to show that elevated plasma CORT increases metabolism in an amphibian.

P13.
SOME LIKE IT HIGH: MINIMIZING FREE GLUCOCORTICOID IS NOT THE ONLY OPTION
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The “Free Hormone Hypothesis” posits that only free, unbound hormone is biologically active and available for use by the tissues. Conventional wisdom in the biomedical literature proposes that corticosteroid-binding globulin (CBG) normally binds 90-95% of blood glucocorticoid, rendering it unavailable to tissues. Under chronic stress, free levels exceed this resulting in reduced fitness as brain function, energy balance, immune function and reproduction are compromised. However, under normal conditions in northern and southern flying squirrels, more than 90% of their cortisol is free. This presents a major challenge to the known consequences of the hypothesis. To assess the generality of these findings, we compared all vertebrate species (89) with known CBG and glucocorticoid levels. We found that more than 60% have less than 90% bound and thus many species deviate from expectation. Similar extremes to that found in flying squirrels are also seen in new world monkeys, yet both groups evolved from ancestors that followed the normal convention. We speculate as to how this state evolved and persisted through time. (Supported by an OGS to LD, an NSTP award to BD, and NSERC grants to RB and JTW).

P14.
STRESS HORMONES, RESPONSES TO A PROLONGED STRESSORS AND ADAPTATIONS TO URBAN LIFE: A COMMON GARDEN EXPERIMENT IN EUROPEAN BLACKBIRDS (*TURDUS MERULA*)
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Environmental conditions differ greatly between forest and city habitats, creating a number of challenges for urban dwelling animals to overcome. The functioning of the hormonal stress axis, the hypothalamo-pituitary-adrenal (HPA) axis, may serve as a possible physiological mechanism to allow individuals to cope with the human-altered city habitat. The European blackbird (*Turdus merula*) provides a model to begin to address whether, and where in the HPA axis city and forest populations may differ. This species is a common forest bird, and only recently (~150 years ago) began to colonize and thrive within large European cities while also continuing to thrive in forest habitat. A previous report indicated that hand-reared individuals from nests in the city of Munich, Germany displayed an attenuated glucocorticoid response to restraint stress compared with hand-raised birds from nearby forest habitat,

suggesting either genetic or early developmental differences between these two populations and habitats. In the current study, nestlings from Munich, Germany or from a forest site were hand-raised and maintained on a simulated natural photoperiod throughout their lifetime. An assessment of multiple levels of the HPA axis was performed both prior to and following a prolonged experimental exposure to multiple stressors. Specifically, glucocorticoid levels were assessed from baseline and post-restraint stress samples. Further animals received an injection of dexamethasone to assess the negative feedback system and an injection of adrenocorticotropin hormone (ACTH) to measure the capacity of the adrenals to produce glucocorticoids. The data presented will elucidate whether differences in the make-up of the HPA axis and its responsivenss to prolonged exposure to stressors may allow for adaptation to city life.

P15.
THE EFFECTS OF PREDATOR CUES ON BEHAVIOR, CORTICOSTERONE AND WHITE BLOOD CELL DIFFERENTIAL IN THE TERRESTRIAL SALAMANDER *DESMOGNATHUS OCHROPHAEUS*
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A stressor is typically defined as a real or perceived threat to homeostasis and is often unpredictable. Stressors trigger changes in behavior and physiology that allow an animal to cope with the stressor, while suppressing functions not essential to immediate survival. Stress responses are usually mediated by glucocorticoid hormones, such as corticosterone (CORT). A common stressor experienced by wild animals is that of a predator attack. Cues emanating from a predator are also considered potent activators of stress responses. In this study, *D. ochrophaeus* salamanders were exposed to kairomones, chemical cues emitted by predators and detected by prey. It was hypothesized that both acute and repeated kairomone exposure would lead to increased predator avoidance, decreased mating behavior and altered plasma CORT and white blood cell differentials. Kairomones consisted of body rinses from a predatory species of salamander. Subjects were placed on substrates moistened with kairomones one time (acute exposure) or every day for 2 weeks (repeated exposure). Compared to control animals, acute exposure to kairomones caused a decrease in locomotion (a predator avoidance behavior) and mating behaviors, but no changes in plasma CORT. Repeated exposure to kairomones produced no change in baseline locomotion, mating activity, or plasma CORT. The lack of response to repeated exposure was not due to habituation. Contrary to expectation, repeated exposure to kairomones resulted in a decreased blood neutrophil-lymphocyte ratio. To summarize, kairomones were potent modulators of behavior, but repeated exposure to kairomones did not alter baseline expression of behavior. The effects of kairomones did not appear to be associated with changes in plasma CORT.
Acknowledgements: I would like to thank Dr. Sarah Woodley for her help, as well as Rachel Michael and Katie Ratay for their help in counting white blood cells. I would also like to thank the entire Department of Biology at Duquesne for their help and use of resources.

P16.
EARLY NUTRITIONAL STRESS IMPAIRS A TADPOLE'S ABILITY TO RESPOND TO SUBSEQUENT WATER REDUCTION STRESS IN *PELOBATES CULTRIPES*
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In nature, animals are exposed to multiple stressors and the direct or indirect effects of these stressors collectively influence their fitness. However, our understanding is mostly limited to the effect of singular stressor on the animal physiology and its fitness. Here we examined the effect of successive stressors on a stress response ability of a tadpole. Specifically, we tested if experiencing nutritional stress early during tadpole development affects its ability to respond to consecutive water reduction stress later during metamorphosis. To test this we raised tadpoles of *Pelobates cultripes* until Gosner stage 35 at three different food levels, low (LF), medium (MF) and high (HF). Animals from HF treatment were bigger and metamorphosed earlier than LF treated animals at stage 35. Release from food stress and subsequent exposure to high (HW) and low (LW) water at stage 35 showed that only tadpoles from HF treatment accelerated development in LW. To mimic elevated corticosterone (CORT) levels due to starvation in MF and LF treatments that may have affected the ability to accelerate the rate of metamorphosis under LW condition, we treated tadpoles daily with 500 nM CORT from stage 32 for 12 days, which resulted in smaller stage 35 tadpoles. Subsequent exposure to water treatments at stage 35 showed that only control group accelerated development in LW. In addition, to connect results from laboratory experiments with natural settings, we collected naturally occurring large and small [mean (16 vs 4 gms)] stage 36 *P. cultripes* tadpoles from the same pond. Smaller tadpoles may represent a history of early-life stress. We treated both groups with LW treatment, and the larger tadpoles metamorphosed significantly earlier. Our data show that an initial stressor attenuates an animal's ability to mount an appropriate stress response to a subsequent stressor. Thus, experiencing stressors consecutively may be more harmful than expected based on studies on isolated stressors. (JEB Travelling fellowship to SSK).

P17.
REGULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-1 IS INDEPENDENT OF CORTICOTROPIN-RELEASING FACTOR RECEPTOR ACTIVATION
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TCAP-1, a 41-residue peptide with about 20% sequence identity with CRF is widely expressed in the central nervous system. This peptide has been previously shown to attenuate CRF-induced behaviors elicited in a number of tests including the elevated plus maze, open field and acoustic startle test. Recently, this peptide was also shown to block CRF-induced cocaine-seeking behavior following cocaine withdrawal. Moreover, acute intracerebroventricular (icv) administration of TCAP-1 blocks CRF-mediated cfos labelling in the prefrontal cortex, septum, hippocampus, amygdala and dorsal raphe nucleus of rats. Surprisingly, repeated icv injections show a decreased ability of TCAP-1 to block CRF-mediated cfos activity in these same regions, even though the same TCAP-1 administration regimen is more effective at attenuating CRF-induced behaviors, relative to acute TCAP-1 administration. Because the effect of TCAP-1 on CRF-induced behaviors differs in vivo depending on whether the peptides are administered centrally or peripherally, one possibility is that TCAP-1 acts to modulate central mechanisms governing CRF and glucocorticoid signal processing differentially. In order to test this hypothesis, the interactions of TCAP-1, CRF and glucocorticoids were investigated in a number of in vitro models. These studies indicate that TCAP-1 acts independently of either CRF–, glucocorticoid– or mineralcorticoid–mediated signal transduction and transcription, and confers a number of regulatory changes on neurons, which consequently impact on the function of HPA elements. (This work was supported by grants from NSERC and Protagenic Therapeutics.)

P18.
CRF NEURONS AT THE INTERFACE BETWEEN SENSORY AND MOTOR PROCESSING
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Corticotropin-releasing factor (CRF) is a 41 amino acid peptide that is best known as the principle hypophysiotropic hormone regulating the pituitary-adrenal axis during stress. CRF also regulates many stress and anxiety related behaviors including food intake, and over-expression of CRF is thought to be a main causative agent in anxiety related eating disorders such as anorexia nervosa. Recent data collected in our laboratory using amphibian models indicate that, in addition to affecting appetite, CRF may modulate visual sensory pathways involved in detecting and responding to food. Here we examine the hypothesis that CRF directly modulates sensorimotor processing in the optic tectum, the major site for integration of visually guided behavior in the amphibian brain. In the African clawed frog *Xenopus laevis*, RT-PCR revealed that cells in the optic tectum express mRNA for CRF and the CRF R1 receptor but not the CRF R2 receptor. In vitro studies revealed that both basal and depolarization-induced release of CRF, determined using a homologous radioimmunoassay, was greater from the optic tectum relative to the telencephalon, hypothalamus or brainstem. These findings most likely reflect regional differences in the inhibitory regulation of CRF, as tectal content of CRF is actually lower than that of the hypothalamus in the two anuran species that have been studied to date. Radioligand binding studies indicate that specific binding of [^{125}I-Tyr]-oCRF to tectal cell membranes can be displaced by the CRF R1 antagonists antalarmin or NBI 27914. We conclude that the optic tectum possesses an intrinsic CRF signaling system that may be involved in modulating communication between sensory and motor pathways involved in food intake.

P19.
RAPID NONGENOMIC EFFECTS OF CORTISOL ON MEMBRANE FLUIDITY AND PHOSPHORYLATION IN RAINBOW TROUT LIVER
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While rapid steroid signaling is thought to play a key role in cellular function, little is known about the nongenomic role of cotisol in hepatic tissue. Therefore, we studied the effects of cortisol on the biophysical properties of the liver plasma membrane, and investigated the effect of this corticosteroid on the phosphorylation status of p38 and p44/42 mitogen-activated protein kinases (MAPK) as well as substrate proteins for several protein kinases in trout hepatocytes. The effect of cortisol on membrane fluidity was examined by measuring fluorescence anisotropy of the membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH) in isolated liver plasma membrane fractions. In vitro, physiological (100 ng/ml) and pharmacological (500 and 1000 ng/ml) cortisol concentrations were shown to rapidly increase hepatic membrane fluidity. Moreover, cortisol rapidly modulates the hepatic phosphorylation profile but does not alter the phosphorylation status of either p38 or p44/42 MAPK. These results indicate for the first time that stress-induced plasma cortisol levels may modulate liver membrane fluidity and will lead to rapid intracellular signaling cascade in rainbow trout.

P20.
NEUROPROTECTIVE ACTIONS OF TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-1 IN VITRO: REGULATION OF METABOLIC AND APOPTOTIC PATHWAYS
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TCAP-1 is a paralogous member of a highly conserved family of four peptides associated with the four teneurin transmembrane proteins. The peptide has been found to be highly neuroprotective against high alkaline, high calcium and low oxygen stressors. Protection from alkaline stress has been found to be attributed, in part, to the upregulation of reactive oxygen species (ROS) scavenging systems such as superoxide dismutase, superoxide dismutase copper chaperone, and catalase. In addition to oxidative stress protection, TCAP-1 also rescues cells from hypoxic stress. This was demonstrated through increased cell viability and a reduction in the expression of HIF-1α, a characteristic marker of hypoxic stress. Moreover, cell viability in cultures of immortalized embryonic mouse neurons is significantly reduced in shRNA knockdowns of TCAP-3, the embryonic neuron paralog. Thus, this study was aimed at understanding the cellular mechanisms by which TCAP-1 confers neuroprotection through a series of in vitro studies using immortalized mouse hypothalamic cell cultures. These data indicate that the neuroprotective actions of TCAP-1 operate, in part, by a p90rsk mediated mechanism, which may regulate downstream actions on cellular metabolism and apoptotic pathways. Metabolic adaptation through upregulation of glycolytic enzymes, expression of favourable isozymes, altering of mitochondrial functioning and reorganization of key metabolic enzyme allocation would allow for sustained energy production despite various cellular stressors. Furthermore, the activation of anti-apoptotic pathways, through TCAP-1 mediated signalling cascades, could work synergistically with metabolic adaptation to increase cell viability. (Supported by grants from NSERC and Protagenic Therapeautics).

P21.
CIRCULATING CORTICOSTERONE CONCENTRATIONS INCREASE WITH BREEDING SEASON PROGRESSION IN A LONG DISTANCE MIGRANT, FRANKLIN'S GULL (*LEUCOPHAEUS PIPIXCAN*)
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Environmental cues can influence the physiology of a variety of animals, particularly birds. Many of these cues are experienced seasonally and have significant effects on fitness, reproduction, and overall condition. Considerable evidence indicates that reproducing later during the breeding season results in lower reproductive success (and therefore lower fitness). Additionally, the stress response, as mediated by the hypothalamopituitary-adrenal axis (HPA) appears to be sensitized due to temporal constraints on fledging young and the impending migration. In this study, we characterized the stress response of Franklin's gull (*Leucophaeus pipixcan*) adults nesting early and late in the breeding season to evaluate whether changes in the sensitivity of the HPA axis are associated with seasonal changes in physiological condition. Using a previously validated radioimmunoassay (RIA), we quantified peak corticosterone (CORT) levels in these birds. Additionally, we performed a handling stress to measure changes in the stress response following 20 and 30 minutes of handling. We also quantified heterophil-to-lymphocyte ratios to better understand the effects of stress on the innate and acquired immune systems. Peak CORT concentrations were higher in birds nesting later in the season, as compared to birds nesting earlier in the breeding season. Interestingly, heterophil-to-lymphocyte ratios did not vary across the season, despite the increase in peak CORT concentrations. These results may suggest that this long distance

migrant has evolved a more tightly regulated stress response with respect to immunity, a consequence possibly stemming from its short breeding period and long migration. (Supported by ND Game and Fish Department)

P22.
VARIATION IN GLUCOCORTICOID REGULATION AMONG INVASIVE KENYAN HOUSE SPARROWS (*PASSER DOMESTICUS*)
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House sparrows (*Passer domesticus*) are one of the most widely distributed species in the world, but *how* this species has come to occupy so many areas remains unclear. Despite extensive phenotypic variation among introduced and native populations, genetic evolution (i.e., adaptation) is an unlikely explanation for these differences, particularly within the invasive range, given the short time scales over which invasions typically occur. Rather, phenotypic plasticity, especially plasticity mediated by stress hormones (e.g. glucocorticoids), is more likely to have facilitated behavioral changes that foster colonization success in some species such as neophilia and innovation. In the present study, we compared baseline, stress-induced, as well as negative feedback-induced levels of corticosterone during molt and reproduction among six Kenyan house sparrow populations along a gradient of old (~60 years) to new (<5 years). We predicted that individuals at the invasion front (newest populations) would release less corticosterone in response to a stressor and reduce levels of corticosterone more rapidly in response to negative feedback. Whereas we found no significant differences among populations in any of the parameters during the molting season, this outcome may be due to a damped adrenal response seen in other populations during this life-history stage. During reproduction, however, we expect to see the predicted patterns among populations (analysis is ongoing). (Work was supported by NSF grant IOS 0920475 to LBM.)

P23.
TEMPERATURE AS AN ENDOCRINE AND CELLULAR STRESSOR IN BROOK TROUT
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Global warming presents many challenges for the conservation and management of wildlife, yet the mechanisms by which temperature affects populations are often unknown. Although somatic growth is a key aspect of population persistence, our understanding of the means by which temperature impacts growth and stress physiology is limited. In the present study, brook trout (*Salvelinus fontinalis*) exposed to constant temperatures (16, 18, 20, 22, or 24 °C) were monitored for growth and tissue samples were collected at 8 and 24 days for physiological analysis. Through 24 d, growth rate was highest at 16 °C and decreased with temperature to a low at 24 °C. Plasma cortisol levels were lowest at 16 °C (1.3 $ng \bullet ml^{-1}$) and increased with temperature to a peak of 23.4 $ng \bullet ml^{-1}$ at 24 °C. Abundance of the inducible isoform of heat shock protein (Hsp)-70 in gill tissue increased with temperature and was 10- and 56-fold higher at 22 and 24 °C than at 16 °C. In brook trout exposed to constant 21 °C or daily temperature fluctuations of 4 or 8 °C with mean of 21 °C), growth rate was highest at constant temperature and decreased with increased magnitude of temperature fluctuation. We did not detect an effect of temperature fluctuation on plasma cortisol levels. Gill Hsp-70 was 40- and 700-fold higher at 4 and 8 °C fluctuation than at constant temperature. There was no effect of temperature on plasma glucose in either of these experiments. A field study was conducted in summer 2010 at 8 sites in western Massachusetts. There was no significant relationship between temperature and plasma cortisol, but plasma glucose ($p = 0.002$, $r^2 = 0.11$) and gill Hsp-70 ($p < 0.001$, $r^2 = 0.51$) both increased with temperature. These data suggest that sublethal yet stressfully elevated temperatures limit growth in brook trout and may provide a mechanism by which this species is ecologically limited. Gill Hsp-70 may serve as a valid biomarker for exposure to stressful temperatures in wild brook trout populations. (This project was supported by USGS and a predoctoral fellowship to JG Chadwick.)

P24.
EFFECTS OF INDIAN HERBS ON THE MODULATION OF STRESS AND IMMUNE RESPONSE IN TILAPIA
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Consumers are now able to understand the negative effects of chemicals and are shifting to natural products. Natural products such as plant products are now being used to reduce stress, promote growth, and increase immune response in fish and shellfish. Some of these products are alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oils. In this experiment, we wanted to examine few Indian herbs such as, turmeric, garlic, and chili *in-vi*vo to modulate stress and immune response in fish, tilapia.Fish were fed with these herbs as additives and treated in two different conditions: controlled and stressed. In the stressed group the fish were crowded in tanks so that their optimal density for space is exceeded. Different physiological and biochemical parameters such as, condition factor, blood glucose, spleen somatic index (SSI), plasma protein, packed cell volume (PCV) and macrophage respiratory burst activity were measured. The data indicate that these herbs have positive effects on the reduction of stress. These neutraceuticals can be used in aquaculture industry to reduce stress related complexities such as, low growth rate, disease susceptibility, and high mortality.

P25.
RECEPTOR-MEDIATED REGULATION OF MYOSTATIN BY CORTISOL IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*
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Myostatin (mstn) is a well-characterized inhibitor of muscle growth in mammals. However, elucidating the function of mstn in fishes has not been achieved. Three putatively functional mstn isoforms have been identified in rainbow trout (*Oncorhynchus mykiss*, Rodgers *et al.* 2007). These isoforms (mstn-1a,-1b, and -2a) are ubiquitously expressed in rainbow trout, suggesting a wider and more divergent range of function in this species. Previous research has demonstrated differential expression of mstn isoforms in response to acute handling stress in several fish species, suggesting a link between the stress response and myostatin. We hypothesize that cortisol, the main stress hormone in fish, will differentially regulate mstn mRNA expression in a receptor-mediated manner. An *in vivo* study using rainbow trout was utilized to characterize the interaction between cortisol and mstn mRNA expression. Fish were allocated to one of the following treatment groups: control group, cortisol (2µg/g fish), RU-486 (2µg/g fish), combination of cortisol and RU-486 (2µg/g fish), vehicle control (2µl safflower oil/g fish). Fish were sampled at 12hrs and 24hrs post injection, tissues were harvested for total RNA isolation, and mstn-1a, -1b, and 2a mRNA expression was analyzed using quantitative PCR. Results indicate that cortisol does not directly regulate mstn expression, as seen in mammals. Interestingly, treatment with cortisol significantly increased mstn-2a mRNA expression in white muscle compared to the control group,

but not the vehicle control group. Mstn-2a mRNA levels were not affected by the oil-based vehicle control compared to the absolute control group. This lack of true response is consistent with the lack of glucocorticoid response element in the mstn gene promoters, and suggests an interesting and divergent regulation of stress and growth.
Reference: D. Garikipati, S.A. Gahr, E.A. Roalson and B.D. Rodgers (2007). Endocrinology 148(5):210-15

TOPIC: *ENDOCRINE DISRUPTION*

P26.
INVESTIGATING THE SUBLETHAL EFFECTS OF METAL NANOPARTICLES ON FROG METAMORPHOSIS WITH A CULTURED TAILFIN APPROACH
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Amphibian metamorphosis is a thyroid hormone (TH)-dependent, highly coordinated developmental event that is sensitive to environmental perturbations, both natural and anthropogenic. TH-inducible nuclear receptors (TRα, TRβ) act to regulate the transcription of target genes in both the absence and presence of TH, causing varying tissue responses. Also, *Rana* larval keratin 1 (RLK1), a novel type 1 keratin, is constitutively expressed in tailfin tissues but decreases in the presence of TH. The C-fin assay uses cultured tailfin biopsies from *Rana catesbeiana* (American Bullfrog) tadpoles and exposes them to increasing concentrations of test chemicals. Specifically, relative expression of mRNA levels of TRα, TRβ, and RLK1 were examined in the presence and absence of TH to identify disruption of TH signaling due to exogenous chemicals. Furthermore, three stress-responsive genes: catalase (CAT), superoxide dismutase (SOD) and heat shock protein 30 (Hsp30), were also examined to elucidate oxidative or chemical stress. The purpose of this research is to investigate endocrine disruption and stress response of sublethal concentrations of titanium dioxide (TiO_2) and silicon (Si) nanoparticles (NPs) with the C-Fin assay. NPs are used extensively in health care, food products, and consumer products, and physico-chemical properties distinct from their bulk counterparts. Furthermore, different functional coatings on the NPs allow multiple industrial applications, but also can alter their interaction with biological organisms. With the C-Fin assay, we found slight differences in thyroid signalling and cellular stress between different functionalized TiO_2 NPs, and perturbations in both due to Si NPs. Due to the high conservation of THs and TRs among vertebrates, care should be taken to characterize the effects of sublethal concentrations of these NPs fully.

P27.
THE C-FIN ASSAY: A NOVEL APPROACH TO CHARACTERIZING ESTROGEN/THYROID HORMONE CROSSTALK IN A WASTEWATER EFFLUENT CONTEXT
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Disruption in the health of wildlife populations has become increasingly prominent in recent history. One factor attributed to this decline has been the marked increase in the environmental loading of pharmaceuticals and personal care products (PPCPs) into our water systems. Many PPCPs are known to act as endocrine disrupting chemicals (EDCs). Frogs are environmental sentinels and serve as indicators of disruption for estrogen and thyroid hormone activities. We have developed a method to screen for endocrine disruption in *Rana catesbeiana* tadpoles: the cultured tail fin (C-fin) assay. One C-fin assay uses 8 athyroid premetamorphic tadpoles. Twelve tail fin biopsies from each tadpole are cultured in serum-free medium. Each biopsy is exposed to different treatment conditions with or without TH or estrogen. After 48 h, the biopsies are collected, the RNA is isolated, and quantitative real time polymerase chain reaction (QPCR) is performed. Transcripts encoding proteins in hormone- and stress-signaling pathways are used as the first indicators of endocrine perturbations. The repeated measures design of the C-fin assay allows for the screening of multiple conditions in one individual while maintaining biological variation and complex tissue structure. We are using effluent from pilot wastewater treatment plants to elucidate the actions of EDCs on tadpoles while simultaneously clarifying the mechanisms of potential estrogen/TH crosstalk. Using the C-fin assay we have observed some transcriptional perturbations in hormonal responses to municipal wastewater effluents. Thus the C-fin assay is a novel method to characterize the interplay of estrogen and TH all within the context of a relevant wildlife species.

P28.
ACTIVITY OF STEROID-CONJUGATING ENZYMES IN HATCHLING RED-EARED SLIDER TURTLES EXPOSED TO BISPHENOL-A
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Endocrine disrupting chemicals (EDCs) are a class of compounds that impact the functioning of the endocrine system. To more fully understand how EDCs affect an organism, it is important to study not only the endpoint effects of a compound, but also the transient physiological response of the organism to exposure such as the upregulation of sulfotransferases (SULT) and glucuronyltransferases (UGT), two superfamilies of hepatic enzymes. In vertebrates, SULT and UGT are the two main families of enzymes involved in phase II steroid metabolism and these same enzymes are also utilized by the body for clearance and removal of xenobiotics such as EDCs. This study aims to understand which detoxifying pathway is more prominent in EDC metabolism and whether or not the activity of these enzymes is affected by exposure to an EDC. To investigate this aim, red-eared slider (*Trachemys scripta*) hatchlings were injected with either Bisphenol-A or a vehicle control and livers were harvested 12, 24, or 48 hours post-injection. Liver homogenates (S9 fraction) were used for enzyme assays of SULT and UGT to quantify and compare enzyme activity. Preliminary analyses show a trend for increased enzyme activity of both SULT and UGT by 24 hours post-injection, however there appears to be a great deal of individual variation in ability to respond to exposure through increased enzyme activity. Investigating changes in enzyme activity will provide insight into mechanisms of endocrine disruption, allowing for a more complete understanding of potential threats of endocrine disruptors on humans and wildlife. (Supported by EPA STAR fellowship F09G11129 to S.G.C.)

P29.
IMPACTS OF BISPHENOL-A EXPOSURE DURING LARVAL DEVELOPMENT IN CONTAINER DWELLING MOSQUITOES
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Endocrine disrupting compounds (EDCs) have been implicated in a variety of biological problems from population decline to various cancers. Bisphenol-A (BPA) is one such endocrine disruptor that is widely used in manufacturing and is nearly ubiquitous in the environment. BPA has been shown to have estrogenic (feminizing) and toxic effects in developing organisms from a wide range of vertebrate taxa; however, little is known about effects of this compound on invertebrates. This study investigates endpoint effects and physiological responses of larval mosquitoes to BPA exposure, and the results will inform our understanding of EDC effects on invertebrates. To study the endpoint effects of BPA, larvae of two species of container-dwelling mosquitoes, *Aedes aegypti* and *Aedes albopictus*, were reared individually in one of eight concentrations of BPA (serial dilution; 100ppm-0.01ppb) or in a control lacking BPA. We recorded time to adulthood, survival, mass and sex. We found that BPA did not affect sex ratios, time to adulthood or mass in either species; however there was a significant negative effect of BPA on survival in both species at 100ppm. To assess the larval response to BPA we will test for the presence of BPA metabolites; preliminary trials indicate that a detectable amount of water-soluble metabolites (presumably BPA conjugates) are present in the system 48 hours after exposure. In a larger follow up study, larvae will be initially reared individually in water free of BPA. At each instar, 15 larvae will be exposed to tritiated BPA and after 48 hours we will test for the presence of metabolites using thin-layer chromatography. Understanding how mosquitoes respond to exposure to EDCs may help to inform the conservation or control of other invertebrate species with similar life histories. This could subsequently lead to a better understanding and therefore greater effectiveness of pest control. (Supported by EPA STAR fellowship F09G11129 to S.G.C.)

P30.
SUBLETHAL CADMIUM EXPOSURE IMPACTS THE STRESS PERFORMANCE IN RAINBOW TROUT
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Cadmium is widely distributed in the aquatic environment and is toxic to aquatic organisms even at sublethal concentrations. Although cadmium is sequestered predominately within the liver and kidney, and rainbow trout are able to adapt to chronic exposure, the impact of this metal on animal performance is poorly understood. We tested the hypothesis that sublethal concentrations of cadmium alter the metabolic status and impact the stress response to a secondary stressor in rainbow trout. To test this, immature rainbow trout were exposed to 0 (control), 0.75µg/L (low) or 2.0µg/L (high) concentrations of cadmium using a flow-through system for 28d. Fish were sampled for blood and tissues at 4h, 1d, 7d and 28d after exposure. There were no changes in plasma cortisol or the head kidney steroid biosynthetic capacity in response to cadmium exposure over the 28d period. The liver metabolic capacity was impacted at the high, but not low cadmium concentrations over the 28d period. However, subjecting these fish to a secondary acute stressor after 28d exposure resulted in the attenuation of plasma cortisol and glucose levels in the cadmium groups compared to the control fish. Overall, the results suggest that environmentally relevant concentrations of cadmium will disrupt the evolutionarily conserved cortisol stress response to secondary stressors in trout. (Supported by NSERC discovery grant to MMV).

P31.
EFFECTS OF PRENATAL BISPHENOL-A ON GOAL-DIRECTED BEHAVIOR AND INSULIN RESISTANCE IN SUFFOLK SHEEP
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Bisphenol-A, an endocrine disrupting chemical that mimics estrogen, is found in commonly used containers. Low doses of exposure in human fetuses are of concern during periods of development. Previous research shows that prenatal BPA exposure elevated the risk of insulin resistance in mice; this current study investigates whether insulin responses are predictive of behavioral responses in tests of goal-directed behaviors in a sheep model. Goal-directed behavior was determined by measuring consumption of palatable food (PFT) and the rate of learning in a conditioned place preference test (CPP), in male and female peripubertal animals exposed to 0.5 mg/kg/day BPA or vehicle daily between days 30-90G. Insulin responses were determined using a glucose tolerance test. Control males demonstrated greater goal directed behavior for food than control females. BPA and control males show no difference in the amount of food consumed in the PFT. BPA females and injection controls (IC) are consuming more food than uninjected control females, 89.77% and 86.09% respectively, which was significantly higher than uninjected control females (57.77%). BPA males and BPA females show no difference in time spent in the conditioned side of a CPP from same-sex controls. There is no effect of BPA on male motivation for food. In females, daily injection of the ewe has masculinized the motivation for food. This study demonstrates the sex differences in food-induced goal directed behavior is not affected by BPA doses commonly found in humans.

P32.
POLYBROMINATED DIPHENYL ETHERS CAN DISRUPT MOLTING IN *DAPHNIA MAGNA* NEONATES
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Polybrominated diphenyl ethers (PBDEs) are flame-retardants, which can bioaccumulate and biomagnify and are found worldwide despite their banned usage in some countries. In recent years the possibility that PBDEs may disrupt endocrine functions in vertebrates has been well investigated, but little attention has been paid to the endocrine disrupting potential of these persistent contaminants in aquatic invertebrates. The current study aimed to investigate whether PBDEs affect molting in *Daphnia magna*. Prior to molting studies, 48-hr LC50 values of 5 environmentally prevalent PBDEs were determined. A series of concentrations of PBDEs-28, -47, -99, -100 and -209, and water and vehicle (0.1% DMSO) controls, were prepared in triplicates of 10 daphnids (<18 hours old) per treatment, and survival assessed at 48 hours. For molting studies, sublethal concentrations of these PBDEs and controls were prepared for 20 daphnids per treatment, and molts recorded every 24 hours, then every 6 hours, until all neonates reached 4 molts. The 48-hr LC50s were 110.7, 7.9, 2.6, and 11.1 ng/ml for PBDEs-28, -47, -99, -100 respectively. The highest concentration of PBDE-209 tested (2.5 µg/ml) did not affect survival. PBDE-28 at 12 ng/ml significantly increased the time it took to complete 4 molts (198.2 $\pm$ 8.0 hours) compared to controls (145.8 $\pm$ 6.6 hours), $P < 0.01$. PBDE-99 at 6.7 ng/ml also increased the time to 4 molts (133.5 $\pm$ 2.6 hours c.f. 153.0 $\pm$ 5.1 for controls), but the trend was not statistically significant. No other PBDEs affected this endpoint at the concentrations tested. However, during the study there were times when the number of molts was significantly lower than controls for PBDE-47 (20 ng/ml) and PBDE-99 (6.67 ng/ml), a pattern which is likely to reflect metabolism of PBDEs during the treatment period. This

study found that some PBDEs can delay molting at environmentally relevant concentrations, and raises concern for disrupted molting in crustaceans exposed to PBDEs.

TOPIC: *GONADAL DEVELOPMENT AND GAMETE MATURATION*

P33.
LOCAL EXPRESSION AND STEROIDOGENIC EFFECTS OF GROWTH HORMONE (GH) IN THE CHICKEN OVARY AND FOLLICULAR GRANULOSA CELLS (GC)
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Preovulatory follicular development (PFD) is mainly regulated by the gonadotropins (FSH, LH), steroids and intraovaric factors. Here we analyzed the role that local GH may exert on the regulation of steroidogenesis and proliferation in granulosa cells (GC) of the hen's ovary. Follicles from 35-48 week-old hens were used at different developmental stages (F4 at the beginning and F2 at the end of development). Both GH mRNA (by *in situ* hybridization) and protein (by immunohistochemistry) were expressed mainly in the GC, and to a lesser extent in the theca cells of the follicular layers. Sequence of a GH cDNA 690-bp fragment obtained from F2 follicular wall was basically identical to that obtained from the pituitary. Although several GH variants were observed by SDS-PAGE and Western blot, the main isoform showed a MW of 17 kDa, at all developmental stages. Addition of GH (0.1, 1, 10 nM) stimulated the synthesis of progesterone (P4) in primary GC cultures in a dose-dependent manner (1.5, 2.9, 5.4 times, respectively). GH also stimulated the expression of cholesterol side-chain cleavage enzyme (cytochrome P450scc) mRNA, a rate-limiting enzyme during P4 synthesis (2.9, 4.6, 4.9 times, respectively), whereas the synthesis of 3ß-hydroxysteroid dehydrogenase (3ß-HSD) (a constitutive enzyme) was not changed. The locally expressed GH stimulated the proliferation of cultured GC and their P4 production (1.2, 2.2, 4.4 times at 4X, 6X and 8X concentrated conditioned media, respectively). These data suggest that locally produced GH may modulate follicular development through autocrine/paracrine effects in the chicken ovary. (Supported by CONACYT (60296N & 161791) and PAPIIT-UNAM (IN210209) grants.)

P34.
CHARACTERIZING AQUAPORIN 1B EXPRESSION DURING OVARIAN FOLLICULAR DEVELOPMENT IN THE ZEBRAFISH *DANIO RERIO*
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The resumption of meiosis in teleost ovarian follicles is accompanied by an increase in follicle volume due to the uptake of water; a process referred to as oocyte hydration. Recent studies have identified a teleost-specific water channel protein, aquaporin 1b (Aqp1b) as responsible for facilitating oocyte hydration in a number of marine species. Surprisingly little is known of the involvement of Aqp1b in oocyte maturation and the hormones that regulate its expression. This study investigated the role of Aqp1b in oocyte hydration in the zebrafish by evaluating temporal patterns of Aqp1b expression and examining the regulation of expression by reproductive hormones and intracellular signalling factors *in vitro*. Using RT-qPCR, it was demonstrated that Aqp1b is expressed in all stages of ovarian follicles, and expression is significantly higher in midvitellogenic follicles than full grown follicles. *In vitro* incubations with human chorionic gonadotropin (hCG), 17α,20β-dihydroxy-4-pregnen-3-one (17,20βP), forskolin and phorbol 12-myristate 13-acetate (PMA) had no effect on the level of Aqp1b expression in full grown follicles, whereas hCG significantly decreased expression and PMA significantly increased expression in midvitellogenic follicles. Contrary to expectations, Aqp1b appears to be constitutively expressed in the ovary, and the level of expression remains constant during oocyte maturation. These findings suggest the possibility that Aqp1b is regulated at a post-transcriptional level, as has been documented in other teleost species. Complementary analyses of Aqp1b synthesis and trafficking would be valuable in producing a comprehensive understanding of the molecular mechanisms of Aqp1b and its role in the zebrafish ovarian cycle.

P35.
REGULATION OF OVARIAN PROSTAGLANDIN SYNTHESIS IN THE ZEBRAFISH: ACTIONS OF GONADOTROPIN, 17A, 20B-DIHYDROXY-4-PREGNEN-3-ONE, AND INSULIN-LIKE GROWTH FACTOR -1
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Oocyte maturation and ovulation are sequential events leading to the release of an oocyte that is ready to be fertilized. While much is known of the hormonal control of oocyte maturation, surprisingly little is known of the control of prostaglandin production which mediates ovulation. 17α, 20β-dihydroxy-4-pregnen-3-one (17,20β-P) and the insulin-like growth factor (IGF) system are key regulators of oocyte maturation in the zebrafish (*Danio rerio*). The goal of this study was to determine if 17,20β-P and IGF-1 affect the gene expression of two enzymes involved in prostaglandin biosynthesis, phospholipase A_2 ($cPLA_2$) and cyclooxygenase 2 (COX-2), and the levels of PGE_2 and $PGF_{2\alpha}$ produced by full grown zebrafish ovarian follicles. Addition of 17,20β-P caused an increase in $cPLA_2$ (*cpla2*) gene expression, whereas IGF-1 increased both $cPLA_2$ and COX-2 (*ptgs2*) gene expression. There was an additive increase in $cPLA_2$ gene expression when both hormones were combined. Measurement of prostaglandin levels from the incubation medium revealed that both 17,20β-P and IGF-1 increased $PGF_{2\alpha}$ production, and IGF-1 also induced PGE_2 synthesis. Furthermore, the effects of IGF-1 on gene expression and prostaglandin production where enhanced when combined with the gonadotropin analog human chorionic gonadotropin (hCG). These results suggest that the regulation of prostaglandin biosynthesis in fish ovarian follicles involves the coordinated actions of multiple hormones.

P36.
REGULATION OF THE NOVEL INSULIN-LIKE GROWTH FACTOR 3 LIGAND IN THE OVARY OF ZEBRAFISH
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The insulin-like growth factor system has garnered recent attention for its potential role in ovarian development in fish with the discovery of the gonad specific IGF-3. It has been shown that recombinant human IGF-1 and -2, as well as recombinant zebrafish IGF-3 are potent stimulators of oocyte maturation

in zebrafish. Previous studies from our lab demonstrated that IGF-3 gene expression was stimulated by the gonadotropin analog human chorionic gonadotropin (hCG). The goal of this study was to further investigate the actions of various hormones and signal transduction pathways in the regulation of IGF-3 gene expression in the zebrafish ovary. Real-time PCR was used to quantify IGF-3 gene expression in full grown (FG) and mid-vitellogenic (MV) follicles. Growth hormone, a common regulator of the IGF system, had no effect on IGF-3 gene expression in either follicle size class. The adenylate cyclase activator forskolin caused a substantial increase in IGF-3 gene expression in FG and MV follicles. The increase in IGF-3 gene expression in follicles treated with hCG was abolished by the addition of PKA inhibitor H-89. PMA and A23187, stimulators of protein kinase C (PKC), both significantly repress IGF-3 gene expression in both size classes of zebrafish follicles. Interestingly, melittin, a stimulator of arachidonic acid (AA) release and prostaglandins, downstream products of AA metabolism, both cause a small reduction in IGF-3 gene expression in FG follicles. Taken together these results demonstrate that IGF-3 gene expression is up-regulated by hCG through a cAMP-dependant pathway, whereas IGF-3 gene expression is down-regulated by the AA pathway possibly in a PKC-dependant manner.

P37.

SECRETION AND PROLIFERATIVE EFFECTS OF CHICKEN GROWTH HORMONE IN TESTICULAR CELL CULTURES TREATED WITH GHRH

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Recent findings showed the cytoplasmic and nuclear distribution of growth hormone (GH) and its receptor (GHR) in chicken testicular germ cells, suggesting an intracrine, autocrine and/or paracrine role for this hormone during spermatogenesis. The expression of testicular GH may be modulated by a variety of factors, including growth hormone releasing hormone (GHRH) and Pit-1. We analyzed the effect of GHRH (1, 10 and 100 nM, for 2 h) on GH release into the media of primary cell cultures derived from chicken testes at two developmental stages: during puberty (20 weeks, PC) and sexual maturity (32 weeks, MC). Released GH increased after GHRH treatment both in PC media (10.8±1, 13.4±2 and 14.5±2 ng/ml) and MC media (5.83±2.05, 7.6±0.6 and 8.5±2 ng/ml) in comparison to the corresponding controls (7.4±1 and 3.2±0.7 ng/ml, respectively). Western blot analyses were performed to characterize whether GHRH modulation involved secretion of particular GH isoforms. Interestingly, 15 kDa and 26 kDa GH variants were differentially modulated after 100nM GHRH treatment, resulting in a 2.5 fold-increase in 15 kDa GH secretion, whereas a significant decrease was found for the 26 kDa variant release. On the other hand, GHRH (1, 10, 100 nM) stimulated proliferation of testicular cells in culture (2.7-, 3.6- and 2.0-fold, respectively, in comparison to the controls) as determined by the PCNA assay. Using the ^{3}H-thymidine incorporation assay GH also increased the proliferation of testicular cells (by 118.7±18.07%, 170.79±28% and 102.1±15.7% at 1, 10 and 100 nM, respectively, when compared to 96.06±12.4% from the untreated controls). Altogether, these results suggest a role of GHRH and GH during chicken testicular cell proliferation, probably through autocrine/paracrine mechanisms. [This work was supported by grants CONACyT (F1-60296) and PAPIIT-UNAM (IN-210209), graduate fellowships from CONACyT (185024) and PDCB-UNAM and Alejandro Bayón Fund established by CA.]

P38.

IDENTIFICATION OF OVARIAN GENE EXPRESSION PATTERNS DURING VITELLOGENESIS IN ATLANTIC COD (*GADUS MORHUA*)

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One of the most pervasive problems in aquaculture is reproductive failure due to inferior egg quality. To alleviate this problem, more information is needed on the intraovarian factors that regulate oocyte development. The purpose of this study was to identify molecular markers during oocyte growth that could be used as predictors of maturational competence, using Atlantic cod (*Gadus morhua*) as a model species. Cultured cod in their first reproductive season were sampled during mid and late stages of vitellogenic growth (474-587 and 736-814 µm oocyte diameter, respectively). Representational difference analysis and real time quantitative PCR were used to identify differences in gene expression from intact ovarian follicles and follicle cell layers. A type IV ice-structuring protein (similar to apolipoproteins) and gephyrin 2 were upregulated among oocyte-derived transcripts during late vitellogenesis. Within follicle cells, luteinizing hormone receptor, aromatase, and serotonin receptor 1E were also upregulated during late vitellogenesis, while a conserved G protein-coupled receptor (GPR27) was downregulated. Several cytoskeletal genes (cysteine-rich protein 1, myosin-2, transgelin) in follicle cells were also upregulated in late vitellogenesis and likely represent thecal cell transformation to smooth muscle-like tissue prior to ovulation. These results indicate that changes in gene expression during vitellogenesis influence lipid binding, maturational competence and ovulation and may serve as potential markers to improve spawning procedures used in aquaculture. (Research supported by NH Sea Grant and travel supported by UNH Center for Comparative and Molecular Endocrinology.)

TOPIC: *GROWTH AND AGING*

P39.

T_3 AND 3,5 -T_2 PARTICIPATE IN TILAPIA GROWTH THROUGH A DIFFERENT SIGNALING PATHWAY

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Considered the biologically active thyroid hormone (TH); triiodothyronine (T_3) actions involve TH-dependent *de novo* gene transcription and are mediated by TH nuclear receptors (TR). Recent studies from our laboratory have shown that in some teleosts, 3, 5-T_2, a TH formed through the outer-ring deiodination of T_3 is also bioactive. The effects of 3, 5-T_2 appear to be mediated by a different isoform of TRβ1 that contains a 9 amino acid insert in its ligand binding domain, and which we have denominated long TRβ1 (L-TRβ1). To further understand 3, 5-T_2 bioactivity and its action mechanism, we used body growth as an endpoint and analyzed 3, 5-T_2 participation in this process, as well as the possible differential regulation of the signaling pathway of both iodothyronines. To this end, we treated tilapia fingerlings (~0.8 ±0.02 g) by immersion with 1.3 nM of one of the following TH: T_3, 3, 5-T_2, the prohormone T_4 and the inactive isomer rT_3. A negative control group was treated with methimazole (MMI: 4.5 mM). All experimental groups were treated for 8 hours, three times week for a month. We recorded growth (body weight) and measured the expression of GH, IGF-1, S-TRβ1 (short TRβ1: without the 9 aa insert) and L-

TRβ1. These genes are involved in growth and/or are part of TH signaling pathway. Growth rate of fish exposed to T_4, T_3 or 3, 5-T_2 was significantly higher (average of 26%) than that of the control (untreated) group ($p < 0.001$), while growth rate of tilapia exposed to rT_3 was similar to control fish. Tilapia treated with MMI showed the lowest growth rate ($p < 0.001$). Intrahepatic expression of GH and IGF-1 was up-regulated by both, 3, 5-T_2 and T_3, while the expression L-TRβ1 increased only in the 3, 5-T_2-treated fish and S- TRβ1 was up-regulated only by T_3. Our results support the notion that although T_3 and 3, 5-T_2 seem to participate in the growth process, the signaling pathways of both hormones are regulated differently. (Tilapias were kindly provided by SAGARPA, Querétaro, Qro., and kept in laboratory facilities by Miguel Angel Maqueda. This work was partially supported by: CONACYT 080420 and PAPIIT IN203409).

P40.

MOLECULAR CLONING OF HEPATIC INSULIN-LIKE GROWTH FACTOR-1 CDNA AND SEQUENCE ANALYSIS IN LIZARDS

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The insulin-like growth factor (IGF) hormone family is well conserved in structure and function across all vertebrates. This high degree of conservation is likely due to the critical roles that these hormones play in development, growth, and metabolism. To date, the structure and function of insulin-like growth factors have been described in a wide range of vertebrate taxonomic groups, however, lizards have received little attention. Therefore, the objective of this study was to indentify IGF-1 cDNA sequence data from five lizard species. Primers designed against conserved regions of IGF-1 were used to generate cDNA from *Sceloporus undulatus*, *S. jarrovii*, *Anolis sagrei*, *Coleonyx elegans*, and *Goniurosaurus lichtenfelderi*. The cDNA was cloned into the pCR™4-TOPO® TA vector and sequenced. The resulting cDNA base sequences were converted to amino acid (AA) sequences. *Sceloporus undulatus* IGF-1 contained 55 AA and *S. jarrovii*, *A. sagrei*, *C. elegans*, and *G. lichtenfelderi* IGF-1 contained 43 AA. Comparison of the deduced AA sequences of IGF-1 to human IGF-1 confirms high sequence identity: *S. undulatus* (80%), *S. jarrovii* (74%), *A. sagrei* (72%), *C. elegans* (74%), and *G. lichtenfelderi* (77%). High IGF-1 sequence identity was observed between these species of lizards (86-100%). Although these species belong to the same order, variation occurred in the C domain, which is likely due to the fact that Iguania and Gekkota diverged approximately 240 million years ago. The percent of sequence identity is associated with the degree of relatedness between species and progressively decreases with evolutionary distance. To our knowledge, we are the first to clone IGF-1 from any species of reptile. The partial IGF-1 sequences cloned from lizards contribute to the growing body of literature that indicates that the high percent of sequence identity is associated with the essential involvement of IGF-1 in growth regulatory mechanisms among vertebrates. (Supported by the Society for Integrative and Comparative Biology, Graduate School-New Brunswick, and School of Environmental and Biological Sciences.)

P41.

EFFECTS OF CORTISOL ADMINISTRATION ON *ONCORHYNCHUS MYKISS* MYOBLAST PROLIFERATION AND MYOSTATIN ISOFORM EXPRESSION

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In mammals, myosatellite cells and the resulting myoblasts appear to be affected by the primary stress hormone cortisol. Specifically, glucocorticoids appear to enhance myoblast proliferation in primary murine and human myoblast models and decrease proliferation rates in C2C12 cells. Additionally, a potent negative regulator of muscle tissue, myostatin, appears to be up-regulated in response to increasing concentrations of cortisol, at least *in vitro*. In salmonid fishes, however, the actions of cortisol on myoblast proliferation and myostatin expression have been little studied. In addition, the *myostatin* promoters of several salmonid species do not appear to contain glucocorticoid response elements (GRE). Here, we present data indicating that primary myoblasts isolated from juvenile rainbow trout (*Oncorhynchus mykiss*) exhibit a differential response to cortisol as compared to mammalian systems, like C2C12 cells. In rainbow trout primary myoblasts treated with cortisol, no changes in proliferation rates were detected; however, a decrease in proliferation occurred upon administration of the glucocorticoid receptor antagonist mifepristone (RU-486). Similarly, a direct effect of cortisol on the expression of the three putatively functional *myostatin* isoforms in rainbow trout, *myostatin-1a, -1b and -2a,* was not detected. These data suggest evolutionary changes in the regulation of salmonid myoblast proliferation by glucocorticoids, a phenotype that may be linked to the indeterminate growth of these animals. Likewise, the absence of mRNA transcription changes in the presence of cortisol supports the *in silico* finding that the promoters of the three functional rainbow trout *myostatin* isoforms do not contain glucocorticoid response elements. (Supported by ND EPSCoR #0447679, NIH NCRR #2P20RR015566 (NDSU CPR) and NIH NIAMS #1R03AR055350 to PRB.)

TOPIC: *DEVELOPMENTAL ENDOCRINOLOGY*

P42.

APPARENT ECDYSONE 20-MONOOXYGENASE ACTIVITY IN THE ADULT PARASITES *ASCARIS SUUM* (NEMATODA) AND *HYMENOLEPIS DIMINUTA* (CESTODA)

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Arthropods and nematodes share common developmental patterns in which their growth exceeds the capacity of their exoskeleton/cuticle. Thus, the animals will undergo the process of molting, where the exoskeleton/cuticle is shed and a new larger one is synthesized in its place. Polyhydroxylated keto-steroids, i.e., ecdysteroids, are critical regulators of molting in arthropods. The conversion of ecdysone (E) to the active molting hormone, 20-hydroxyecdysone (20E), at the target tissues for molting hormone action elicits molting. In arthropods the enzyme which converts E to 20E is a cytochrome P450-dependent steroid hydroxylase, ecdysone 20-monooxygenase (E20M), an enzyme highly conserved amongst arthropods. The parasitic nematode *Ascaris suum* undergoes four molts in its life cycle although the regulation of these molts is poorly understood. Studies with *A. suum* and another parasitic helminth, viz., the cestode *Hymenolepis diminuta*, revealed that ecdysteroids including E and 20E are present in tissue extracts of these animals, and that exogenous application of ecdysteroids elicits physiological events. These data suggest that ecdysteroids may play a role in parasite development, so an investigation of the presence of E20M-like activity in *A. suum* and *H. diminuta* was initiated. Utilizing a radioenzymological assay for E20M activity in insect tissues, *A. suum* muscle, reproductive tissue, and whole *H. diminuta* homogenates were subjected to differential centrifugation and the resulting subcellular fractions assayed. All *A. suum* muscle and reproductive tissue fractions displayed significant levels of E20M-like activity. Initial evaluations indicated that all *H. diminuta* fractions also displayed significant activity levels. These appear to be the first indications that the enzyme needed for forming active molting hormone is present in these helminths and that more study is warranted.

P43.

TEMPORAL AND SPATIO-REGULATION OF SOX3 BY THYROID HORMONE SUGGESTS A ROLE FOR SOX3 IN EPITHELIAL PROGENITOR DEVELOPMENT DURING INTESTINAL METAMORPHOSIS IN *XENOPUS LAEVIS*

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During amphibian metamorphosis, the small intestine is extensively remodeled from the larval to adult form under the control of thyroid hormone (TH). The remodeling of the small intestine during amphibian metamorphosis comprises first the apoptosis of the larval epithelia and then a burst of cell proliferation followed by differentiation. The resulting adult intestine is analogous to the mammalian intestine. We are using the intestinal metamorphosis in *Xenopus laevis* as a model to study vertebrate intestinal development. Previous studies have suggested that the cell fate change during TH-dependent frog intestinal metamorphosis is under the control of TH through tissue-specific gene regulation. To understand how TH-regulated tissue-specific gene expression affects cell fate during frog intestinal metamorphosis, we performed cDNA array analysis on intestinal tissue-specific RNA (epithelium or non-epithelium, mainly connective tissue of the intestines) of premetamorphic (stage 56), climax (stage 61), and the end of metamorphosis (stage 66) tadpoles and identified a number of genes specific to different tissues. Most interestingly among them, the transcription factor Sox expresses specifically in the proliferating epithelial progenitor/stem cells at the climax of metamorphosis. These results suggest that Sox3 may play an important role in the development and/or maintenance of the epithelial progenitor/stem cell population during the intestinal remodeling in *Xenopus laevis.*

P44.

ECDYSONE CONTROLS THE PROGRESSION OF IMAGINAL DISC PATTERNING AND REGULATION OF SIZE

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The essential question of how size is regulated, from organelles to organisms, remains unsolved. A fundamental developmental process, if size regulation fails it can affect organ function as well as whole body integrity and fitness. We aim to determine how mechanisms regulating nutrition-dependent development, developmental timing and tissue patterning are integrated to produce an organism of the appropriate size and proportions. To achieve this, we have established a temporal patterning map of the presumptive adult tissues, the imaginal discs, which we will use both as a measure for developmental timing and to better understand growth termination. This map includes the description of gene expression patterns representative of most of the major patterning cascades in the imaginal disc throughout the third instar larva, the stage at which most growth occurs. Next, we are exploring the mechanisms allowing ecdysone signalling to regulate the growth and differentiation of imaginal tissues and larval body. We can eliminate ecdysone in the larval body by specifically ablating the gland that produces the hormone, the prothoracic gland (PGX), in the third instar. Our preliminary data shows that PGX larvae grow at normal rates but their imaginal discs pattern slowly and grow at significantly reduced rates. To discover how ecdysone differentially regulates the growth of imaginal versus larval tissues, we will compare the expression of known growth regulators like dMyc, Insulin Receptor and cyclin D in the wing imaginal discs and the larval fat body. Using this approach, we hope to generate a more comprehensive view of growth control.

P45.

EXPRESSION AND FUNCTIONAL ANALYSES OF ERYTHROPOIETIN IN THE TRANSITION OF RED BLOOD CELLS FROM THE LARVAL TO ADULT TYPE IN *XENOPUS LAEVIS*

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In anuran amphibians, the larval RBCs are replaced by adult RBCs during metamorphosis. The molecular mechanisms by which the larval blood cells are specifically removed from circulation are not yet understood. We identified *Xenopus* tumor necrosis factor-related apoptosis-inducing ligand 1 (xTRAIL1) as ligand of *Xenopus* death receptor-Ms (xDR-Ms) and suggested that xTRAIL1 can cause apoptosis, probably mediated through xDR-Ms, in larval RBCs, but may not kill adult RBCs, presumably owing to Protein kinase C (PKC) activation, as part of the mechanism for RBC switching. In this study, we investigated whether erythropoietin (Epo) can contribute to survival of the adult RBCs during metamorphosis, because Epo induces PKC activation in mammalian erythroblasts. We first examined the expression levels of *epo* mRNAs during development. Quantitative RT-PCR revealed that the *epo* mRNA was most abundant in the lung at stage 62. We next examined an effect of Epo in xTRAIL1-induced apoptosis of the adult RBCs. Treatment of them with Epo significantly inhibited the apoptosis. These results implied that Epo might participate in survival of the adult RBCs in the transition of RBCs. Now, we examine whether the survival signaling induced by Epo is specific for the adult RBCs during metamorphosis. (This work was supported by Kitasato University Research Grant for Young Researchers (K.T.))

P46.

EXPRESSION LEVELS OF TRα, LAT1, AND PKM2 ALTER THE RATE OF METAMORPHIC CHANGE

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Thyroid hormone (TH) plays a key role for most physiological and developmental changes during metamorphosis. TH exerts its function by binding thyroid hormone receptors (TRs) in the nucleus to initiate TH-dependent gene expression. Intracellular TH levels are regulated by thyroid hormone transporters (THTs) and cytosolic thyroid hormone binding proteins (CTHBPs). Intracellular TH levels and TR expression levels likely regulate the timing of transformation of different tissues during metamorphosis. We hypothesized that altered TR, LAT1 (a THT), and PKM2 (a CTHBP) expression affects the rate of metamorphosis when overexpressed. In this study, we overexpressed TR α, LAT1, and PKM2 in tail muscle cells by intramuscular plasmid DNA injection and quantified the disappearance of tail muscle cells during T3-induced metamorphosis. In cells overexpressing TR, the rate of cell death was significantly faster compared to controls.. Moreover, cell death was accelerated or delayed in muscle cells overexpressing LAT1 or PKM2, respectively, compared to controls. These results show that the expression levels of TR and proteins affecting intracellular TH levels can affect the rate of metamorphic changes.

P47.

INHIBITOR OF GROWTH (ING) FAMILY TUMOR SUPPRESSOR PROTEINS ARE NOVEL MODULATORS OF THYROID HORMONE ACTION IN *XENOPUS LAEVIS* TADPOLES

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A striking example of thyroid hormone (TH) effects on postembryonic development is found in frog tadpole metamorphosis, during which a wide variety of cellular processes take place in different tissues. This process is completely dependent upon THs (T_3 and T_4), which primarily exert their effects through high-affinity binding to nuclear TH receptors (TRs). TRs, bound to DNA in the promoter regions of certain genes, modulate their transcription by recruiting cofactors and interacting with basal transcription machinery. Proper TH signaling through TRs is critical to the growth and development of most vertebrates. Serious growth and cognitive defects occur when TH signaling is altered. There is a great need to identify the cellular effectors of TH action. Inhibitor of Growth (ING) tumor suppressors are members of the plant homeodomain (PHD) finger protein family, which regulate chromatin structure and gene expression. ING proteins are important regulators of proliferation and apoptosis. Several ING transcript variants exist in *Xenopus laevis* frogs, most of which have previously been shown by our group to be TH-responsive in a tissue-specific manner. We now expand on these data by determining the expression profiles of newly discovered *X. laevis* ING isoforms. We have also revealed a novel function for ING proteins: modulation of TH action. Here, we examine the effects of manipulating ING transcript and protein levels on TH action in *X. laevis* tadpoles. We demonstrate that ING overexpression causes TH-dependent morphological and transcript-level perturbations in transgenic *X. laevis* premetamorphic tadpoles exposed to exogenous T_3. Using a *X. laevis* oocyte model, we also show that ING overexpression enhances the TH-dependent transcription of a reporter gene. ING family members may thus be part of a regulatory feedback mechanism of TH action in frogs. (This work was funded by NSERC and a NIH intramural research program).

P48.

ABNORMAL CORTISOL LEVELS AFFECT ZEBRAFISH EARLY EMBRYOGENESIS

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The role of the glucocorticoid receptor (GR) signaling in the stress response has been well studied in adult vertebrates. In general, the stress response is activated after hatching in fishes, and in late stage fetal development in mammals. However, until recently, the function of GR in early development was unknown. Our prior research detailed a critical role for the glucocorticoid receptor in initial embryogenesis using zebrafish (*Danio rerio*) as a model. We have shown that levels of cortisol, the primary GR ligand in teleosts and most mammals, decrease temporally after initial maternal hormone deposition and prior to hatching. We hypothesized that this pre-hatch time period represents a phase of tightly regulated cortisol signaling and that low cortisol levels maintain the proper developmental patterns mediated by the glucocorticoid receptor. To test this, we microinjected cortisol (32pg/egg) into the yolk of one-cell zebrafish embryos and observed morphological changes during embryogenesis. We observed accelerated growth over the first 24 hours post fertilization (hpf), as measured by developmental staging and the development of somites from 12-15 hpf. In addition, a significant percentage of 48 hpf embryos develop heart defects, with some embryos exhibiting cardiac edema and others developing without a heart. Overall, abnormal cortisol levels during early development lead to growth and cardiac defects in zebrafish. (This study was supported by a Natural Sciences and Engineering Research Council of Canada discovery grant to MMV.)

P49.

ONTOGENY OF NESFATIN-1 AND PROHORMONE CONVERTASES IN THE GASTROENTEROPANCREATIC TISSUES OF SPRAGUE-DAWLEY RATS

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Nesfatin-1 is a novel metabolic peptide encoded in the precursor protein, nucleobindin-2 (NUCB2). Here we report, for the first time, NUCB2 expression in the gastroenteropancreatic tissues during the development of Sprague Dawley rats. The ontogeny of nesfatin-1 and PCs (PC 1/3 and PC 2) was compared in rat stomach, duodenum and pancreas by immunohistochemical studies at embryonic day 21 and postnatal day 1, 6, 13, 21, and 27. Nesfatin-1 immunoreactivity was abundant in the islet beta cells at postnatal day 13, 20, 27, but at embryonic day 21 and postnatal days 1 and 6, only a small number of islet cells were nesfatin-1 immunopositive. To further characterize nesfatin-1 positive cells in the pancreas, we performed double immunofluorescence with nesfatin-1 and insulin. Insulin and nesfatin-1 colocalized at all development stages. Nesfatin-1 immunoreactivity was found in the stomach from postnatal day 13, 20 and 27, but was relatively less in embryonic day 21, postnatal day1 and postnatal day 6. We found colocalization of nesfatin-1 with prohormone convertases 1 in adult pancreatic islet cells and at postnatal
day 13, 20, 27. At embryonic day 21 and postnatal days 1 and 6, only a small number of islet cells were immunoreactive for nesfatin-1 and PC1. Pancreatic islets cells at embryonic day 21, postnatal day 1 and 6 were immunoreactive for PC 2, but did not colocalize with nesfatin-1. The levels of NUCB2 mRNA expression increased in the heart, duodenum and pancreas; although no developmental mRNA expression changes were observed in the liver and stomach. Plasma nesfatin-1 levels increased with age, reaching adult levels at postnatal day 27. Together, synthesis and secretion appears to gradually increase during development. These results collectively suggest that nesfatin-1 has important age- and tissue-specific roles in the developmental physiology of rats. [This work was funded by an operating grant from the Canadian Institutes of Health Research (CIHR) and a Research Tools and Instruments Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to SU. SU is a CIHR New Investigator and is a recipient of the Early Researcher Award from the Ontario Ministry of Research and Innovation.]

P50. IGF SIGNALING REGULATES SPATIAL AND TEMPORAL ORGANIZATION OF NEWBORN GNRH NEURONS THROUGH THE PI3 KINASE PATHWAY IN ZEBRAFISH EMBRYOS

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The initiation of puberty and functioning of the reproductive axis depends on the proper spatial and temporal organization of GnRH neurons. GnRH neurons are born in the nasal compartment during embryogenesis, and migrate into the forebrain hypothalamus.How their spatial and temporal organization is controlled during embryogenesis remains poorly understood. In this study, we show that insulin-like growth factor (IGF) signaling, a central growth promoting signal, regulates normal spatial and temporal organization of newborn GnRH neurons in zebrafish. Blockage of IGF signaling by temporally-controlled expression of a dominant negative form of IGF receptor (IGF1R) or specific IGF1R kinase inhibitors delayed the timing of GnRH2 and GnRH3 neuron emergence. Furthermore, genetic or pharmacological blockage of IGF signaling in early embryos resulted in abnormal spatial distribution of GnRH3 neurons later in life. This action of IGF signaling is developmental stage-dependent because blockage of IGF signaling in advanced embryos had no such effect. This spatial regulation appears to be specific to GnRH3 neurons, because blockage of IGF signaling did not alter distribution of olfactory sensory neurons and midbrain GnRH2 neurons and had no effect on brain patterning. Real-time in vivo imaging of transgenic embryos, which express EGFP in GnRH3 neurons, revealed that the ectopic GnRH3 neurons emerge at the same time as the normal GnRH3 neurons, suggesting that IGF acts on GnRH precursor cells. In agreement, blockage of IGF signaling inhibited the expression several cranial neural crest marker genes. Finally, we showed that pharmacological inhibition of PI3 kinase pathway by LY294002 or wortmannin phenocopied the IGF signaling deficient embryos, while inhibition of MAPK signaling had no effect on GnRH3 neurons. These results suggest that IGF-IGF1R-PI3 kinase signaling pathway regulates precise timing and spatial distribution of newborn GnRH neurons in early embryos.

P51.

P52.

DEVELOPMENTAL AND THYROID HORMONE-INDUCED EXPRESSION OF DNA METHYLTRANSFERASES AND METHYL-CPG BINDING PROTEINS IN *XENOPUS* TADPOLE BRAIN DURING METAMORPHOSIS

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Postembryonic brain development is critically dependent on thyroid hormone (T_3), which influences neural cell proliferation, migration, differentiation, morphology and function. Thyroid hormone is known to promote neurogenesis, and recent findings showed that proteins that methylate the genome, DNA methyltransferases (DNMTs), and proteins that bind to methylated DNA, methyl-CpG binding proteins (MBDs), also play critical roles in cell lineage specification and neurogenesis in the developing brain. We hypothesized that T_3 promotes neurogenesis through its regulation of DNMT and MBD expression. To test this we investigated the developmental expression and T_3 regulation of DNMT (*dnmt1* and *dnmt3a*) and MBD (*mecp2*, *mbd1-4* and *kaiso*) genes in the brain of tadpoles of *Xenopus laevis*. We microdissected tadpole brains and analyzed mRNAs in the preoptic area/diencephalon by RTqPCR. We found that mRNAs for *dnmt1*, *dnmt3a*, *mbd3* and *kaiso* were upregulated during spontaneous metamorphosis. Treatment of premetamorphic tadpoles with T_3 (5nM) caused a time-dependent upregulation of each of these genes. *Dnmt3a* and *mbd3* mRNAs were upregulated by 12 hr; whereas, *dnmt1* and *kaiso* mRNAs were upregulated by 24 hr after T_3 treatment. Using *in situ* hybridization histochemistry, we confirmed the developmental and T_3-dependent increase in *dnmt3a* and *mbd3* mRNAs in the tadpole brain, and we investigated their regional expression. *Dnmt3a* mRNA is widely expressed throughout the tadpole brain in regions where cells are undergoing migration and differentiation, but is excluded from the ventricular and subventricular zones (VZ/SVZ) where cell proliferation occurs. By contrast, the expression of *mbd3* mRNA is restricted to the periphery of the VZ/SVZ throughout the tadpole brain. Our findings support that T_3 may control neurogenesis through regulation of expression of DNMTs and MBDs. (Supported by NSF grants IBN 0235401 and IOS 0922583 to RJD)

TOPIC: *THYROID*

P53.

ROLE OF THYROID HORMONES IN GONADAL SEX DIFFERENTIATION OF ZEBRAFISH

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An earlier study from our laboratory showed that treatment of larval zebrafish with perchlorate, a thyroid hormone (TH) synthesis inhibitor, biased the sex ratio toward females. The purpose of the present study is to determine and compare the effect on sex ratios of a different TH synthesis inhibitor, methimazole. Three-day postfertilization (dpf) larvae were reared for 30 days in control (reconstituted water), 0.82 mM perchlorate, 0.15 and 0.3 mM methimazole, and 1 and 10 nM TH (T4) solutions. Sex ratio, standard length (SL), head depth, head length and pectoral fin length (PFL) were determined at 45 and 60 dpf. Sex ratios were biased toward males in the TH treatments and toward females in the perchlorate treatment at both sampling times. Methimazole treatments biased sex ratios toward females only at 45 dpf. TH synthesis inhibitors trended to reduce, and T4 to increase, PFL (corrected for SL) at both sampling times. At 60 but not 45 dpf, SL was reduced in all treatments relative to control. Other SL-corrected morphometric traits were not affected. Histopathological analysis of thyroid follicles at 45 dpf indicated that both TH synthesis inhibitors similarly disrupted the negative feedback of the thyroid endocrine system. In conclusion, thyroid endocrine status dictates the direction of gonadal sex differentiation in zebrafish but, unlike their effect on development (PFL), the longevity of the effect of TH synthesis inhibitors on sex ratio differed between the inhibitors. This differential response may provide a useful tool to further study TH-dependent mechanisms of gonadal sex differentiation in zebrafish. (Supported by intramural funding from Texas Tech University and Texas Cooperative Fish and Wildlife Research Unit).

P54. WHERE IS THE DEIODINASE SELECTIVITY FOR ORD OR IRD LOCATED? A STRUCTURE-FUNCTION APPROACH

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Iodothyronine deiodinases (Ds) are a group of reductive dehalogenases that belong to the family of selenoenzymes and comprise three distinct isotypes: D1, D2 and D3. Ds control thyroid hormone (TH) intracellular availability and bioactivity by sequentially and stereo-specifically removing I_2 atoms from the TH molecule. In this way, T_4 outer-ring deiodination (ORD) generates the bioactive T_3 and 3,5-T_2, while its inner-ring deiodination (IRD) produces the inactive metabolite rT_3. While D2 and D3 exclusively catalyze the ORD and IRD pathways, respectively, D1 catalyzes both; however, the identification of the protein region that confers this catalytic selectivity is to date unknown. Protein modeling studies suggest that the molecular arrangement of the three paralogous includes four functional domains: TM (transmembranal), H (hinge), L (linker) and G (globular). Our aim is to gain insights on the structure-function characteristics of Ds. For that purpose, we initially aligned Ds protein sequences (n=67: D1=22; D2=23; D3=22) and found that the G domain that includes the catalytic region, is very similar between the 3 paralogous (60% identity), while the TM, H and L domains stand as the most variable (20%) domains among paralogous, but interestingly as relatively conserved domains among orthologous (D1 50%; D2 55% and D3 60%). This information lead us to divided Ds sequences into 2 major regions: the first half or "variable region" (VR) includes the TM, H and L domains, while the second half corresponds to the "conserved region" (CR) and contains the G domain. We hypothesized that the catalytic selectivity of Ds depends, in some extend, on the VR arrangement. In order to test this hypothesis we are currently constructing D2 and D3 chimeras interchanging VR. The kinetic characterization of the expressed enzymes will give clues on the structure-function characteristics of this important family of selenoenzymes. (This work was partially supported by: CONACYT 080420 and PAPIIT IN203409.)

P55.

CHARACTERIZATION OF SODIUM IODIDE SYMPORTER ACTIVITY IN THYROID AND EXTRATHYROIDAL TISSUES OF AFRICAN CLAWED FROG (*XENOPUS LAEVIS*) TADPOLES

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Iodide (I^-) is required for thyroid hormone (TH) synthesis and I^- deficiency severely impacts development in vertebrates. I^- is transported into thyroid follicle cells by the sodium iodide symporter (NIS). Transport of I^- by the NIS can be disrupted by I^- transport blockers such as perchlorate, which out-competes I^- for the NIS on thyroid follicle cells. Given that many tissues require TH during critical periods of development, even a brief exposure to perchlorate can be sufficient to adversely affect development and growth. Data from our laboratory suggest that there is a dramatic decrease in the sensitivity of *Xenopus laevis* larvae to perchlorate inhibition of thyroid function and metamorphosis between Nieuwkoop-Faber stages 49-55. Presently, nothing is known about the physiological mechanisms underlying developmental changes in the sensitivity to perchlorate. We hypothesize that the availability of I^- from non-thyroid I^- transporting tissues increases between stages 49-55 and that increased I^- delivery to the thyroid mitigates the adverse effect of perchlorate. To test this hypothesis, we re-evaluated which epithelial and non-epithelial tissues express the NIS gene and protein based upon RT-PCR and perchlorate-sensitive 24 h [^{125}I] whole animal uptake studies in stage 58-60 *X. laevis* tadpoles. RT-PCR analysis revealed that NIS was expressed in stomach and small intestine in addition to the thyroid gland. NIS mRNA was not detected in lung, kidney, skin, gill, muscle, heart or liver. Perchlorate sensitive ^{125}I uptake was found in stomach, lung, kidney, and small intestine but not gill, muscle, liver, or heart. Perchlorate-sensitive ^{125}I uptake by stomach was 6-10 times greater than in any other non-thyroidal tissue, suggesting that developmental changes in gastric NIS gene expression may affect I^- availability to the thyroid gland. Current work is focused on determining changes in NIS gene expression in stomach during metamorphosis.

P56.

DEIODINASE TYPE II AND THE PERIPHERAL REGULATION OF THYROID HORMONE HOMEOSTASIS IN LAMPREY (*PETROMYZON MARINUS*) DURING METAMORPHOSIS AND FOLLOWING A THYROID CHALLENGE

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Larval lamprey are unique among vertebrates as they lack thyroid follicles and maintain very high serum thyroid hormone (TH) levels which must decline dramatically for the normal progression of metamorphosis, goitrogens can induce precocious metamorphosis and exogenous thyroid hormones can disrupt natural metamorphosis. To help elucidate the mechanisms of maintaining TH and the nature of the decline in serum TH concentration at metamorphosis, the peripheral regulators of the thyroid axis were invested. We have isolated a 1.8 kb cDNA of the sea lamprey deiodinase type II (D2), consisting of the full-length coding region complete with selenocysteine; however, we have yet to identify the selenocysteine insertion sequence (SECIS) in the 3' UTR. D2 expression was detected in the intestine, liver, kidney, and brain with the highest expression levels in the intestine. Consistent with the notion that TH levels/activity must be minimized for lamprey metamorphosis to proceed, real-time PCR showed DII expression levels are reduced at times of tissue morphogenesis (i.e. metamorphosis), suggesting a reduced capacity to activate TH. The role of D2 in maintaining TH homeostasis in the face of a thyroid challenge was tested. These experiments indicate that TH treatment results in the downregulation of D2 and that D2 expression is upregulated in response to the decline in serum TH levels associated with $KClO_4$ treatment. Thus, as is the case in higher vertebrates, D2 is an important regulator of thyroid homeostasis in lamprey.

P57.

KRÜPPEL-LIKE FACTOR 9 ENHANCES THYROID HORMONE RECEPTOR β AUTOINDUCTION IN TADPOLE BRAIN *IN VIVO*, INCREASING TISSUE SENSITIVITY TO THYROID HORMONE AND ACCELERATING METAMORPHOSIS

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Thyroid hormone (T_3) receptor β (TRβ) is a direct T_3 target gene that is strongly induced (autoinduced) during tadpole metamorphosis. TRβ autoinduction is thought to be critical for hormone action during metamorphosis. The transcription factor Krüppel-like factor 9 (KLF9) is the most rapidly responding T_3 target gene thus far identified. Previously, we showed in the *Xenopus* cell line XTC-2 that KLF9 associates with the TRβ promoter to accelerate and enhance TRβ autoinduction. Here we show that KLF9 promotes TRβ autoinduction in tadpole brain *in vivo*. We used electroporation-mediated (EM) gene transfer to transfect plasmids into the brains of early prometamorphic tadpoles to express wild type or mutant forms of KLF9. Forced expression of KLF9 increased basal TRβ mRNA expression in euthyroid tadpoles, but had no effect in methimazole-treated, hypothyroid animals, suggesting that KLF9's major action is to enhance liganded TR action. Similar to our findings in XTC-2 cells, forced expression of KLF9 accelerated TRβ autoinduction in tadpole brain *in vivo* and

enhanced T_3-dependent induction of the TR target gene TH/bZip. Consistent with our previous mutagenesis experiments conducted in XTC-2 cells, the actions of KLF9 *in vivo* did not depend on its DNA binding activity, but required the second transactivation domain 'B'. Chronic, forced expression of KLF9 in tadpole brain accelerated metamorphosis, which we hypothesize is due to KLF9 increasing TRβ expression, and thereby enhancing tissue sensitivity to T_3. In support of this hypothesis, we found that forced expression of TRβ in tadpole brain *in vivo* by EM gene transfer increased expression of TR target genes. We conclude that the immediate early transcription factor KLF9 participates in a transcriptional regulatory network to promote TRβ autoinduction *in vivo*, forming a positive feedback loop that sensitizes the cell to further hormonal stimulation owing to the production of more TRβ. (Supported by NIH grant R01 NS046690 and NSF grant IOS 0922583 to RJD)

P58.

THYROTROPIC ACTIVITY OF SUPERACTIVE HUMAN GLYCOPROTEIN HORMONE ANALOGS AND MAMMALIAN GONADOTROPINS IN GOLDFISH (*CARASSIUS AURATUS*): INSIGHTS INTO THE EVOLUTION OF THYROTROPIN RECEPTOR SPECIFICITY

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Thyrotropin (TSH) is a pituitary glycoprotein hormone (GPH) hetero-dimer that binds to its G-protein coupled receptor at the thyroid to promote the synthesis and secretion of thyroid hormone. Very little is known about TSH-TSH receptor (TSH-R) interactions in teleost fish. Mammalian gonadotropins (GTHs) have been reported to have an intrinsic ability to activate teleost fish TSH receptors, suggesting the TSH-R in teleost fish is more promiscuous than in other vertebrates. In this study we utilized the goldfish T_4 release response and human TSH (hTSH) analogs as *in vivo* tools to evaluate the structural constraints on hormone-receptor interactions in a fish species. We found that four positively charged amino acids substituted for neutral or negatively charged amino acids at the n-terminal portion of the GPH subunit α (GSUα) significantly increased biological activity of hTSH, as in mammals. Since the positively-charged amino acids that increase thyrotropic activity are in the shared GSUα subunit, we further hypothesized that these positively charged amino acids could also increase thyrotropic activity of mammalian GTHs. We found that bovine FSH, whose GSUα subunit contains four positively charged amino acids, was also thyrotropic (1.23 ± .10 mUnit/µg) in goldfish. Though recombinant human FSH did not produce a dose-dependent increase in T_4, thyrotropic activity could be recovered with the addition of positively charged amino acids at the n-terminal portion of the GSUα (0.56 ± .03 mUnit/µg). Bovine LH, however, did not have thyrotropic activity, demonstrating the protein structure of GTH β subunits also contribute to the thyrotropic activity of the GTH dimers in goldfish. These studies demonstrate that mammalian GPH analogs can be effectively utilized to evaluate the conservation of receptor binding mechanisms between fish and mammals, helping to identify the structural characteristics of mammalian hormones that endow them with heterothyrotropic activity in goldfish.

P59.

HORMONAL REGULATION OF THYROID-RELATED GENE EXPRESSION IN THE RED DRUM, *SCIAENOPS OCELLATUS*

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Robust daily rhythms of circulating (thyroxine) T_4 and a daily cycle of thyrotropin (TSH) expression in the sciaenid fish, the red drum, suggest that circulating thyroid hormones may play a dynamic role in feedback regulation of TSH. If thyroid function is regulated through a 3,5,3'-triiodothyronine (T_3) set point, it would be expected that physiological levels of T_4 would be more effective in negative feedback on TSH than T_3. We found that i*n vitro* pituitary incubation in medium containing 10nM T_3 did not significantly alter the expression of either the α or β subunit of TSH but incubation with T_4 at 10nM significantly inhibited the expression of TSHβ To further examine the differential effects of thyroid hormones we developed an immersion technique to administer physiological doses of T_3 and T_4 *in vivo*. Both hormones persist in static tank water for at least 40hrs. Immersion in tank water at 200ng/ml T_4 significantly increased plasma T_4 by 23.5 ng/ml over control by 40 hours while inhibiting plasma T_3 by 2.3 ng/ml below control. Similarly, immersion in 100ng/ml T_3 increased plasma T_3 by 13.2 ng/ml over control by 22 hours while decreasing plasma T_4 by 6.0 ng/ml below control, presumably through inhibition of TSH secretion. Liver expression of type 3 deiodinase was unaffected by T_4 but significantly increased by 22hrs of T_3 static immersion. These result indicate that T_4 negative feedback is a physiological regulator of TSH expression in red drum, but further suggest that hepatic thyroid hormone deactivating pathways are more sensitive to alterations in circulating T_3 than T_4, supporting a role for a peripheral T_3 set point in regulating thyroid hormone delivery to target tissues.

P60.

FORMATION OF THYROID HORMONE FROM PHARYNGEAL AND HEAD KIDNEY PREPARATION OF A TELEOST FISH *COLISA FASCIATUS*

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Cell–free preparation of pharyngeal and head kidney regions from *Colisa fasciatus* effectively catalysed the synthesis of thyroid hormone . Catalysing activity for the formation of monoiodotyrosine, diiodotyrosine and thyroxine was located in the microsomal (105000 g sediment) fraction pharyngeal preparation whereas in the case of head kidney it was located in the soluble supernatant (105000 g supernatant) fraction. Formation of triiodothyronine (T3), however, was not observed. Synthesis of thyroxine in both the cases could be detected in 90min incubated samples and the peaks were observed in 120 min incubated samples. Pharyngeal microsomal fraction was found to be more active than the head kidney soluble supernatant fraction in synthesizing the thyroid hormone. Antithyroid agents like thiourea and thiouracil and reducing agents such as ascorbic acid and reduced glutathione inhibited the catalyzing activity of both the preparations; higher sensitivity of the pharyngeal preparation was observed in this connection.

P61.

ACTION MECHANISM OF 3,5-T_2: RECEPTOR-HORMONE INTERACTION

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T3 is considered the bioactive thyroid hormone (TH) and its actions are mediated by TH nuclear receptors (TR). TR form heterodimers with the retinoid X receptors (RXR) and both interact with the TH responsive elements (TRE) located in the promoter regions of TH-responsive genes. We have described in

fish that like T3, 3,5-T2 regulates gene expression as well as promotes the recruitment of different populations of transcription factors to the TRE. Fish express two isoforms of TRβ1 that differ in the presence of (long: L-TRβ1) or the lack of (short: S-TRβ1) 9 amino acid inserted in their ligand binding domain. We analyzed if T3 and 3,5-T2 preferentially interact with a specific TRβ1 isoform. Two experimental approaches were conducted. In vitro experiments consisted in expressing both TRβ1 isoforms in CV1 cells treated with 10 nM of T3 or 3,5- T2. For in vivo experiments, groups of tilapia fingerlings were treated with: a) 100nM T3 or 3,5-T2; b) 100 nM of retinoic acid (RA) + T3 or 3,5-T2; c) 5 mM methimazole (MMI) + 30 nM T3 or 3,5-T2, and d) 5 mM MMI, + 30 nM RA and T3 or 3,5-T2. In all experiments, the formation of TRβ1-TRE complexes were analyzed by electrophoretic mobility shift assays (EMSA). In vitro experiments showed that 3,5-T2 favors the formation of L-TRβ1-TRE complexes, while the formation of the S-TRβ1 TRE complexes were favored by T3, thus suggesting that the stability of either complex L-TRβ1-TRE or S-TRβ1-TRE depends on the presence of 3,5-T2 or T3. Results from the in vivo experiments showed the formation of complexes with slightly different weights, depending on the TH treatment, while RA increased complex intensity in T3, but not 3,5-T2-treated fish. Furthermore, EMSAs of RA+T3-treated fish showed the formation of homo or heterodimers, while a possible monomeric TRE-TRβ1 interaction was suggested in those fish exposed to RA+3,5-T2. (Tilapias were kept in laboratory facilities by Miguel A Maqueda. Supported by: CONACYT 080420 and PAPIIT IN208511).

TOPIC: *METABOLISM AND FEEDING*

P62.
CORTISOL INHIBITS FOOD INTAKE BY REDUCING GHRELIN SIGNALING IN TILAPIA
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It is well known that following a stressor plasma cortisol levels rise inducing physiological changes within the animal that are directed toward maintaining allostasis. Less well understood is cortisol's role in regulating food intake in teleosts. This study investigated cortisol's role on food intake and regulation of the neuroendocrine appetite stimulating hormones (neuropeptide Y and ghrelin) in tilapia (*Oreochromis mossambicus*). Male and female tilapia were assigned randomly to the following treatments; unhandled, vehicle control, cortisol, or cortisol + RU486. Food intake was determined 24 h post-injection during a 1 h feeding trial. Cortisol significantly reduced food intake which was reversed by RU486 treatment. A second study was then conducted to investigate the effects of cortisol on the orexigenic hormones, NPY and ghrelin. Cortisol treatment significantly reduced plasma and stomach mRNA levels of ghrelin. RU486 treatment blocked cortisol's negative effects on plasma ghrelin but not on stomach ghrelin mRNA levels. In the diencephalon, cortisol significantly reduced GHSR1a-LR mRNA levels; this effect was not reversed by RU486 treatment. Cortisol alone had no effect on ghrelin or NPY mRNA levels in the diencephalon. However, the cortisol + RU486 exhibited opposite effects: significantly increased ghrelin mRNA levels, but significantly reduced NPY mRNA levels in the diencephalon. In the telencephalon, cortisol significantly reduced NPY and GHSR1b-LR mRNA levels, which was blocked by RU486 treatment. These data demonstrate that cortisol inhibits food intake. The negative effects of cortisol on food intake may be mediated by the reduction in plasma ghrelin levels and/or reduction in NPY in the telencephalon. In addition to, cortisol may be altering the activity of anorexigenic (e.g. corticotropin releasing hormone). [This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2010-65206-20615 from the USDA National Institute of Food and Agriculture to LGR.]

P63.
DIFFERENTIAL EFFECTS OF GROWTH HORMONE FAMILY PEPTIDES ON THE EXPRESSION OF INSULIN-LIKE GROWTH FACTOR 1 AND 2 mRNAs
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The growth of vertebrates is primarily controlled by the growth hormone (GH)-insulin-like growth factor (IGF) system. In mammals, postembryonic growth is mediated by IGF-1, produced under the influence of GH, whereas embryonic growth is mediated by IGF-2, the production of which is not influenced by GH. Recent reports of IGF-2 expression in tissues of juvenile and adult fish suggest that it may play a role in regulating post-embryonic growth in this group. In this study, we used juvenile (postembryonic) rainbow trout to examine the influence of GH family peptides on the expression of IGF-1 and IGF-2 mRNAs and to assess the mechanism(s) through which GH exerts its actions. Fish were implanted with mini osmotic pumps containing GH or saline for 21 days. GH significantly increased mRNA levels of IGF-1 and IGF-2 in both liver and muscle over levels observed in saline-implanted fish. The direct effects of GH, prolactin (PRL), and somatolactin (SL) were assessed on isolated hepatocytes incubated in vitro. GH stimulated expression of IGF-1 and IGF-2 mRNAs in a concentration- and time-related manner; GH was more potent and more efficacious in stimulating IGF-2 expression than IGF-1 expression. The ERK pathways inhibitor, U0126, and the PI3K/Akt pathway inhibitor, LY294002, blocked GH-stimulated IGF-1 and IGF-2 expression. PRL had slight but significant effects on the expression of IGF-1 and IGF-2; PRL-stimulated expression of IGF mRNAs also was blocked by U0126 and LY294002. SL had no effect on the expression of either IGF-1 or IGF-2 mRNA. These findings indicate that GH stimulates IGF-2 expression to a greater extent than IGF-1 expression and support a role of IGF-2 in postembryonic growth of fish. These findings also indicate that GH-stimulated IGF-1 and IGF-2 expression involves activation of the ERK and PI3K/Akt signaling pathways. (Supported by NSF IOS 0920116)

P64.
GROWTH HORMONE-STIMULATED LIPOLYSIS IN THE LIVER OF RAINBOW TROUT IS MEDIATED BY THE PI3K-AKT PATHWAY
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Growth hormone (GH) regulates several aspects of metabolism in vertebrates, including the mobilization of stored lipid. In this study, we used hepatocytes isolated from rainbow trout as a model system in which to elucidate the molecular signaling events that underlie GH-stimulated hepatic lipolysis. Fish were particularly advantageous for this study because their liver stores significant amounts of lipid, and the lipolytic processes in their liver have been well characterized. Studies were conducted on hepatocytes isolated from fish fasted 1 week, a nutritional regime previously found to shift animals from an anabolic state to a catabolic state involving activation of lipolysis. GH stimulated lipolysis in hepatocytes in a time- and concentrated-related manner. GH-stimulated lipolysis was manifested by increased activity of the lipolytic enzyme, hormone-sensitive lipase (HSL), as well as by increased expression of the two HSL-encoding mRNAs, HSL1 and HSL2. The activation of HSL was dependent upon phosphorylation of the enzyme. The role of the PI3K-Akt pathway in mediating GH-stimulated HSL activation and HSL mRNA expression was supported by several observations. GH decreased the phosphorylation of Akt, a response similar to that observed in hepatocytes treated with the PI3K inhibitor, LY294002. In addition, LY294002 mimicked the actions of GH, stimulating the activity of HSL and enhancing the expression of HSL1 and HSL2 mRNAs. Moreover, LY294002 augmented the effects of GH on HSL

activity and on expression of HSL-encoding mRNAs. These findings indicate that GH promotes hepatic lipolysis by stimulating both the synthesis and activation of HSL via inactivation of the PI3K-Akt pathway. (Supported by NSF IOS 0920116 to MAS).

P65.

NEUROENDOCRINE REGULATION OF FOOD INTAKE DURING ACUTE STRESS IN THE TILAPIA, *OREOCHROMIS MOSSAMBICUS*

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Fish encounter a number of environmental stressors, including variable water quality, temperature, and food availability. The stress response that ensues is orchestrated by the sympathetic nervous system as well the hypothalamic-pituitary-interrenal (HPI) axis, principally resulting in the release of cortisol. These two mechanisms share the overall effect of mobilizing metabolic energy and redirecting it towards homeostatic maintenance. Consequently, an important factor altered by stress in fish is reduced food intake. We have previously shown reduced food intake in response to an acute stress in tilapia. However, whether cortisol is mediating these effects is unknown. Therefore, the current study investigates the effects of metyrapone, a cortisol synthesis inhibitor, on food intake during an acute stress. Metyrapone was administered via feed in three experimental groups receiving doses of 10, 25, and 50mg/kg body weight for 1 wk prior to a 30 min crowding and handling stress. Following the stressor, fish were allowed to feed for 1 h. Stress reduced ($P < 0.01$) food intake, while elevating mRNA levels of corticotropin-releasing hormone (CRH; $P < 0.001$), the initiator of the stress axis. Additionally, metyrapone treatment dose-dependently blocked the stress-induced reduction in food intake, with the 25 and 50 mg/kg doses reversing the effect of stress on food intake ($P < 0.05$ and 0.001, respectively). The elevation of CRH mRNA levels was also reversed in all metyrapone treatments, suggesting that cortisol and CRH play a role in mediating the observed reduction in food intake during stress in tilapia. (This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2010-65206-20615 from the USDA National Institute of Food and Agriculture to LGR.)

P66.

A NOVEL GHRELIN RECEPTOR IN CHANNEL CATFISH, GHS-R3A, DEMONSTRATES HIGH AFFINITY FOR HOMOLOGOUS LIGAND BUT NOT SYNTHETIC GROWTH HORMONE SECRETAGOGUES

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A novel ghrelin receptor was isolated from channel catfish (*Ictalurus punctatus*) embryo cDNA. Sequence and genomic organization of the receptor have been determined. The novel receptor gene encodes a seven-transmembrane domain receptor, which we have designated cfGHS-R3a. cfGHS-R3a is composed of 388 amino acids and shares 48% sequence identity with cfGHS-R1a and 44% sequence identity with cfGHS-R2a. Upon phylogenetic analysis this new receptor demonstrates only a modest degree of conservation and similarity with vertebrate GHS-R1a and GHS-R2a receptors. Using quantitative RT-PCR analysis, cfGHS-R3a expression was detectable in the central nervous system and several peripheral tissues. The cDNA from all three channel catfish ghrelin receptors were individually transfected into mammalian cells and intracellular calcium mobilization assays were carried out using homologous ligands and synthetic growth hormone secretagogues (GHS). Similar to assays with trout and tilapia GHSR1a-LR, there was no response of catfish GHS-R1a to homologous ligands (cfGRLN -C8 , -C10, -C8gly and -C10gly) or synthetic GHS (GHRP-6 and Hexarelin). However, a dose-dependent increase in intracellular Ca^{2+} concentration was demonstrated in cells transfected with cfGHS-R2a when the cells were treated with both homologous ligands and synthetic GHS. Furthermore, a dose-dependent increase in intracellular Ca^{2+} concentration was demonstrated in cells transfected with the novel receptor cfGHS-R3a when the cells were treated with homologous ligands but not when treated with synthetic GHS. These results indicate cfGHS-R3a has a high affinity for ghrelin in the channel catfish; however, further study is required to determine the physiological significance of these findings.

P67.

CORTICOSTERONE STIMULATES FEEDING BEHAVIOR AND INDUCES EXPRESSION OF HYPOTHALAMIC FEEDING CONTROL GENES IN *XENOPUS LAEVIS* TADPOLES

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The secretion of glucocorticoids (GCs; e.g. corticosterone - CORT) following exposure to a stressor may promote subsequent feeding to replenish energy stores expended during the stress response. Glucocorticoids have been found to act on the hypothalamus to stimulate feeding; however, the molecular signaling mechanisms by which GCs activate hypothalamic orexigenic pathways are still being elucidated. Hypothalamic AMP-activated protein kinase (AMPK) has been shown to play an important role in feeding and metabolism in mammals, and some evidence suggests that GCs induce rapid phosphorylation (activation) of AMPK, which in turn promotes feeding. Activation of AMPK has been shown to increase mRNA expression of neuropeptide Y (NPY), a potent orexigenic factor in vertebrates. We have established an amphibian model for investigating the ontogeny and function of hypothalamic feeding controls using the frog *Xenopus laevis*. We previously showed that NPY and CORT stimulate food intake in juvenile frogs. Here we tested whether CORT has similar effects in tadpoles, and we investigated CORT-induced changes in hypothalamic gene expression. Treatment of early prometamorphic tadpoles with CORT (added to the aquarium water) caused a significant increase in feeding behavior scored by the number of tadpoles present within a tank quadrant containing food. We treated tadpoles with CORT for 0, 1, 3, 6, 12, or 24 hours and measured AMPK and NPY mRNA in the brain by RTqPCR. AMPK mRNA was increased by 6 hours and remained elevated through 12 hours. NPY mRNA was increased by 1 hour and remained elevated through 6 hours of CORT exposure. Our findings show that CORT stimulates feeding in *X. laevis* tadpoles, similar to its action in juvenile frogs. This effect may be explained in part by increased transcription of AMPK and NPY. Studies are ongoing to investigate the role that AMPK plays in feeding in frogs and to determine whether CORT affects AMPK phosphorylation in the amphibian hypothalamus. (Supported by NSF grant IOS 0922583 to RJD)

P68.

P69.
DO NESFATIN-1 AND GHRELIN INTERACT TO REGULATE FOOD INTAKE IN GOLDFISH?
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The gut/brain hormone ghrelin and the nucleobindin 2-encoded novel hormone nesfatin-1 are two endocrine factors that regulate food intake. Ghrelin (orexigen) increases, while nesfatin-1 (anorexigen) reduces food intake. Ghrelin has been extensively characterized in fish. While we recently reported nesfatin-1 and its anorectic effects in goldfish for the first time, the potential mediators and mechanisms of nesfatin-1 regulation of feeding is currently unknown. Nesfatin-1 and ghrelin co-localization was recently detected in the gastric oxyntic cells of rats, suggesting that they are functionally related in mammals. We hypothesized that nesfatin-1 and ghrelin interact to regulate feeding in goldfish. Using immunohistochemistry, co-localization of ghrelin-like and nesfatin-1-like immunoreactivity was detected in the hypothalamus and gut (j-loop), two key food intake regulatory tissues of goldfish. Intracerebroventricular administration of nesfatin-1 inhibited food intake and the expression of preproghrelin and ghrelin receptor mRNAs in the forebrain. Alternatively, central injections of ghrelin stimulated food intake and inhibited nucleobindin 2 mRNA expression in the forebrain but had no affect on the ghrelin receptor mRNA expression. Our results suggest that nesfatin-1 inhibits the ghrelin/ghrelin receptor system, while ghrelin suppresses nesfatin-1 to regulate feeding in goldfish. Collectively, this novel data supports the hypothesis that nesfatin-1 and ghrelin interact to regulate goldfish feeding. [This research was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada through a Discovery Grant, and two Research Tools and Instruments Grants to SU. SU is a Canadian Institutes of Health Research (CIHR) New Investigator and is a recipient of the Early Researcher Award from the Ontario Ministry of Research and Innovation. BK is a recipient of the NSERC Alexander Graham Bell Canada Graduate Scholarship.]

P70.
DEVELOPMENTAL REVERSAL IN NEUROPEPTIDE Y ACTION ON FEEDING IN AN AMPHIBIAN
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Neuropeptide Y (NPY) signaling in the hypothalamus is associated with orexigenic behaviors in many juvenile and adult vertebrates, but it is not known whether NPY influences food intake during earlier life stages. The aim of this study was to examine the role of NPY in regulating feeding at different stages of the life cycle in an amphibian (the spadefoot toad, genus *Spea*). In juvenile toads, intracerebroventricular (i.c.v.) injections of NPY (20ng/g and 200ng/g) stimulated movement towards prey (pinhead crickets), and increased the number of strikes and the number of crickets eaten compared to un-injected or saline-injected controls. By contrast, i.c.v. injection of NPY in prometamorphic tadpoles (Gosner stage 35-37) caused a dose-dependent reduction in foraging time, and an increase in swimming time compared to un-injected or saline-injected controls. Conversely, blocking NPY signaling in the brain with i.c.v. injection of a general NPY receptor antagonist increased foraging and inhibited swimming when administered alone, and partially blocked the anorexigenic/locomotor effects of NPY when co-injected in prometamorphic tadpoles. This result suggests that endogenous NPY signaling exhibits suppressive regulation of the rate of food intake in prometamorphic tadpoles. In addition, we used semi-quantitative PCR to show that expression of NPY mRNA in the diencephalon/ mesencephalon region of the tadpole brain increased with developmental stage, which may indicate changing physiological or behavioral actions of NPY signaling during later tadpole stages or during the process of metamorphosis. Future studies of NPY mRNA and protein expression in specific brain regions of different life stages are needed to reveal the precise mechanisms that underlie the developmental reversal in behaviors associated with NPY signaling in amphibians. (Supported by NSF grants IBN 0235401 to RJD and IOS 0818212 to EJC)

P71.
LEPTIN AND THE REGULATION OF FEED INTAKE IN ANAEMIC AND PARASITE-INFECTED RAINBOW TROUT
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Leptin is generally recognized as a potent anorexigen among vertebrates but little is known about the physiological conditions under which this hormone regulates feed intake in fish. In this study, we characterized the relationships between feed intake, oxygen carrying capacity, hepatic leptin gene expression, plasma leptin and the hypothalamic expression of key appetite-regulating genes in rainbow trout infected with the parasite *Cryptobia salmositica*. A time-course experiment revealed an inverse relationship between feed intake and leptin expression in infected fish. Peak parasitemia was associated with 50% reductions in hematocrit and hemoglobin levels, a 75% reduction in feed intake, a 17-fold increase in leptin expression and a 76% increase in plasma leptin. Anorexia in the *Cryptobia*-infected fish was characterized by a reduction in the mRNA levels of hypothalamic neuropeptide Y and an increase in pro-opiomelanocortin gene expression. A strong positive correlation was also observed between oxygen carrying capacity and feed intake during acute disease. Finally, leptin mRNA and plasma levels of fish pair-fed to infected animals did not differ from those of satiated fish. These findings provide evidence that hepatic leptin expression and secretion are stimulated by hypoxaemic conditions but not by restricted feed intake in rainbow trout. Our results also suggest that leptin contributes to feed intake regulation in fish during conditions of reduced oxygen availability. (Supported by NSERC Discovery grants to NJB and PTKW).

P72.
MELANIN-CONCENTRATING HORMONE AND GONADOTROPIN-RELEASING HORMONE mRNAS IN WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*): CLONING, DISTRIBUTION AND EFFECTS OF FASTING
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Melanin-concentrating hormone (MCH), first described as the primary peptide regulating skin colour change in fish, has been recently shown to act as an orexigenic factor in mammals. In vertebrates, gonadotropin-releasing hormone (GnRH) is a major hypothalamic hormone regulating reproduction and has been shown to be an inhibiting factor in food intake regulation. To date the exact role of these peptides in the regulation of feeding in fishes is still unclear. This study aimed at characterizing the structure and function of MCH and GnRH in the winter flounder, *Pseudopleuronectes americanus*, a bottom dwelling, cold water fish inhabiting the shores of Newfoundland. Two forms of MCH (MCH and MCH2) and two receptors (MCH-R1 and -R2) mRNAs were isolated and shown to be expressed in feeding-centers within the brain, including the telencephalon/preoptic area, optic tectum/thalamus and hypothalamus, as well as the pituitary. Furthermore, mRNAs have been identified in peripheral tissues related to feeding and digestion, such as stomach and intestine, as well as in the gonads, which could indicate a function in reproduction. A food restriction experiment show that fasted flounder have higher MCH and MCH-R1 mRNA expression in the optic tectum/thalamus and hypothalamus, respectively, indicating a possible stimulating role in food intake. Three forms of GnRH

[seabream-GnRH (sbGnRH), chicken-GnRH (cGnRH) and salmon-GnRH (sGnRH)] and two receptors (GnRH-R1 and -R2) were isolated in winter flounder. cGnRH mRNA is predominantly expressed in the optic tectum/thalamus, with little expression in the telencephalon/preoptic area and hypothalamus, while sGnRH is found throughout the brain, with highest expression in the telencephalon/preoptic area. For both cGnRH and sGnRH, fasting decreases mRNA expression in the hypothalamus, suggesting that both forms might play a role in food intake and acts as anorexigenic factors in winter flounder. (Supported by NSERC grants)

TOPIC: *GENERAL NEUROENDOCRINOLOGY*

P73.
MOUSE HYPO E-40 CELLS: A MODEL SYSTEM FOR ANALYZING THE ENDOPROTEOLTYIC CLEAVAGE OF POMC BY PROPROTEIN CONVERTASE 1/3
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During the posttranslational processing of some polypeptide precursors proprotein convertase 1/3 (PC1/3) is an endoprotease that cleaves at sites of paired basic amino acids to excise the chemical signal(s) from the precursor. The proprotein, POMC, is the common precursor for melanocortin-related and opioid-related end-products. *Xenopus (Silurana) tropicalis* POMC has nine potential endoproteolytic cleavage sites, yet in the anterior pituitary PC1/3 will cleavage at only three of these sites. Although PC1/3 was identified nearly 20 years ago, the rationale for the substrate selectivity of this enzyme has been elusive. The ideal test situation to resolve this question would be to express *pomc* cDNA constructs in a cell that only expresses PC1/3, and then to observe how this cell handles the posttranslational processing of constructs of POMC in which the target endoproteolytic cleavage site(s) have been mutated. Mouse HYPO E-40 embryonic neurons are a cell line that appear to be suited for this purpose. Real-time PCR analysis indicates that E-40 cells only express the *pc1/3* gene, but not the *pc2* gene. RIA analysis indicates that E-40 cells do not express the mouse *pomc* gene. E-40 cells can be transiently transfected with a *X. tropicalis* pomc cDNA in the pTARGET expression vector. Two days post-transfection both ACTH-related and β-endorphin related immunoreactivity can be detected in extracts of the transfected cells by RIA. The E-40 cells have a number of advantages over mouse AtT20 cells, the current model for studying PC1/3 substrate selectivity. Projects are ongoing to analyze the amino acid requirements around the paired basic amino acid cleavage sites that flank the sequence of ACTH(1-39) in *X. tropicalis* POMC.

P74. NEUROENDOCRINE EFFECTS OF MERCURY IN SEVERAL FISH SPECIES
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Mercury (Hg) is a potent neurotoxicant. Recent studies in fish have also established that Hg is a neuroendocrine disruptor, though little is known about the sub-clinical effects and the underlying mechanisms. Here we present findings from a series of studies (in vitro, field work, laboratory bioassay) that aimed to assess Hg-associated neurochemical effects in several fish species as follows: 1) studies on wild fish (lemon shark, mako shark, seatrout, South River fish); 2) in vitro screening assays (lemon shark, mako shark, yellow perch, goldfish) on neurochemical receptors and enzymes underlying vertebrate reproduction; 3) studies on laboratory-exposed yellow perch. In the wild fish studies, mean brain Hg levels were: 0.145 ppm dry wt in lemon shark (n = 28; South Florida), 1.69 ppm in mako shark (n = 12; Atlantic coast), 0.577 ppm in seatrout (n = 28; South Florida), and 0.069 ppm in South River fish (n = 28; Virginia). Saturation binding curves were developed from each fish species to obtain B_{max} (195 – 266 fmol/mg for mAChR, 236 – 3065 fmol/mg for NMDAR) and K_d (3.18 – 4.64 nM for mAChR, 28.9 – 368 nM for NMDAR). Based on inhibition constants (*K*i) against $Hg^{2+}$and CH_3Hg^+, goldfish and yellow perch were the most sensitive to mAChR and NMDAR binding, respectively. When brain Hg was related to neurochemical enzymes (MAO, AChE) and receptors (mACh, NMDA), there were no Hg-associated neurochemical changes except in the mako shark where a negative correlation with NMDA glutamate receptors (r = 0.496; $p < 0.05$) was found. Via cell-free in vitro screening assays, we are also assessing Hg-related impacts towards sex hormone receptors, neurotransmitter release and uptake mechanisms, and neurochemical receptors and enzymes that have critical roles in reproduction (e.g, dopamine and GABA systems) and neurobehavior (e.g., glutamate pathway). We will also report on neurochemical data from laboratory-exposed perch.

P75.
SEQUENCING AND DISTRIBUTION OF THE PEJEGONADOTROPIN-RELEASING HORMONE ISOFORM (pjGNRH) IN THE BRAIN OF *CHIROSTOMA HUMBOLDTIANUM*
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The Silverside *Chirostoma humboldtianum* is an endemic fish from central highlands and western of Mexico. Traditionally, *Chirostoma* genus has been used as fed in Mexico. Gonadotropin-releasing hormone (GnRH) is a key neurohormone in reproduction. In many teleost fish have been described three GnRH systems. The system GnRH-1 is located in the preoptic area and is the hypophisiotropic form. System GnRH-2, is in the midbrain tegmentum and the cGnRH-II is the isoform present for this system. The salmon GnRH is the system GnRH-3 and is in the neurons of terminal nerve ganglion. The aim of this work was obtain the partial sequence of pjGnRH and determinates its distribution in the brain of *Chirostoma humboldtianum*. Fish from Zacapu lagoon, Michoacan, Mexico, were collected and sacrificed. The brain was extracted and frozen in dry ice until their arrival to laboratory. Total RNAs were extracted with Trizol. The cDNAs were made through out RT-PCR using First Strand kit. For PCR, two primers sets were used originally designed for search pjGnRH in the Argentine Pejerrey fish.The anneling temperature was 54°C. The products were purified through Wizard kit, and sequenced in an ABY system 3600 sequencer. For localization of the pjGnRH isoform, brains of *Ch. humboldtianum* were cut in sagital and transversal sections and a specific antibody against the GAP of pjGnRH was used . Our results showed an amplified sequence of 164 bp. This includes the isoform pjGnRH, processing site and a portion of GAP sequences, which has high identity with that reported for *O. bonariensis* and other species. The pjGnRH was localized in the preoptic area of the brain, specifically, in the *Nucleus Preopticus periventricularis*. Also, some immunoreactive fibers reaching the pituitary were found. These findings are consistent with another closer member of atherinidopsidae family *O. bonariensis*.

P76.

INTRON RETENTION IN A SALMON GONADOTROPIN RELEASING-HORMONE IN THREE SPECIES OF *CHIROSTOMA* GENUS

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Gonadotropin releasing-hormone (GnRH) is a key hormone in reproduction. In fish, the isoform of salmon (sGnRH) is always present, as either system GnRH-1 or GnRH-3. In the *Chirosotoma* an endemic teleost fish of central Mexico, sGnRH is present as system GnRH-3. In this work, we reported intron B retention in a partial sequence of sGnRH mRNA in the brain of three *Chirostoma* species: *C. humboldtianum, C. estor* y *C. promelas*. In these species the products were of 306 bp, and include the hormone sequence, the processing site, and the GnRH associated peptide, which in turn contains the intron B. Besides, in the three species, the sequence obtained is identical for all of them. The comparison of *Chirostoma*'s partial sGnRH sequence with that of other teleosts is high, once the intron B has been removed.

P77.

NEUROPROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN HYPOXIC ORGANOTYPIC CULTURES OF CHICKEN CEREBELLUM

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Anti-apoptotic effects of growth hormone (GH) have been described in different cell types. In the central nervous system (CNS) GH has a role in neuroprotection, although the molecular mechanisms of these actions have not been fully elucidated. GH mRNA, GH and the GH receptor (GHR) are locally expressed in chicken cerebellum; this structure is particularly sensitive to damage by hypoxia-low glucose (HLG) conditions (95%N_2, 5%O_2, DMEM-Low Glucose at 37°C), leading to neuronal apoptosis and/or necrosis. In the present study, we evaluated the anti-apoptotic effect of GH in organotypic cultures of chicken cerebellum under HLG This model has the advantage of maintaining the cytoarchitecture of normal tissue. Immunohistochemical analysis showed the co-localization of GH with NeuN in granule neurons; with Calbindin in Purkinje cells and, to a less extent, with GFAP in glial cells although the cytoarchitecture of these slices was modified under hypoxia conditions, mainly in the Purkinje layer, because these cells showed disorganization. Locally expressed GH increased under HLG conditions as compared to the control group (596.6±70.8 vs. 311.3±43.8 ng GH/mg protein, respectively, as determined by ELISA). Caspase-3 activity increased importantly in HLG treated cultures when compared to the controls under normoxia (11.6±2.1 vs 3.8± 1.0 U/ml) but was significantly reduced after addition of 1 nM GH (to 6.0 ± 0.1 U/ml). Also, GH treatment (1 nM) provoked an increase of 29.1% in the expression of Bcl2 in cultures exposed to hypoxia when compared to hypoxia alone. These results suggest that GH may act as a survival/neuroprotective factor in this ischemia model through anti-apoptotic mechanisms. (Supported by PAPIIT-DGAPA, UNAM 210209; CONACYT 118353 and 234456)

P78.

PHARMACOLOGICAL AND ANATOMICAL EVIDENCE FOR AN EDINGER-WESTPHAL PREGANGLIONIC (EWPG) CELL GROUP IN THE NORTHERN LEOPARD FROG *RANA PIPIENS*

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The Edinger-Westphal nucleus is best known as a midbrain cholinergic cell group (EWpg) that innervates the ciliary ganglion controlling iris muscle contraction in the eye. Recent studies have suggested a new subdivision of the EW populated by urocortin-1 (UCN-1) expressing neurons that project to other CNS sites involved in homeostasis (EW central projecting, EWcp, neurons, Kozciz et al. 2010). While functional EWpg and EWcp subdivisions in mammalian brain are supported by numerous studies, little is known about the role of the EW in amphibians. In fact, early work suggested that pupil constriction in anurans is not controlled by the parasympathetic nervous system, but rather depends entirely on the photosensitive nature of the iris muscle cells. Here we report evidence of a functional cholinergic component of the EW in the frog *Rana pipiens*. Peripheral administration of oxotremorine, a muscarinic receptor agonist, produced statistically significant pupil constriction. Transmission electron microscopy revealed numerous smaller unmyelinated axons in the oculomotor nerve, consistent with parasympathetic nerve fibers. Examination of choline acetyltransferase (ChAT) immunoreactive cells within the oculomotor nucleus revealed a subdivision of smaller neurons in the caudal part of the nucleus. Unilateral eye ablation caused cell death in both the large somatic motor ChAT neurons and the smaller ChAT neurons in the oculomotor nucleus ipsilateral to the side of the ablation. These smaller ChAT neurons were segregated from UCN-1 immunoreactive neurons that were scattered more dorsally and medially to the oculomotor nucleus. Collectively, our data are consistent with a functional EWpg cell group in the brain of anurans, although the precise location of these cells awaits tracing studies. Our observation of UCN-1 neurons in the vicinity of the oculomotor nucleus is consistent with work from other labs suggesting that a EWcp component of the EW is present in anurans. (JK and ND supported by the TTU Clark's Scholars Program).

P79.

NEURO-PROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN CHICKEN CEREBELLAR CELL CULTURES: A POSSIBLE ANTI-APOPTOTIC ROLE OF GH DURING THE HYPOXIA INJURY

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Recent reports indicate that growth hormone (GH) and its receptor (GHR) are expressed in the Central Nervous System (CNS) of several species, where it may act as a neuroprotective factor in addition to its capacity to stimulate growth and development of the brain. A common insult that can cause severe damage on the CNS is ischemia, which can be induced by hypoxia and low glucose condition (HLG). We studied the possible neuroprotective role of GH in a model of ischemic neuronal injury (HLG) using primary cerebellar neuron cultures. The viability of cerebellar neurons exposed to HLG decreased to 38.4±7.2% compared to the control (97.8%), and it increased to 66±12.8% after GH (1 nM) treatment. Likewise, the addition of GH (1 nM) decreased the number of apoptotic cells marked by TUNEL (15.3±2.2%) when compared with those exposed only to HLG (68.1±12.1%). GH treatment (1 nM) was also capable to reduce 1.5 times (4.8 + 0.9 enzymatic activity units) the activity of caspase 3 in cerebellar neurons exposed to HLG (7.5 + 1.3 enzymatic activity units). Furthermore, addition of GH induced the activation of the PI3K/Akt pathway during the HLG insult, and this activation was blocked by Wortmannin

(a PI3K/Akt inhibitor), suggesting that GH exerts its effects through this signaling pathway. Also, the antiapoptotic Bcl-2 protein increased following GH treatment in HLG exposed cells. On the other hand, the addition of 10 nM 15kDa GH variant (which is the most abundant isoform in the chicken cerebellum) also increased cell viability (63.2±11.9%) compared to HLG exposed cultures (28.4±6.2%), and was able to reduce caspase 3 activity by one third (to 5.1 + 1.2 enzymatic activity units).These results indicate that locally expressed GH may act as an autocrine/paracrine survival factor that preserves cellular viability and inhibits apoptotic cell death.(Supported by PAPIIT-DGAPA, UNAM 210209; CONACYT F1-60296, 118353 and 51044).

P80.

CRUSTACEAN CARDIOACIVE PEPTIDE, ITS RECEPTOR, AND PHYSIOLOGICAL EFFECTS, IN THE BLOOD-GORGING BUG, *RHODNIUS PROLIXUS*

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Crustacean cardioactive peptide (CCAP), a cyclic nonapeptide (PFCNAFTGCamide), has multifunctional roles in insects including the stimulation of visceral and cardiac muscle contraction, and the regulation of ecdysis. Previously, we have cloned the cDNA sequence of the CCAP gene from *Rhodnius prolixus* central nervous system (CNS), and shown its expression in the CNS using *in situ* hybridization. In the current study, we have verified the amino acid sequence of CCAP in *R. prolixus* CNS by matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS) and MALDI-TOF MS/MS, and shown its distribution in the CNS and peripheral tissues using immunohistochemistry. We have also partially sequenced the cDNA of the RhoprCCAP G-protein coupled receptor (GPCR) and examined its expression in the CNS as well as peripheral tissues of *R. prolixus*. Physiologically, CCAP dose-dependently stimulates *R. prolixus* hindgut contractions, with threshold at $5x10^{-9}$ M and maximum response at10^{-7}M CCAP. Also, CCAP increases heartbeat frequency in a reversible, dose-dependent manner, with threshold close to 10^{-11}M and maximum response at 10^{-10}M CCAP. CCAP is therefore present in *R. prolixus*, and acts via GPCRs to modify visceral and cardiac muscle contraction. (This work was supported by the Natural Sciences and Engineering Research Council of Canada.)

P81.

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF A GENE ENCODING THE KININ PEPTIDES IN THE BLOOD-GORGING BUG, *RHODNIUS PROLIXUS*

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The digestive and diuretic activities in the haematophagous insect *Rhodnius prolixus* are under neuropeptide regulation. In *R. prolixus*, the kinin-like peptides, first isolated from *Leucophaea maderae* (i.e. leucokinins), have been shown to co-localize with the corticotrophin-releasing factor (CRF)-like diuretic hormone in some neurosecretory cell bodies and their abdominal neurohaemal sites. In addition, kinins are present in endocrine cells of the midgut of 5th instar *R.prolixus*. Leucokinin I stimulates hindgut contractions and midgut contractions in *R. prolixus*. The purpose of this study is to sequence the kinin transcript from the central nervous system (CNS) of *R. prolixus* and then to study its spatial expression. Thus far, a 1198bp cDNA encoding the open reading frame with a 398 amino acid prepropeptide has been isolated that predicts the processing of 12 Rhopr-kinins. Selected members of these Rhopr-kinins stimulate midgut and hindgut contractions. Northern blot analysis reveals a 1.9kb RNA transcript, suggesting that 700bp of the transcript is still missing. Spatial expression of the kinin transcript using Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR), reveals that the transcript is expressed in the CNS and a variety of peripheral tissues, including anterior and posterior midgut and testes. (This work was supported by the Natural Sciences and Engineering Research Council of Canada.)

TOPIC: *REPRODUCTIVE ENDOCRINOLOGY*

P82.

EXPRESSION AND FUNCTIONAL STUDIES OF TWO NOVEL TYPE III GNRH RECEPTORS (2 AND 3) IN THE SEA LAMPREY, A BASAL VERTEBRATE

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Gonadotropin-releasing hormone (GnRH) is a central regulator of reproduction in vertebrates. Its function is mediated by a GnRH receptor (GnRHR), a class A GPCR. Previously, we identified a unique lamprey GnRH receptor (lGnRH-1) that shares several characteristics of both type-I and type-II/-III vertebrate GnRH receptors (Silver et al., 2005). We will report on our latest findings of the identification, expression and functional studies of two novel GnRH receptors (lGnRHR-2 and -3) in the sea lamprey. The inositol phosphate (IP) and cAMP responses of these two receptors transiently expressed in COS7 cells were examined upon stimulation with increasing doses of lamprey GnRH-I, -II or –III. Lamprey GnRH-II and –III, induced an IP response in lamprey GnRH Receptor-2 and -3. There was no IP response in either receptor when treated with lGnRH-I. Lamprey GnRH-II was a more potent activator of the lamprey GnRH receptor-3 than lGnRH-III. Stimulation of lGnRHR-2 and -3 with increasing doses of each of the three GnRH ligands did not elicit a cAMP response. Lamprey GnRHR-2 precursor transcript was detected in a wide variety of tissues including the pituitary in both male and female adult lampreys. Lamprey GnRHR-3 precursor transcript was not as widely expressed and primarily expressed in the brain and eye of male and female lampreys. In summary, out of three functional GnRH receptors described in the sea lamprey to date only two, lGnRHR-1 and -2, are present in the pituitary. From our phylogenetic analysis, we propose that lGnRHR-1 evolved from a common ancestor of all vertebrate GnRH receptors and lamprey GnRHR-2 and -3 occurred due to a local duplication within the lamprey lineage. These novel GnRH receptors share the structural features and amino acid motifs common to other known gnathostome type II/III-GnRH receptors. (Supported by NSF IOS-0849569, NH AES Hatch 332 and NIH Grant 5R21RR024477-02 to SAS and UNH SURF grant to CM).

P83. OPPOSITE-SEX COHABITATION PROMOTES THE MORPHOLOGICAL MATURATION OF GNRH NEURONS IN TRANSGENIC ANIMALS DEFICIENT IN FGF SIGNALING

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Gonadotropin-releasing hormone (GnRH) is a neuropeptide that drives reproduction in vertebrates. We have shown that fibroblast growth factors (FGFs) are signaling factors crucial for the development of GnRH-secreting neurons. Further, preliminary data using transgenic mice have shown that FGFs are also important in the postnatal maintenance of GnRH neurons. We previously generated a transgenic mouse, dnFGFR mice, which have reduced FGF receptor function at the level of the GnRH neuron. While wildtype (WT) mice maintain the same number of GnRH neurons throughout their lives, dnFGFR mice show an age-dependent decline of these neurons. Interestingly, opposite-sex cohabitation in these mice restores GnRH neuron number to wildtype amounts. Although WT mice do not lose GnRH neurons over time, these neurons undergo extensive morphological remodeling during the pubertal transition. During puberty, the number of complex neurons decreases while the number of unipolar neurons increase. These morphological changes likely indicate a functional maturation of the GnRH system. We examined the morphological distribution of GnRH neurons in dnFGFR mice at postnatal day (PN)35, and found that these mice exhibit a more "juvenile" distribution than WT, with more complex and fewer unipolar neurons. This altered distribution persisted in PN100 males. However, PN100 males housed with females since puberty showed normal distribution of GnRH neuron types. dnFGFR PN100 females also had an altered GnRH neuron morphological distribution, but this alteration also disappeared with opposite-sex housing. Our data show, for the first time, that FGF signaling is crucial for the normal morphological changes in maturing GnRH neurons. Further, environmental cues (i.e. opposite-sex housing) can override defects in the FGF system to drive the morphological maturation of these neurons. These data indicate that the GnRH system is highly plastic and responsive to the external environment throughout adulthood.

P84. LEPTIN MODULATES MATE CHOICE PERMISSIVENESS IN THE PLAINS SPADEFOOT TOAD

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The female Plains spadefoot toad (*Spea bombifrons*) hybridizes with the Mexican spadefoot toad (*Spea multiplicata*) depending on her condition: poor condition females are more likely to prefer heterospecific mates. In mammals, leptin helps signal healthy condition via fat stores, so we initially hypothesized that enhanced leptin signaling might increase preference for conspecifics in poor condition females. Mammalian studies have also indicated that leptin can influence the hypothalamic-pituitary-gonadal axis which regulates reproductive behaviors. However, in amphibians and fish, leptin is expressed by a number of tissue types other than fat including heart, brain, liver, lung, ovary, and testis. The wider expression pattern of this hormone suggests a broader suite of effects in these vertebrate animals. To determine if leptin plays a role in mate choice behavior, we manipulated leptin levels in the Plains spadefoot toad (*Spea bombifrons*) and measured mate preferences in 2-choice phonotaxis tests. Rather than enhance preference for conspecifics, exogenous leptin caused *S. bombifrons* females to be random in their preference between species. We also report that leptin reduced the latency to choose a mate, suggesting that females became less choosy. Together these results suggest leptin increases permissiveness in mate choice. It is known that the level of female gravidity can alter her mate choice permissiveness, and the anuran ovary produces high levels of leptin relative to other tissues. Thus, leptin may signal reproductive status and heighten female motivation to mate. Further investigation of this divergent function will help elucidate whether leptin causes female choice to be random or if it influences a switch in preference.

P85.
TESTOSTERONE RAPIDLY INCREASES MILT OUTPUT IN MALE GOLDFISH (*CARASSIUS AURATUS*)

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In many vertebrates, interactions between males and females stimulate rapid increases in the steroid hormones testosterone (T) and estradiol (E_2). Recent work has demonstrated that both T and E_2 act to rapidly (< 1 hr) facilitate male sexual behaviors. However, whether and how steroids may also rapidly influence reproductive physiology and/or mating success has been less well studied. To address this issue, we investigated rapid T effects on milt (sperm and seminal fluid) production in goldfish. We isolated mature male goldfish in small groups for 2 days. On the third day we selected a focal male from each tank and measured the volume of milt expressed. We then injected males I.P. with either 3 mg T in saline + 0.1% methanol or an equivalent volume of saline vehicle. After 1 hr we measured milt volume and took blood samples from all fish. Compared to pre-injection milt volumes, males injected with T rapidly increased their milt output by 54%. In contrast, saline-treated males decreased milt volume by 44%. We then tested whether T elicited rapid increases in milt via its conversion to E_2 by aromatase. To do this, we isolated males in small groups for 2 days. On the third day we injected focal males with either Fadrozole (an aromatase inhibitor) or saline, and 30 min later we measured milt volume and injected each group with 3 mg T. A third group of males received two saline injections. We measured milt volume at 1 hr post-T injection and found that Fadrozole blocked the T-induced increase in milt production. Together, these results suggest that T, via activation of estrogen receptors in the brain or periphery, can significantly increase sperm output over the short time scales that characterize reproductive encounters. Because goldfish spawn in groups in which males engage in intense competition for access to females, such T-induced increases in milt output may enhance a male's ability to engage in sperm competition and, therefore, influence his reproductive success. (This work was supported by Bowdoin College and NSF IOS 0849102 to R.R.T.)

P86.
GONADOTROPIN-RELEASING HORMONE RECEPTOR IN *APLYSIA CALIFORNICA*

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Gonadotropin-releasing hormone (GnRH) is a universal activator of the vertebrate reproduction. However, a recently elucidated GnRH-like molecule in the mollusk, *Aplysia californica*, does not appear to have a reproductive role. To better understand the function of *Aplysia* GnRH and its target tissues, we attempted to identify candidates for the GnRH receptor (GnRHR) in *A. californica*. Initially, we performed *in silico* analysis of expressed sequence tags and whole genome shotgun sequences to search for *A. californica* sequences similar to other known Trochozoa GnRHRs or GnRHR-like receptors. Four partial sequences with high identity to GnRHRs were found. Attempts were made to expand these sequences using a trace assembler (http://genotrace.niob.knaw.nl/). The only significant match was found between an *A. californica* sequence and the N-terminus of the octopus GnRHR. Using this partial sequence, we designed degenerate primers to amplify a larger segment of the target gene and subsequently isolated its 3' end using the 3' rapid amplification of cDNA ends. The identified receptor transcript maintains the conserved structural features and motifs of other known type II GnRH receptors and has 60% identity with the octopus GnRHR. Interestingly, intracellular loop 3 of the *A. californica* GnRHR-like receptor is approximately 32

amino acids (aa) longer than that of the octopus GnRHR. The C-terminal tail is approximately 44 aa long, which is 35 aa shorter than the octopus GnRHR. Expression of the receptor transcript is confined to the central nervous system. Phylogenetic analysis places the *A. californica* GnRHR-like receptor with the octopus GnRHR in the GnRH / corazonin receptor families. To date, the only GnRHR candidate identified in *A. californica* is the one described here. Future plans are to characterize its expression using *in situ* hybridization and conduct functional studies to establish its identity as a bona fide GnRHR. (Supported by NSF IOS-0743818)

P87.
GONADOTROPIN-RELEASING HORMONE SYSTEM DURING PUBERTAL TRANSITION OF FIBROBLAST GROWTH FACTOR 8-DEFICIENT MICE
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Gonadotropin-releasing hormone (GnRH) is critical for the onset and maintenance of reproduction in vertebrates. Puberty, a significant transition in life involving reproductive onset, is triggered by increasing levels of GnRH synthesis and secretion. The development of GnRH neurons is highly dependent on fibroblast growth factor (Fgf) signaling. In transgenic mice deficient in Fgf8 signaling (Fgf8 hypomorphs), the genesis of GnRH neurons is disrupted, leading to a 50% decrease in the number of postnatal GnRH neurons. However, the initiation of pubertal onset appears unaffected in these mice, suggesting the presence of compensatory mechanisms. In this study, we sought to understand the nature of these compensatory mechanisms by examining GnRH neuron number and hypothalamic GnRH content in Fgf8 hypomorphs and wildtype (WT) mice within ages encompassing pubertal transition (postnatal day (PN) 10, 20, 25, 30, 35, to 40). Our results showed that GnRH neuron numbers were significantly reduced in Fgf8 hypomorphs compared to WT in all age groups examined. In parallel, significant decreases in hypothalamic GnRH content in Fgf8 hypomorphs were observed in all age groups except PN35. Interestingly, during the pubertal transition at PN35, the GnRH content of Fgf8 hypomorphs increased to a level no longer different from that seen in WT mice. These data indicate that, during puberty, the GnRH system compensates for the loss of GnRH neurons by increasing its overall GnRH production. This speaks to the extraordinary ability of an organism's pubertal drive to overcome pre-existing deficiencies in order to ensure its reproductive onset. (Supported by NIH 042634 to PST)

P88.
LOCALIZATION OF THE EXPRESSION OF GONADOTROPIN-RELEASING HORMONE LIKE-MOLECULE IN A GASTROPOD MOLLUSK, *APLYSIA CALIFORNICA*
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Gonadotropin- releasing hormone (GnRH) is a key neuropeptide for regulating reproduction in vertebrates. The recent discoveries of GnRH-like molecules in non-chordate animals suggested GnRH may have arisen in an ancestral bilaterian that gave rise to both protostomes and deuterostomes. Our laboratory has previously isolated a full-length cDNA of a GnRH-like molecule, named ap-GnRH, from a gastropod mollusk, *Aplysia californica*. Immunocytochemistry revealed the presence of neurons positive for ap-GnRH in only 2 central ganglia, the pedal and cerebral ganglia, of *A. californica*, but a specific radioimmunoassay revealed that all 5 central ganglia contained immunodetectable ap-GnRH, leading to some confusion regarding the source of this peptide. The goal of the present study is to localize ap-GnRH transcript in central and peripheral tissues of *A. californica* by in situ hybridization (ISH). Using a 604 nt-cRNA probe, we detected the strong presence of ap-GnRH transcript in neurons of the pedal ganglia. In addition, many positive neurons were localized in the abdominal ganglia, followed by 2-3 neurons in the cerebral ganglia. No staining was observed in the remaining central ganglia (buccal and pleural ganglia) or in the peripheral tissues (the ovotestis and atrial gland). These data are largely consistent with our previous immunocytochemical data and support the presence of both ap-GnRH peptide and transcript in the pedal and cerebral ganglia. However, the presence of ap-GnRH mRNA in the abdominal ganglia suggests transcriptional activity also occurs in these ganglia. As such, our inability to detect abdominal ap-GnRH immunoreactive neurons may reflect either alternative processing or exceptionally fast turnover of the peptide in these ganglia. Overall, our data suggest that ap-GnRH is produced by multiple ganglia and support the notion that ap-GnRH may assume functions beyond reproduction. (Supported by NSF IOS 0743818 to PST.)

P89.
IMPACT OF RETINAL DEGENERATION ON MELATONIN AND GONADOTROPIN INHIBITORY HORMONE LEVELS DURING PHOTOSTIMULATION IN SMOKY JOE CHICKENS
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In avian species the reproductive axis is controlled by stimulatory (GnRH) and inhibitory (GnIH) inputs. Light stimulates the synthesis and release of GnRH via hypothalamic photoreceptors, and indirectly controls GnIH production by altering melatonin (Mel) synthesis from the retina and pineal gland. Recently, we reported that blind roosters from the Smoky Joe strain of chickens mature sexually earlier than sighted animals. Thus, in the present study we investigated if the lack of functional retina impacts Mel and GnIH levels. Smoky Joe roosters were maintained on a short-day photoperiod (8h/SDP) until 18 wks of age then transferred to a long-day photoperiod (14h/LDP). At 18 (SDP) and 24 (LDP) wks of age, blood samples were collected every 4h for 24h from 10 blind and 10 sighted birds. Additionally, for both collection ages, pineal, retina and diencephalons were collected from 5 blind and 5 sighted birds in the middle of the dark and light phases. In blind birds, retinal Mel levels were low in all samples while in sighted birds, they were significantly increased during the dark phase under SDP but not LDP. Pineal Mel content was significantly higher during the dark than the light phase for both blind and sighted animals under SDP. Interestingly, under LDP, pineal Mel content in sighted roosters was significantly lower than those observed under SDP. Despite the difference observed in the retina and pineal, plasma Mel profiles were identical between blind and sighted birds with a significant increase during dark phases under both SDP and LDP. Levels of GnIH in the diencephalon mirrored those observed for Mel in the pineal gland with decreased levels during light phases in both blind and sighted birds, and lower levels during dark phase in sighted roosters under LDP. Our data indicate that although Mel and GnIH follow a circadian rhythm in both blind and sighted roosters, a functional retina results in decreased pineal Mel and hypothalamic GnIH during LDP dark phases. (This work was supported in part by the Poultry Industry Council, the Natural Sciences and Engineering Research Council of Canada, and the Ontario Ministry of Agriculture, Food and Rural Affairs.)

P90.
REPRODUCTIVE HORMONES AND RECEPTORS OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN THE KOALA AND ECHIDNA: NEW SEQUENCES AND PHYLOGENY
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Studies of the hypothalamic-pituitary-gonadal axis in marsupials are limited to in vivo studies of the brushtail possum and in silico studies of the sequenced genome for the grey short-tailed opossum, so that knowledge of the mechanisms that regulate reproduction in this taxon is incomplete. Although the platypus genome has been recently sequenced, the mechanisms that govern the reproductive axis in the egg-laying monotremes are also poorly understood. To further appreciate the unique reproductive biology of marsupials (koala) and monotremes (echidna), there is a need to develop tools that allow the measurement and alteration of the hypothalamic and adenohypophysial hormones and receptors. This report presents sequence data for the gonadotropin-releasing hormone (GnRH) peptides, follicle stimulating hormone (FSH), luteinizing hormone (LH), and their respective receptors in the koala (*Phascolarctos cinereus*) and echidna (*Tachyglossus aculeatus*). Phylogenetic analysis of these reproductive hormones and their receptors, along with those from other vertebrates, allows identification of homologs in the vertebrate lineage and clarifies the evolutionary relationship for these three subclasses of mammals. [Supported by a Canadian NSERC grant to NMS and an Australian Dreamworld Research Grant to SDJ].

P91.
INHIBITION OF CORTICOSTERONE SYNTHESIS INDUCES THE TRANSITION FROM COURTSHIP TO FEEDING BEHAVIOR IN RED-SIDED GARTER SNAKES (*THAMNOPHIS SIRTALIS*)
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Seasonal modulation of glucocorticoids plays an important role in supporting critical life-history events such as reproduction and migration. In a well-studied population of red-sided garter snakes (*Thamnophis sirtalis*), glucocorticoids are elevated during the brief mating season. Elevated glucocorticoids likely facilitate energetically expensive courtship behavior, as snakes do not eat during the mating season and must migrate up to 17 km to forage at feeding grounds. We previously demonstrated that dispersing male red-sided garter snakes have significantly lower baseline corticosterone than courting snakes, suggesting that elevated corticosterone is necessary to support reproductive behavior. To test this hypothesis, we collected courting snakes and randomly assigned them to one of the following hormone implant treatments: control, 250 or 1000 mg metyrapone, a corticosterone synthesis inhibitor. Males were then tested on a y-maze and allowed to choose between a female or worm trail (i.e., a courtship or feeding cue). Significantly more snakes receiving 1000 mg of metyrapone (8 of 10) chose worm trails at 14 days post-treatment than snakes receiving the control implant (1 of 10; $P = 0.007$). These results indicate that a decrease in plasma corticosterone regulates the behavioral switch from reproduction to foraging in the spring. In a second experiment, we examined whether the differences in plasma corticosterone between courting and foraging snakes result from differences in corticotrophin releasing factor (CRF). We collected actively courting snakes from the den and foraging snakes during dispersal ($n = 4$ in each) and processed the brains for CRF immunohistochemistry. There were no differences in the distribution or numbers of immunoreactive cells between snakes. These preliminary results suggest that some other factor, such as a change in the sensitivity of the hypothalamus-pituitary-adrenal axis, may underlie the activation of feeding behavior during spring.

P92.
THE EFFECT OF FOOD AVAILABILITY ON THE REPRODUCTIVE SYSTEM OF A SONORAN DESERT SONGBIRD
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Many bird species exhibit large seasonal changes in their hypothalamo-pituitary-gonadal (HPG) axis. This requires time and energy and so birds must time their seasonal reproductive activity to coincide with peak food abundance. To do this, they track environmental cues that predict future conditions conducive to reproduction. Most birds prioritize the use of environmental cues in a hierarchical fashion, with the annual change in day length serving as the cue that begins or ends reproductive activity and other supplementary cues, such as food availability, fine tuning reproductive phenology to local environmental conditions. However, the mechanism(s) by which food influences the HPG axis remain(s) poorly understood. We examined the influence of food availability on the body mass and HPG axis of adult male Abert's Towhees, *Melozone aberti*. Birds caught during winter 2011 were exposed to long days to initiate reproductive development and were randomly assigned to one of three groups: (1) *ad libitum* food, (2) restricted food availability, in which they receive 70% of their *ad libitum* food consumption, for four weeks, or (3) two weeks of food restriction followed by two weeks of *ad libitum* food. Two weeks of food restriction decreased body mass, furcular fat, and cloacal protuberance (CP; an androgen-sensitive secondary sexual characteristic) width. Reinstating *ad libitum* food increased CP width. However, at the end of the study the three groups did not differ in their testis masses. Thus, food availability influenced the HPG axis, but this influence varied at different points on the axis. To elucidate the nature of this influence we are currently measuring the circulating concentrations of reproductive hormones (luteinizing hormone and testosterone), the amount of hypothalamic gonadotropin-releasing and -inhibitory hormones, and association between these neuropeptides and the brain expression of nutritionally sensitive neuropeptide Y. (Supported by CAP LTER grant to SD).

P93.
SOCIAL REGULATION OF KISSPEPTIN SIGNALING IN *ASTATOTILAPIA BURTONI*
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The neural inputs that relay environmental cues to the vertebrate reproductive axis are largely unknown. Kisspeptins are neuropeptides known to be potent stimulators of Gonadotropin Releasing Hormone 1 (GnRH1) neurons in the preoptic area (POA), which control pituitary release of the gonadotropins in all vertebrates. To investigate the role of kisspeptins in environmental control of reproduction, we use a cichlid species, *Astatotilapia burtoni*, in which the reproductive capacity of adult males is dependent on social status. Subordinate males can be experimentally manipulated to ascend to dominant status and this transition produces dramatic increases in circulating testosterone, GnRH1 neuron size, and testes size within several days. We have identified the *A. burtoni* mRNA sequence of a kisspeptin gene, *Kiss2*, and its putative receptor, *GPR54a*. Male *Kiss2*exhibits high expression in the hypothalamus, where *GPR54a* is also localized. Ascending males had higher *GPR54a* expression in the POA compared to both stable subordinate and dominant males.

Complementary studies for *Kiss2* are currently underway. Our findings indicate that neuronal populations participating in kisspeptin signaling are quickly responsive (30 minutes) to reproductively-significant social cues. Furthermore, our results suggest that a shift in kisspeptin signaling may participate in environmentally-driven augmentation of the reproductive axis.

P94.
SYSTEMIC RNA INTERFERENCE AS A TOOL FOR UNRAVELING THE NEUROHORMONAL REGULATION OF DESERT LOCUST (*SCHISTOCERCA GREGARIA*) REPRODUCTION
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The desert locust (*Schistocerca gregaria*) is a fearsome pest insect. Swarms consisting of billions of individuals can seriously damage the crop production in large areas of the world. An interesting target for pest control strategies is the reproductive process and this fact stimulates fundamental research of locust reproduction. We recently produced an EST database derived from desert locust central nervous system (Badisco *et al*., 2011). Homology searches resulted in the identification of multiple transcripts encoding neuropeptides that may be candidate key players in desert locust reproductive physiology. The availability of this novel transcript sequence information stimulated us to employ RNA interference (RNAi) to further investigate the possible biological role(s) of these neuropeptides. A selection was made of assessable parameters that could provide insight in their involvement in the desert locust's reproductive physiology. Desert locusts display a very robust systemic RNAi response upon injection of double stranded RNA (even when administered in the order of magnitude of a few nanograms) into the haemocoel and they generally display at least 80 % knock-down of the targeted transcript. Therefore, RNAi has become an indispensable technique in our investigation of the molecular and neurohormonal regulation of desert locust reproduction. We recently analyzed the effects on reproductive physiology of a knock-down of locust insulin-related peptide, neuroparsins, ovary maturating parsin and juvenile hormone biosynthesis enzymes.

P95.
LINKING STEROID HORMONES TO PRIMARY AND SECONDARY SEXUAL CHARACTERS IN ALTERNATIVE REPRODUCTIVE TACTICS OF CHINOOK SALMON
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The proximate mechanisms that underlie the evolution of within-sex variation in mating behaviour, secondary sexual characters and reproductive investment patterns are still poorly understood. Species exhibiting alternative reproductive tactics are ideal model systems to examine these mechanisms. Chinook salmon (*Oncorhynchus tshawytscha*) exhibit two distinct alternative reproductive tactics; hooknoses (derived from the exaggerated snout, which develops prior to spawning), that are large males that establish spawning dominance hierarchies via intense male-male competition (using secondary sexual characters such as large kypes (jaws) and dorsal humps) and jacks, that are small precocious sneaking males that steal fertilizations (via sperm competition) from hooknoses while they spawn with females. In this study, we examine androgen profiles (11-ketotestosterone & testosterone) of spawning hooknoses and jacks. Furthermore, we also examine relationships between androgen levels and primary (testes mass) and secondary (hump, kype, and body size) sexual characters. We found that hooknoses and jacks did not significantly differ in terms of either 11-ketotestosterone or testosterone concentrations. Moreover, we found significant positive relationships between levels of both androgens within each alternative reproductive tactic, with the relationship being stronger for jacks than hooknoses. Testosterone and 11-ketotestosterone levels during the spawning season covaried positively with gonad investment in jacks. In hooknoses, 11-ketotestosterone was positively related to hump depth and body size. Overall, our findings suggest that there are differential androgen effects for each of the alternative reproductive tactics. [Supported by grants from the Natural Sciences and Engineering Research Council, Canadian Foundation for Innovation, Ontario Research Foundation, and an Ontario Ministry of Research and Innovation Postdoctoral Fellowship (to IAEB).]

P96.
THE ORIGIN OF TESTICULAR SOMATIC CELLS IN THE GONAD DURING SEX CHANGE IN THE PROTOGYNOUS WRASSE, *HALICHOERES TRIMACULATUS*
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The three-spot wrasse (*Halichoeres trimaculatus*) changes sex from female to male. Oocytes in the ovary disappear completely, and male germ cells and somatic cells comprising testicular tissue arise anew during the process of sex change. In general, the differentiation of germ cells is controlled by somatic cells that surround the germ cells. Thus, we believe that it is essential to understand how testicular somatic cells differentiate during sex change. Therefore, the purpose of this study was to identify the origin of testicular somatic cells in the gonads by analyzing cellular behavior during sex change. Apoptosis in the sex changing gonad was analyzed by immunodetection of Caspase-3 which is one of the key mediators of apoptosis. However, few Caspase-3 immunoreactive somatic cells were found to be present during sex change, which suggests that ovarian somatic cells survive during the regression of ovarian tissue. Cell proliferation was investigated by 5-bromodeoxyuridin (BrdU) uptake in sex changing gonads. Interestingly, BrdU immunoreactivity (BrdU-ir) was detected in many granulosa cells surrounding the degenerating oocytes at an early stage of sex change. In addition, BrdU-ir was also detected in a few epithelial cells covering ovigerous lamella and somatic cells associated with gonial germ cells. To define the fate of proliferating cells, BrdU pulse chase analysis was performed. At an early stage, BrdU immunoreactive somatic cells were of three types, granulosa cells, epithelial cells and somatic cells associated with gonial germ cells. In spermatogenic gonads at 2 weeks after BrdU injection, BrdU-ir was detected in the somatic cells in the central region of the lamella, Sertoli cells surrounding the cyst of spermatogenic germ cells, and somatic cells associated with gonial germ cells. These results suggest that some testicular somatic cells originate from functional somatic cells of the ovary. (Supported by a Grant-in-Aid for Science Research from the Ministry of Education, Science, Sports and Culture of Japan and SORST, JST)

TOPIC: *MOLECULAR EVOLUTION*

P97.

CHARACTERIZATION OF PITUITARY GROWTH HORMONE IN THE GREEN IGUANA (*IGUANA IGUANA*)

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Although growth hormone (GH) has been studied in many vertebrates, it is poorly characterized in reptiles, where its structure has been described only in a turtle, a crocodile and, more recently, a snake. Here we studied the morphology and ultrastructure of pituitary somatotrophs in the green iguana (gi, Order *Squamata*) and characterized the giGH structure, which allowed an evolutionary analysis. The green iguana pituitary is constituted by a *pars distalis* and a *pars nervosa*, divided by a *pars intermedia* which, in contrast to other reptiles, is more developed in this species. The cellular distribution of giGH was analyzed by *in situ* hybridization and immunohistochemistry, using a specific-riboprobe and an antibody against chicken GH, respectively. The somatotrophs were found mainly in the caudal region of the *pars distalis*. Electron micrographs showed that they are 6.5-10 µm cells containing a good amount of granules (250-300 nm) where giGH is stored. The pituitary giGH was purified by immunoaffinity chromatography using a heterologous immunosorbent prepared with a polyclonal anti-chicken GH (cGH) antibody. SDS-PAGE and Western blotting of the purified preparation showed a main monomeric giGH variant with an apparent MW of 26 kDa and a dimeric molecular variant of 52 kDa (under reducing conditions). Furthermore, giGH showed at least four charge variants (pIs between 6.2-7.4) by isoelectric focusing. Additionally, the cDNA of giGH was amplified by PCR and 3' & 5' RACE. The sequence obtained consisted of 1016 bp that encoded a pro-hormone of 218 aa, containing a signal peptide (27 aa) and the mature protein corresponding to 191 aa. An identity pattern of 81%, 82%, and 84% was obtained when giGH was compared with chicken, crocodile and turtle GHs, respectively. The phylogenetic analysis showed that giGH goes in a different branch than turtles and crocodiles, which are closer to birds. These findings will contribute to better understand the evolution of vertebrate GH. (Supported by CONACYT (F1-60296, 220839) and PAPIIT (IN205406-3, IN21029) grants.)

P98.

CORTICOTROPIN-RELEASING HORMONE (CRH) FAMILY MEMBERS AND THEIR RECEPTORS IN LAMPREY (*PETROMYZON MARINUS*): POTENTIAL INSIGHTS INTO THE EVOLUTION OF THE CRH FAMILY

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The CRH superfamily of peptides has a long evolutionary history. In vertebrates the superfamily consists of four paralogues, corticotrophin-releasing hormone (CRH), urotensin I (UI) [also included urocortin (UC) and sauvagine (SV)], urotensin II (UII), and urotensin III (UIII). These paralogues likely arose from two separate lineages: the CRH/UI,UC,SV lineage and the UII/UIII lineage. It is currently unclear if the gene duplications which gave rise to the two CRH family lineages (and subsequently the 4 paralogues) occurred in basal vertebrate ancestors or if one of these duplications occurred after the divergence of gnathostomes and agnathans. To aid in the determination of the evolutionary history of the CRH superfamily we set out to identify CRH family members and their receptors in the lamprey, *Petromyzon marinus*, one of two extant agnathans. We also wanted to use these data to better understand the general aspects of the CRH hypothalamic-pituitary axis in this ancient vertebrate. Our data indicate that there are only three members of the CRH family in lamprey which we have designated CRHA, CRHB, and CRHC. In addition we have isolated partial cDNAs for two distinct CRH receptors with homology to CRH-R1 and CRH-R2. These sequence data will be discussed with respect to their relevance to the evolution of the CRH family and the gene duplications which gave rise to the 4 CRH paralogues present in gnathostomes. Preliminary expression analysis of these lamprey genes in a variety of tissues will also be presented. The long-term goals of this research are to investigate the hypothalamic-pituitary axis in lamprey and the potential for cross regulation between the stress (CRH), reproductive and thyroid axes. Cross-regulation between the CRH and thyroid axes is well-established in numerous vertebrates and there are data to suggest interactions between the reproductive and thyroid axes.

P99.

ANNOTATION OF THE NEUROENDOCRINE-ASSOCIATED GENES OF THE LAMPREY *PETROMYZON MARINUS* AND THE IMPLICATIONS FOR EVOLUTION OF VERTEBRATE GONADOTROPIN-RELEASING HORMONES

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The development of the hypothalamic-pituitary axis was a seminal event in the evolution of vertebrates. Study of the ancestral jawless vertebrates is key to understanding this event, with the gonadotropin-releasing hormones (GnRHs) deserving particular attention given their pivotal placement in the hierarchy of the neuroendocrine system. Previous data incorporating several lines of evidence showed all known vertebrate GnRHs were grouped into four paralogous lineages: GnRH 1, 2, 3 and 4; with proposed evolutionary paths. Using the currently available lamprey genome assembly, we have identified many neuroendocrine system genes and analyzed in greater detail the evolutionary history of the GnRHs based on the conserved syntenies between lamprey and gnathostomes. Our analysis corroborates recent views that GnRH3 was lost in the tetrapod lineage and did not arise in the teleost lineage as a result of a third round of whole genome duplication. With respect to the agnathans and GnRH, our analysis of the synteny agree with the previous proposal in that lamprey GnRH-I and –III resulted from a duplication event within the lamprey lineage. However, the data now suggest a substantially different view of the evolutionary history of the GnRH family in vertebrates. Significantly, the current evidence suggests that all of the genome duplication events that generated the different fish and tetrapod paralogous groups likely took place before the divergence of the ancestral agnathans and gnathostome lineages and that the type IV GnRHs in lamprey (GnRH-I and -III) share a more recent common ancestry with GnRH2 and 3. Given the single amino acid difference between lamprey GnRH-II and GnRH2 we propose that a GnRH2-like gene existed before the lamprey/gnathostome split and that paralogous genes (GnRH-I/III and GnRH 3) evolved divergent structure/function in lamprey and gnathostome lineages. The synteny analysis offers a new view on the evolution of the GnRHs. (Supported by NSF IOS-0849569, NH AES Hatch 332 and the Lamprey Genome Project Consortium.)

P100.
ARE NOVEL RFAMIDE PEPTIDES NEUROREGULATORS OF THE LAMPREY NEUROENDOCRINE SYSTEM?
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The synthesis and secretion of gonadotropin-releasing hormone (GnRH) is the key neuroendocrine function in the hypothalamic regulation of the hypothalamic-pituitary-gonadal axis. In lampreys, there are three hypothalamic GnRHs that regulate the pituitary-gonadal axis. In addition, there are several important brain neurohormones/factors that have been shown to stimulate/modulate gonadotropin releasing hormone (GnRH) and gonadotropin in vertebrates. Gonadotropin-inhibitory hormone (GnIH) is a dodecapeptide having a C-terminal LPLRF-amide motif first identified in quail and shown to inhibit the synthesis and release of gonadotropins (Tsutsui et al. 2000). Subsequently, GnIH orthologs which belong to the LPXRF-amide peptide family (X = L or Q) have been described in fish but studies on the functions on the hypothalamic-pituitary axis are limited. We recently identified three RFamide peptides (lamprey RFa-1a, RFa-1b and RFa-2) from the lamprey brain. Here we will report on the effects of these lamprey RFamide peptides on the concentrations of lamprey GnRH-I, -II, and -III in adult lamprey brain and on RNA expression of gonadotropin hormone and GnRH receptor-I in the lamprey pituitary. These peptides were tested in two different adult lamprey reproductive seasons at different water holding temperatures. Lamprey RFa-1a, RFa-1b or RFa-2 each was tested both *in vivo* and *in vitro*. Concentrations of lamprey GnRH-I, II, and III were determined by HPLC and specific radioimmunoassays. Lamprey RFamide-2 at 50 and 100 µg/kg fish significantly stimulated brain GnRH-III and only at 100 µg/kg fish significantly stimulated GTHβ expression (real time PCR) in female lamprey when held at temperatures above 15 C and below 15 C, respectively. In summary, we provide evidence of a novel RFamide peptide that has a stimulatory neuroregulatory function on the neuroendocrine axis in a basal vertebrate mediated by the environmental cue of temperature. (Supported by NSF IOS-0849569, NH AES Hatch 332 to SAS, UNH SURF to KG, DK and by Grants-in-Aid for Scientific Research (16086206 and 18107002 to KT) from the Ministry of Education, Culture, and Science, Tokyo).

P101.
THE OXYTOCIN/VASOPRESSIN RECEPTOR FAMILY HAS AT LEAST FIVE MEMBERS IN THE GNATHOSTOME LINEAGE
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The vertebrate oxytocin and vasopressin receptors form a subfamily of G-protein coupled receptors (GPCRs) that mediate a large variety of functions, including social behavior and the regulation of blood pressure, water balance and reproduction. In mammals four family members have been identified and studied, three that respond to vasopressin (VP) named V1A, V1B and V2, and one that is activated by oxytocin (OT), called the OT receptor. The corresponding receptors have been identified in chicken but received different names. Until recently only V1-type receptors have been described in several species of teleost fishes. A thorough phylogenetic and evolutionary analysis of the family has not yet been reported. We have identified family members in several vertebrate genomes and performed phylogenetic analyses in order to create a family tree that may serve as a means of classifying OT/VP-family receptors as well as deducing orthology relationships within the family. We report here the existence of five distinct ancestral gnathostome receptor subtypes in the OT/VP receptor family: V1A, V1B, V2A, V2B and OT receptors. The identification of distinct V2A and V2B receptors has not been previously recognized. We have found these two subtypes in all examined teleost genomes and conclude that the V2A type is orthologous to mammalian V2 receptors whereas the V2B type is orthologous to avian V2 receptors. Thus, there have been differential losses in birds and mammals. Some teleost fish species have had additional gene duplications resulting in up to eight receptor family members. The consequences of this complexity for endocrine functions in fishes, particularly the regulation of water balance, offers to be an interesting avenue of research. (Supported by the Swedish Research Foundation and Carl Trygger's Foundation)

TOPIC: *MEMBRANE RECEPTORS AND CELL SIGNALING*

P102.
DIFFERENTIAL INVOLVEMENT OF PHOSPHOINOSITIDE 3-KINASE IN ENDOGENOUS GONADOTROPIN-RELEASING HORMONE SIGNALLING IN GONADOTROPES AND SOMATOTROPES OF GOLDFISH, *CARASSIUS AURATUS*
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In goldfish, *Carassius auratus*, two endogenous gonadotropin-releasing hormones (salmon (s)GnRH and chicken (c)GnRH-II) control maturational gonadotropin (LH) and growth hormone (GH) secretion via Ca^{2+}-dependent intracellular signalling pathways. We investigated the involvement of phosphoinositide 3-kinase (PI3K) in GnRH-stimulated LH and GH release and associated intracellular Ca^{2+} increases ($[Ca^{2+}]_i$) in morphologically identified goldfish pituitary somatotropes and gonadotropes. Immunoreactive PI3K p85α, the regulatory subunit for conventional Class IA PI3Ks, was detected by Western blot in goldfish pituitary and brain tissue extracts and both endogenous GnRH isoforms modulated phosphorylation of PI3K p85α in excised pituitary fragments. Selective PI3K inhibitors wortmannin (100 nM) and LY294002 (10 µM) significantly reduced sGnRH- and cGnRH-II-elicited LH release responses from primary cultures of mixed pituitary cells and $[Ca^{2+}]_i$ increases in identified gonadotropes. Surprisingly, wortmannin and LY294002 inhibited GnRH-evoked GH release but only attenuated the $[Ca^{2+}]_i$ response in identified somatotropes to cGnRH-II, but not sGnRH. The effects of PI3K inhibition on hormone release responses were specific to GnRHs and not the result of general decreases in the releasable hormone pool or sensitivity to $[Ca^{2+}]_i$ changes as both wortmannin and LY294002 had no effect on Ca^{2+} ionophore-evoked LH and GH secretion. These results indicate that PI3K is involved upstream of $[Ca^{2+}]_i$ increases in mediating LH and GH responses in a cell- and GnRH isoform-specific manner and is the first to implicate PI3K in GnRH-induced LH and GH release in any primary pituitary cell system. [This research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) individual Discovery Grants to J.P. Chang and J.L. Stafford. Financial support for J.G. Pemberton was provided by the Province of Alberta Queen Elizabeth II Graduate Scholarship, NSERC Alexander Graham Bell Canada Graduate Scholarship (CGS M), and the University of Alberta as a recipient of the Dr. Richard E. Peter Memorial Scholarship, Walter H. Johns Graduate Fellowship and Department of Biological Sciences Teaching Assistantship. Y. Yu was a recipient of a F.S. Chia PhD Recruitment Scholarship from the University of Alberta. We thank Alan Mar and Herman Cortes for their assistance with this study.]

P103.
TESTOSTERONE ACTS AT THE CELL SURFACE TO INDUCE TELEOST GRANULOSA/THECA CELL DEATH VIA AN APOPTOTIC PATHWAY
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The teleost ovarian follicle undergoes extensive remodeling and regression during the reproductive cycle—a process involving apoptosis and cell death. However, the hormonal regulation of these processes remains unclear. In the current study the role of testosterone in regulating regression of Atlantic croaker (*Micropogonias undulatus*) ovarian follicles was investigated in co-cultured granulosa/theca (G/T) cells. Testosterone (T) treatment enhanced serum starvation-induced cell death and apoptosis of G/T cells. This effect was mimicked by a cell-impermeable T conjugate, T-bovine serum albumin, indicating that this androgen action is initiated at the cell surface. Previously, an androgen binding moiety with the features of a membrane androgen receptor was biochemically characterized on croaker ovarian membranes. Mibolerone, a nuclear androgen receptor agonist, was ineffective in promoting apoptosis and cell death, which suggests that T actions are independent from the nuclear receptor. Together, the data suggests that T-induction of apoptosis and cell death are through a novel membrane androgen receptor in the croaker ovary. T treatment also increased expression of a pro-apoptotic member of the Bcl-2 gene family, Bax, and two Bax upstream regulators, JNK and p53. These results suggest that T induces cell death of G/T cells in croaker through the apoptotic pathway involving JNK, p53 and Bax. By examining the role of T in croaker follicle cell death and elucidating the corresponding basic mechanisms of androgen action, we are learning more about the regulatory components involved in the breakdown and remodeling stages of the teleost reproductive cycle.

P104.
EFFECT OF NMDAR ON JUVENILE HORMONE BIOSYNTHESIS IN THE COCKROACH, *DIPLOPTERA PUNCTATA*
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Juvenile hormone (JH), produced by the corpora allata, regulates development, metamorphosis and reproduction in most insect species. The regulation of JH biosynthesis is a critical process in the life of all insects. N-methyl-D-aspartate (NMDA) receptors, a subtype of ionotropic glutamate receptors, mediate the majority of excitatory neurotransmission in the central nervous system of vertebrates. Evidence suggests that NMDAR also play a role in regulating JH biosynthesis in insects. However, the mechanism of this regulation is poorly understood. To date, one NMDAR1 (NR1) gene and one NMDAR2 (NR2) gene with six isoforms have been identified in *Drosophila* whereas there are few reports of NMDAR in cockroach species. In the current study, two subunits of *D. punctata* NR1 gene have been identified. Although the rodent NR1 gene encodes eight functional isoforms of the NR1 subunit which appear to originate by alternative splicing, the two subunits of *D. punctata* NR1 identified in our study may be generated by gene duplication. The effect of the agonist NMDA, and the antagonist MK-801 on JH biosynthesis were determined at different concentrations of calcium. The data suggest that the regulation of JH biosynthesis by NMDAR is related to calcium. Further investigations including the profiling of NMDAR expression in the brain and nervous system as well as NMDAR function characterization will be performed to provide information for the study of the points of regulation of the JH biosynthetic pathway (Supported by Natural Sciences and Engineering Research Council of Canada and China Scholarship Council).

P105.
MODELING THE ACTIVATION OF THE MELANOCORTIN 2 RECEPTOR
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Two features that distinguish the melanocortin 2 receptor (MC2R) from the other four mammalian melanocortin receptors (MCRs) are: a) MC2R requires the accessory protein, MRAP1, for trafficking to the plasma membrane and for activation by ACTH; and b) MC2R can only be activated by ACTH, but not by any of the other melanocortin (e.g., α-MSH, β-MSH, γ-MSH). This study will test the hypothesis that a two step mechanism is required for activation of MC2R following an ACTH binding event. For this study a human MC2R cDNA construct with an N-terminal V-5 epitope tag was functionally expressed in CHO cells that were co-transfected with a mouse MRAP1cDNA and the cAMP reporter construct, CRE/Luc. Cells were incubated with analogs of ACTH(1-24), SYSMEHFRWGKPVGKKRRPVKVYP. Alanine substitution of the entire HFRW motif resulted in a complete loss of activation activity, and alanine substitution of the complete KKRRP motif resulted in a significant drop in activation activity. Alanine substitution of the entire GKPVG motif resulted in a 500 fold drop in activation activity. Finally co-incubation of ACTH(1-24) with the analog KKRRPVKYP at a concentration of 10^{-7} M completely blocked activation of MC2R. Based on these observations, our operating assumption is that ACTH(1-24) will first bind to MC2R through the KKRRP motif. This binding event coupled with the secondary structure at the GKPVG region of ACTH will orient the HFRW motif into a binding pocket that induces the activation of the receptor. (This research was supported by NSF grant IOB 0516958.)

P106.
ACTH ANTAGONIST: THE ACTIVATION OF MELANOCORTIN 2 RECEPTORS EXPRESSED IN CHO CELLS BY ACTH(-24) CAN BE BLOCKED BY CO-INCUBATION WITH ACTH(15-24)
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A human MC2R cDNA construct was transiently co-transfected with a mouse MRAP1 gene into CHO cells. Two days later transfected CHO cells were incubated with either ACTH(1-24) or analogs of ACTH(1-24) at concentrations ranging from 10^{-6} to 10^{-12}M. After a 15 minute incubation a direct cAMP EIA kit (Assay Designs Ann Arbor, MI) was used to measure cAMP production in the CHO cells. While ACTH(1-24) produced a robust standard curve, the analog SYSMEHFRWGKPVGAAAAAVKVYP was 1000 fold less potent. Although these data indicated a role for the KKRRP motif in the activation of the receptor, the KKRRPVKVYP analog when incubated alone did not activate hMC2R. However, when concentrations of ACTH(1-24) ranging from 10^{-6} to 10^{-12}M were co-incubated with either 10^{-6} or 10^{-7}M KKRRPVKVP there was complete blockage of hMC2R activation; whereas co-incubation with KKRRPVKVP at 10^{-8}M resulted in a 34±5% drop in activation. By contrast the analog RRPVKVP (10^{-6}M) did not block the activation of hMC2R by ACTH(1-24). These results point to the importance of K^{15} and K^{16} for the activation of hMC2R. In conclusion, the analog ACTH(15-24) [KKRRPVKVP] can function as an ACTH(1-24) antagonist in our *in vitro* system, and this analog may be useful as an *in vivo* antagonist of ACTH activation of MC2R under conditions in which it would be desirable to lower the levels of circulating cortisol. (This research was supported by NSF grant IOB 0516958.)

P107.
ACTIVATION OF THE MELANOCORTIN 2 RECEPTOR OF *XENOPUS TROPICALIS*: TRENDS AMONG TETRAPODS
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The objective of this study was to determine whether the melanocortin 2 receptor (MC2R) of *Xenopus (Silurana) tropicalis* requires co-expression with the accessory protein MRAP1, and whether this receptor can only be activated by ACTH, but not by α-MSH. Although the genome of this amphibian has been sequenced, to date no gene corresponding to mammalian MRAP1 has been detected. In our initial experiments, an xtMC2R cDNA construct with an N-terminal V-5 epitope tag was expressed in CHO cells. Immunocytochemical staining indicated that trafficking of xtMC2R to the plasma membrane did not occur. However, when xtMC2R was co-expressed with mouse MRAP1, xtMC2R immunoreactivity was detected on the plasma membrane. Next CHO were co-transfected with xtMC2R, mouse MRAP1cDNA and the cAMP reporter construct, CRE/Luc and stimulated with hACTH(1-24) or mammalian α-MSH. As expected only ACTH(1-24) activated the receptor in a dose dependent manner. We then tested an analogs of ACTH(1-24) in which the HFRW motif in ACTH(1-24) was replaced with alanine residues (A4) and an analog in which the KKRRP motif in ACTH(1-24) was replaced with alanines. Neither analog was able to activate xtMC2R. An earlier study had predicted that the HFRW binding site on melanocortin receptors involves key residues in TMs 2,3,6, and 7 [Pogozheva et al. (2005) Biochemistry 44, 11329-11341]. Based on these observations we predicted that a second binding site for the KKRRP motif in ACTH(1-24) might be located in TM4, extracellular loop 2, or TM5. We found that alanine substitutions in these regions caused a significant drop in activation when the mutant xtMC2R was stimulated with concentrations of ACTH(1-24) ranging from 10-11M to 10-6M. Immunocytochemical analysis of the mutant constructs indicated that the receptors were trafficking to the plasma membrane. These results will be compared to studies on human MC2R. (This research was supported by NSF grant IOB 0516958.)

P108.
CHARACTERIZATION OF AN ALLATOTROPIN-LIKE PEPTIDE RECEPTOR IN THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*, AND ITS HOMOLOGUE IN THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*
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Following a reverse pharmacology approach, we identified an allatotropin-like peptide receptor (ATR) in *Tribolium castaneum*. Allatotropins are multifunctional neuropeptides initially isolated from the tobacco hornworm, *Manduca sexta*. They have been shown to be myoactive, to be cardio-acceleratory, to inhibit active ion transport, to stimulate juvenile hormone production and release and to be involved in the photic entrainment of the circadian clock. A tissue distribution analysis of the *T. castaneum* ATR by means of qRT-PCR revealed a prominent sexual dimorphism, the transcript levels being significantly higher in the male fat body and reproductive system. The endogenous ligand of the receptor, Trica-ATL, is able to increase the frequency and tonus of contractions in the gut and in the reproductive tract of mature red flour beetles. A homologous receptor was found in the EST-database of *Schistocerca gregaria*, which made a more thorough tissue distribution and additional experiments possible. (This research was supported by the Interuniversity Attraction Poles programs (Belgian Science Policy Grant (P6/14)), the Research Foundation of Flanders (FWO-Flanders G.0405.09) and the K.U. Leuven Research Foundation (GOA/11/02))

P109.
NOVEL PROPERTIES OF THE MELANOCORTIN 5 RECEPTOR OF *SQUALUS ACANTHIAS* AND THE MELANOCORTIN 2 RECEPTOR OF *CALLORHINCHUS MILII*: EVOLUTION OF MELANOCORTIN RECEPTORS IN CARTILAGINOUS FISHES
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The presence of 5 melanocortin receptor genes in tetrapods is the result of the two genome duplication events that occurred during the early evolution of the chordates, and a local gene duplication that occurred during the early radiation of the gnathostomes. Hence, it is surprising that only three MCR orthologs have been characterized from the genome of the elasmobranch, *Squalus acanthias* (MC3R, MC4R, & MC5R) and the gene of the holocephalan, *Callorhinchus milii* (MC1R, MC2R, & MC3R). Synteny studies provide evidence that MC2R and MC5R were most likely the result of the local gene duplication. This study analyzes the ligand selectivity and MRAP (melanocortin receptor accessory protein) requirements of *S. acanthias* MC5R and *C. milii* MC2R. The results of this study reveal that these MCR paralogs have properties that are rather quite distinct from their mammalian counterparts. (This research was supported by NSF grant IOB 0516958.)

P110.
IDENTIFICATION OF A PUTATIVE PLASMA MEMBRANE GLUCOCORTICOID RECEPTOR IN THE MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS*)
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Glucocorticoids elicit a wide variety of physiology actions, many of them associated with the stress response. Through the classical mode of action, glucocorticoids bind intracellular receptors to regulate gene transcription and production of new proteins. We, and others have demonstrated that glucocorticoids can act rapidly in a nongenomic fashion to elicit various cellular responses via membrane-associated cell signaling pathways. Glucocorticoids rapidly suppress prolactin secretion from the tilapia pituitary through a nongenomic mechanism of action. We therefore, evaluated if the tilapia might possess a putative membrane glucocorticoid receptor associated with nongenomic actions of cortisol. Competitive receptor binding assays were employed using semipurified liver and kidney plasma membrane preparations that provide considerably more protein than pituitary preparations. Glucocorticoids also elicit nongenomic actions in these tissues. We show that cortisol binds with high affinity to tilapia liver (Kd=2.44 nM and Bmax=0.06791 nM) and kidney (Kd=30.08 nM and Bmax=0.04690 nM) membranes. The association and dissociation were rapid with $t_{1/2}$ of 1.71-2.66 minutes and reached equilibrium within 20 minutes. The rate of association/dissociation was 30 times faster than that of the intracellular glucocorticoid receptor characterized in other teleosts ($t_{1/2}$ of 62.4-72 minutes). The kinetics of the putative GC membrane binding site is similar to that exhibited by other characterized plasma membrane steroid binding sites. Evidence here demonstrates the first characterization of a putative glucocorticoid membrane binding site in fishes. Future research aims to evaluate the regulation of this binding site in the context of physiological adaptation to environmental stimuli, including salinity and metabolic state.

P111. TWO CALCITONIN-LIKE RECEPTORS EXIST IN THE CHAGAS' DISEASE VECTOR, *RHODNIUS PROLIXUS*

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Rhodnius prolixus undergoes a period of rapid diuresis after ingesting large blood meals. Neurohormones with either diuretic or anti-diuretic activity control the process of diuresis by acting on several tissues including the anterior midgut, hindgut and Malpighian tubules. They do so by binding to their receptors. Identification and functional analysis of diuretic factors' receptors is important as they serve as potential targets for mimetic agonists or antagonists that could be used to disrupt diuresis and ultimately prevent the spread of Chagas' disease. One of the neurohormones that potentially plays a role in diuresis is Rhopr-diuretic hormone 31 (Rhopr-DH_{31}) which belongs to the insect calcitonin-like family of diuretic hormones. Interestingly, two Rhopr-DH_{31} receptor-encoding genes exist in *R. prolixus* (*Rhopr-DH_{31}-R1* and *Rhopr-DH_{31}-R2*), as has been described for several other insect species. Here we report the cDNA sequences of these two receptors and characterize their expression in fifth-instar *R. prolixus*. Preliminary RT-PCR results demonstrate that *Rhopr-DH_{31}-R1* is expressed in multiple tissues, but not the hindgut, in unfed fifth-instar *R. prolixus*. Highest expression for *Rhopr-DH_{31}-R1* is observed in testes, suggesting a potential role for Rhopr-DH_{31} in male reproduction. However, *Rhopr-DH_{31}-R2* is only expressed at very low levels in the central nervous system. Two DH_{31} receptors have been identified in several insect species and phylogenetic analysis reveals that these receptors could be a result of a gene duplication event since they form two distinct clusters. (Supported by NSERC discovery grant to IO)

TOPIC: *ENDOCRINE-IMMUNE SYSTEM INTERACTIONS*

P112.

CHANGES IN THYMIC GROWTH HORMONE DURING CHICKEN DEVELOPMENT

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It has been shown that growth hormone (GH) is expressed in lymphoid tissues where it may have a role as an autocrine/paracrine factor. Here we studied developmental changes in the GH expression, content, distribution and isoform pattern in the chicken thymus. The expression and distribution of GH mRNA and GH were analyzed by *in situ* hybridization using a full-length DIG-labeled riboprobe (690 bp) and by immunohistochemistry. GH-positive signals were observed mainly in lymphocytes within the cortex, although some epithelial, dendritic-like cells and macrophages within the medulla also showed signal. While the GH cDNA obtained from the immune system was identical in sequence to that in the pituitary, the thymic GH immunorreactivity (GH-IR) was associated with proteins of different molecular size 10, 17, 26, 28, 30, 34, 36, 40, 42, 44, 48, 50 and 58 kDa. Variants of 34-40 kDa (50%) were abundant in embryonic thymus, whereas the 17 kDa fragment (60%) was predominant in post-hatching chicks (under reducing conditions), except at 2 weeks (30%). GH concentration (as determined by ELISA) in embryonic stages was 35 ng/mg protein and a significant increased was detected at 2-4 weeks (80 ng/mg protein), GH concentration decreased again at 10 (50 ng/mg protein) and 20 (40 ng/mg protein) weeks. GH concentration showed changes in the thymus, plasma, and pituitary 8 h after 4 week-old chickens were treated with an i.v. injection of lipopolysaccharide (LPS, 5 mg/kg). The results showed a significant increase in thymic GH against control (2-fold), which indicates that local expression of GH can be regulated during the immune response against this endotoxin. These results suggest that local expression of GH may be involved as an autocrine/paracrine factor during development and functional response in the thymus. (Supported by CONACYT (60296N & 161791) and PAPIIT-UNAM (IN210209) grants.)

P113.

LYMPHOCYTE ACTIVATION AND PREDISPOSITION TO HIGH-FAT DIET INDUCED OBESITY IN MICE

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A proinflammatory phenotype is associated with obesity and insulin resistance in type 2 diabetes. Previous data suggest that a genetic link may exist between diet, myostatin expression and potential for generating an inflammatory response. High-fat diet induced obesity (HFDIO) susceptible mice (C57BL/6) present with evidence of an increased inflammatory response resulting from consumption of a high-fat diet in comparison with HFDIO resistant mice (SWR/J). However, it is unclear how the activation of the immune system in these models is affected by high-fat diets. Here, we present data further describing the relationship between metabolism, inflammation and myostatin expression in splenocytes. To challenge mice, lipopolysaccharide (LPS) from *Salmonella enterica* was administered via intraperitoneal injection and splenocytes were isolated five hours post-challenge. We then examined subpopulations of lymphocytes by flow cytometry. Results indicate that HFDIO resistant mice exhibit less activation of T- and B-lymphocytes while HFDIO susceptible mice appear to display a more heightened immune response. Overall, these data further support a close relationship between propensity for high-fit diet induced obesity and dysregulation of the immune system.

P114.

GROWTH HORMONE DIFFERENTIALLY ACTS ON THE INSULIN-LIKE GROWTH FACTORS AND TNF-A IN LIVER AND IMMUNE ORGANS OF TILAPIA *(OREOCHROMIS NILOTICUS)*

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The enlargement of aquaculture as a world-wide food production factor goes along with rearing of fishes at high densities. This has led to increasing problems caused by infectious diseases and, thus, strongly enhanced the interest in fish immune system. Recent evidence suggests that the growth hormone (GH)/insulin-like growth factor (IGF) axis may play a role in fish immune organs. Furthermore, data on potential changes in IGF-I, IGF-II and the GH receptor (GHR1) after experimental treatment are scarce and nothing is known about a potential influence of GH on tumour necrosis factor (TNF)-α. Thus, in tilapia we investigated the influence of GH on GH, IGF-I, IGF-II, GHR1 and TNF-α gene expressions at different levels of the hierarchy with special emphasis on the major immune organs head kidney and spleen by real-time PCR. Endocrine IGF-I served as positive control. Two consecutive intraperitoneal injections of bream GH upregulated liver IGF-I mRNA to the 1.84-fold and raised the IGF-I serum concentration (11.63 ± 0.71 ng/ml vs.

7.86 ± 0.75 ng/ml). Liver IGF-II mRNA was enhanced to the 4.87-fold. In brain, no change occurred in the gene expression levels of all genes investigated. GH gene expression was exclusively detected in the pituitary. At the pituitary level GH injections elevated GH expression to the 1.69-fold which coincided with higher (1.89-fold) IGF-I and (1.6-fold) TNF-α mRNA. GH raised IGF-I mRNA in head kidney to the 8.08-fold while IGF-II and GHR1 gene expressions were not affected. In spleen, no change occurred in GHR1 mRNA while IGF-I mRNA was elevated to the 2.98-fold and IGF-II mRNA to the 2.24-fold. In correlation, in situ hybridization showed markedly more cells in head kidney and spleen containing IGF-I mRNA after GH injection. The obtained stimulation of the IGF-I and in part also IGF-II expression in fish immune organs by GH which likely is not mediated by the GHR1 indicates a local role of the IGFs in organ constitution and sustainment. [This study was supported by Swiss National Science Foundation (Project Nos. 111028, 118165) and the Hartmann Müller-Foundation for Medical Research (Grant 1115).]

P115.
ELEVATED OOCYTE CORTISOL LEVELS INDUCE INNATE IMMUNE RESPONSE IN EARLY RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* EMBRYONIC CELLS
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Naturally-ovulated rainbow trout oocytes were incubated in cortisol-enriched ovarian fluid for 3 hours to examine the effects of the cortisol exposure on the ontogeny of innate immunity in rainbow trout embryos. Egg cortisol content was elevated from ~4 ng/oocyte (controls - CC) to ~5 (C1) and ~7.5 ng/oocyte (C2) prior to fertilization. Lysozyme activity, *intelectin*, *TLR-5M* and *TLR-5S* gene expression were measured and intelectin localization was examined in embryos using immunohistological [IH] methods. Lysozyme activity was significantly elevated in the C2 treatment group from the zygote until 13-days post fertilization (dpf), but was not affected at 21-dpf. Intelectin was present in 12-hours post-fertilization (hpf) (2-cell stage) embryos and levels were elevated in both cortisol treatment groups at 12-hpf and then suppressed between 36- and 48-hpf. The distribution of intelectin was on the cell surface of the early embryonic cells. *Intelectin* mRNA was detected in oocytes and transcript levels were significantly elevated in both cortisol treatment groups relative to the controls after the 3 h incubation. There were no differences among the three treatment groups at 1- and 5-dpf, but *intelectin* mRNA levels were lower in the cortisol treatment groups at 13-dpf, and the transcript levels in the C2 treatment group were lower than in the CC and C1 treatment groups at 26-dpf. mRNA transcripts for the two *TLR-5* genes were present in oocytes; transcript levels were significantly higher in both cortisol treatment groups relative to the controls after the 3 h incubation; *TLR-5S* mRNA was more abundant than that of *TLR-5M* mRNA. The ontogeny of the gene expression patterns, and the gene, intelectin and lysozyme responses to increased oocyte cortisol content emphasize the importance of innate immune properties of the early embryonic cells prior to the differentiation of the embryonic cells. (Supported by NSERC and OMAF to JFL and JSL; NSERC Post-Doctoral Fellowship to SR; NSERC Doctoral Scholarship to ML).

P116. VARIATION IN THE REGULATION OF INFLAMMATION ALONG A HOUSE SPARROW RANGE EXPANSION
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Organisms introduced outside their native range, or those at the edge of expanding populations, should damp inflammatory responses for at least two reasons. First, sacrifice of such energetically costly defenses would be beneficial in novel areas where infection risk is low (i.e., enemy release). Second, many parasites that invaders would encounter in novel areas would too be novel, and morbidity and mortality to novel infections is often associated with overly robust inflammation. Previous studies in house sparrows (*Passer domesticus*), one of the world's most broadly distributed species, have supported these ideas, finding lower prevalence of some parasites and damped inflammatory responses in introduced populations. However, the molecular bases of these physiological differences and whether similar patterns are detectable at finer spatiotemporal scales are unknown. Here, we asked whether adjustments in the regulation of inflammatory responses facilitated the colonization of Kenya, one of the world's most recent invasions. We predicted that the newest (western-most) Kenyan populations would exhibit the weakest whereas the oldest (eastern-most) populations would exhibit the strongest expression of microbial detector (Toll-like receptors 2 and 4; TLR2 and TLR4) and/or pro-inflammatory cytokine (i.e., interleukins-1β and 6) mRNA as well as rates of clearance of an exogenous inflammatory stimulus (lipopolysaccharide (LPS), a component of Gram-negative bacteria). Although populations differed dramatically in gene expression, even when separated by less than 20km, only when parasite (i.e., coccidian) effects were accounted for were predicted gradients in immune variation revealed, and even then patterns were suggestive but not statistically significant. Ongoing analyses are exploring the impacts of other parasites on immune gene expression and attempting to link such variation with clearance of LPS. (This work was supported by NSF-IOS 0920475.)

TOPIC: *HYDROMINERAL BALANCE*

P117.
EXAMINING PHYLOGENETIC HISTORY OF THE EXTRACELLULAR CALCIUM-SENSING RECEPTOR: A KEY SENSOR IN CALCIUM HOMEOSTATIC SYSTEMS
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Ionic calcium (Ca^{2+}) supports essential physiological functions in animals and, consequently, its concentration is homeostatically regulated within narrow bounds. The extracellular calcium-sensing receptor (CaSR) is a Family C G protein-coupled receptor (GPCR) that acts as a Ca^{2+} sensor, and as a nutrient sensor and perhaps in fishes as a salinity detector. Based on cDNA sequencing and genome database mining efforts, CaSRs are restricted to the chordate-vertebrate lineage. Using a data set comprising nucleotide and deduced protein sequences of vertebrate CaSRs, non-vertebrate chordate CaSR-like molecules and related Family C GPCRs, evolutionary relationships were inferred through phylogenetic tree estimation. Using statistical evaluation of non-synonymous (amino acid residue-altering; dN) versus synonymous (silent; dS) codon changes, differences in evolutionary selection among phylogenetic tree branches were demonstrated. Generally, within the major vertebrate clades there is strong evidence from this branch analysis for purifying selection during CaSR evolution from ancestral forms. But, notably, there is evidence of adaptive selection at the roots of the cartilaginous fish, bony fish, and tetrapod clades. This adaptive, or diversifying, evolutionary selection is interpreted in the context of skeletal biology (cartilaginous vs. mineralized bony skeleton) and natural history (aquatic vs. terrestrial lifestyles relative to the availability of environmental Ca^{2+}). Homology modeling of three-dimensional protein structures and evolutionary trace (ET) analysis were applied to map functionally-important, conserved amino acid residues to the receptor's molecular structure. ET

analysis showed strong conservation among all vertebrates of amino acid residues at predicted Ca^{2+}-binding sites in the receptor's extracellular domain. Site analysis of dN/dS ratios revealed relaxation from purifying selection at some codon positions, suggesting different functional roles.

P118.

THE REGULATION OF BRANCHIAL IONOREGULATORY PATHWAYS BY PROLACTIN AND GROWTH HORMONE: A COMPARATIVE APPROACH INVESTIGATING EURYHALINE AND STENOHALINE TELEOSTS

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In teleosts, prolactin (PRL) and growth hormone (GH) are known to regulate the activities of osmoregulatory tissues (e.g. gill and kidney), but the cellular mechanisms underlying ion homeostasis remain poorly understood. We have begun a comparative approach to understanding the role of pituitary hormones in regulating mitochondrion-rich cells (MRCs) in the gill of fishes with different osmoregulatory abilities. In euryhaline Mozambique tilapia (*Oreochromis mossambicus*), hypophysectomy blocked the recruitment of MRCs that express a Na^+/Cl^- cotransporter (NCC) during freshwater (FW) acclimation; NCC expression was restored by PRL replacement therapy. Surprisingly, hypophysectomy did not impact the recruitment of MRCs that express a $Na^+/K^+/2Cl^-$ cotransporter (NKCC) during seawater (SW) acclimation, nor did replacement therapy with GH or cortisol. When less SW tolerant Nile tilapia (*O. niloticus*) were hypophysectomized and acclimated to brackish water (23 ppt), plasma osmolality was elevated in parallel with diminished branchial gene expression of NKCC and Na^+/K^+-$ATPase_{\alpha 1b}$ when compared with sham-operated controls. This is consistent with pituitary-based regulation of SW-type MRCs. These patterns suggest contrasting dependence of SW-type MRCs on pituitary control in *O. mossambicus* and *O. niloticus*, whereas expression of NCC in both species requires an intact pituitary. To identify the molecular mechanisms underlying pituitary regulation of MRCs in gill, we turned to the genetically accessible zebrafish (*Danio rerio).* Zebrafish are tolerant of low ion conditions, allowing us to test our hypothesis that PRL plays a conserved role in recruiting NCC-expressing MRCs during acclimation to ion-poor conditions. Consistent with this idea, preliminary data suggest that gene expression levels of PRL and PRL receptors change in parallel with gill ionoregulatory responses to low ion conditions. (Supported by NSF (IOB05-17769), USDA (2008-35206-18785 and 18787), NIH (T32-MH020051-07) and the Pauley Foundation.)

P119.

A GUT NEUROPEPTIDE REGULATING RENAL FUNCTION IN DROSOPHILA: STIMULATION OF MALPIGHIAN TUBULE PUMPING BY VISCERAL PIGMENT DISPERSING FACTOR

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In *Drosophila*, brain neurons expressing the neuropeptide pigment-dispersing factor (PDF) regulate circadian rhythms in a manner highly similar to that of vasoactive intestinal peptide (VIP) in mammals. Like VIP, PDF is expressed outside the circadian network by neurons innervating the gut. In this study we investigate the possible visceral functions of PDF. PDF neurons located in the abdominal ganglia of the fly CNS project to the gut and innervate it superficially at the junction of the midgut and hindgut. Loss of abdominal PDF does not affect intestinal motility or circadian clock gene expression. Instead, we find that bath-application of PDF onto dissected viscera induces persistent contractions of the Malphigian tubules, which emerge from the junction of the midgut and hindgut, in a dose-dependent manner. In-situ hybridization studies indicate that the PDF receptor is expressed within the muscles surrounding the base of the tubules. Live-imaging experiments using the FRET sensor Epac1-camps reveal that these muscles respond to bath applied PDF with increases in intracellular cAMP. Both the Pdf-induced contractions and the increase in intracellular cAMP are abrogated in han^{5304} PDF receptor mutants, and can be rescued by reintroduction of PDF receptor in to han^{5304} muscles. These results indicate that gut-innervating PDF neurons regulate renal function at a distance and do so in a non-circadian manner. This work further highlights the highly parallel physiological roles of fly PDF and mammalian VIP.

P120.

IGF BINDING PROTEIN-5 IS EXPRESSED IN IONOCYTES AND REGULATES CALCIUM UPTAKE IN ZEBRAFISH

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Insulin-like growth factor binding proteins (IGFBPs) are high affinity binding partners for IGFs and play important roles in regulating IGF availability and actions. IGFBP-5 is the most conserved member of this gene family. Recently, we have shown that zebrafish have two distinct IGFBP-5 genes (termed as *igfbp-5a* and *-5b*, likely resulted from a gene duplication event during teleost evolution (Dai et al., FASEB J, 2010). These two genes exhibited distinct spatial and temporal expression patterns. Interestingly, one of them, *igfbp-5a*, is specifically expressed in epidermal ionocytes surrounding the yolk sac and in gill arches. Fluorescent double-labeled *in situ* hybridization analysis revealed that *igfbp-5a* mRNA is co-localized with *trpv6* mRNA but not with the H^+-ATPase *atp6v1a*l mRNA. The Ca^{2+} channel protein *Trpv6* expressing ionocytes are known to be important for calcium uptake. Acclimation of zebrafish embryos to artificial water with altered ion concentrations showed that *igfbp-5a* mRNA levels are increased in response to reduced calcium concentrations. Low Ca^{2+} water also increased the number of the *igfbp-5a/trpv6*-expressing ionocytes, while it had no effect on the number of *atp6v1al*-expressing ionocytes. To test the role of Igfbp-5a in these cells and in calcium uptake, antisense morpholinos were used to knockdown Igfbp-5a. Compared with the control embryos, Igfbp-5a knocked down embryos had increased calcium content and calcium influx. Knocking down of Igfbp-5a also resulted in an increase in the number of *Trpv6*-expressing ionocytes. These findings suggest that *igfbp-5a* is specifically expressed in the *trpv6*-expressing ionocytes and its expression is regulated by environmental calcium concentrations. Our study in zebrafish has also unraveled a novel role of IGFBP-5 in calcium homeostasis.

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Call for Nominations for the Bargmann-Scharrer and the Pickford Medal Lectures for the 17th International Congress of Comparative Endocrinology

The Council of the International Federation of Comparative Endocrinological Societies (IFCES) requests nominations for the Bargmann-Scharrer Lecture and for the Pickford Medal Lecture to be given at the 17th International Congress of Comparative Endocrinology in Barcelona, Spain, July 15-19, 2013. The Bargmann-Scharrer lecturer should be a prominent comparative neuroendocrinologist. The Pickford medalist should be a comparative endocrinologist under the age of 45. Please send by October 31, 2011, a nomination letter and a CV of the nominee to Prof. Robert J. Denver, Department of Molecular, Cellular and Developmental Biology, The University of Michigan, 830 N University Ave., Ann Arbor, MI 48109-1048 USA, E-mail: rdenver@umich.edu.

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