General and Comparative Endocrinology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Integrative and comparative reproductive biology: From alligators to xenobiotics

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A R T I C L E I N F O

Article history:
Received 16 December 2015
Revised 14 March 2016
Accepted 19 March 2016
Available online xxxx

Keywords:
Louis J. Guillette Jr.
Endocrine disrupting chemicals
Variation
Reproductive biology
Evolution of placenta
Temperature-dependent sex determination

A B S T R A C T

Dr. Louis J. Guillette Jr. thought of himself as a reproductive biologist. However, his interest in reproductive biology transcended organ systems, life history stages, species, and environmental contexts. His integrative and collaborative nature led to diverse and fascinating research projects conducted all over the world. He doesn't leave us with a single legacy. Instead, he entrusts us with several. The purpose of this review is to highlight those legacies, in both breadth and diversity, and to illustrate Dr. Guillette's grand contributions to the field of reproductive biology. He has challenged the field to reconsider how we think about our data, championed development of novel and innovative techniques to measure endocrine function, helped define the field of endocrine disruption, and lead projects to characterize new endocrine disrupting chemicals. He significantly influenced our understanding of evolution, and took bold and important steps to translate all that he has learned into advances in human reproductive health. We hope that after reading this manuscript our audience will appreciate and continue Dr. Guillette's practice of open-minded and passionate collaboration to understand the basic mechanisms driving reproductive physiology and to ultimately apply those findings to protect and improve wildlife and human health.

1. Introduction

To leave a legacy is a great achievement for any person, but Dr. Lou Guillette was such a scientific force and a true collaborator that he leaves us several diverse legacies in the field of reproductive biology. Dr. Guillette regularly challenged dogma, and promoted the idea that conducting basic research to understand "normal" was essential for defining "abnormal". He also helped pioneer the use of cutting-edge technologies to investigate the effects of endocrine disrupting chemicals (EDCs) and to discover emerging contaminants.

One of Dr. Guillette's legacies is the idea that measures of variation in the response of an animal or population to environmental change are as important, or potentially more important, than measures of mean responses. This insight likely originated from his deep understanding of the dynamic nature of the endocrine system and importance of natural hormonal fluctuations for controlling normal reproduction. Indeed, his interest in reproductive biology facilitated vast contributions to the field of endocrinology. He contributed to our understanding of normal seasonal variation in hormone profiles of many different species and conducted diverse studies to understand basic reproductive physiology. He is also well known for helping to define the field of endocrine disruption, having contributed significantly to our appreciation of the diverse ways in which EDCs affect wildlife and human health.

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http://dx.doi.org/10.1016/j.ygcen.2016.03.026
0016-6480/

Please cite this article in press as: McCoy, K.A., et al. Integrative and comparative reproductive biology: From alligators to xenobiotics. Gen. Comp. Endocrinol. (2016), http://dx.doi.org/10.1016/j.ygcen.2016.03.026

Dr. Guillette also made broad contributions to the field of evolutionary biology. He increased our understanding of temperature-dependent sex determination and showed that the endocrine chorioallantois is not unique to mammals. He worked tirelessly to translate his knowledge into advances in human reproductive health. In addition to his creative scientific pursuits, Dr. Guillette brought together scientists of multiple disciplines to compare and integrate perspectives. Ultimately, his creativity, deep knowledge, collaborative skill, and willingness to share credit and ideas in the building of scientific relationships are what lead to the breadth of his contributions.

2. Variation should be explored, not ignored

Living systems are inherently variable, and this variation allows organisms to respond to environmental change at both individual and population levels. In many studies, variance is viewed as an inconvenient feature of imperfect systems, and attempts are made to minimize or equalize variance among groups so that it can be ignored and attention can be focused on comparing measurements of central tendency. One of Dr. Guillette's enduring legacies will be his recognition that measures of central tendency often do not provide sufficient information about the response of an animal or population to environmental change. He and his colleagues argued that the distribution and variance of a dataset can provide insights into mechanisms and responses that are not apparent in a simple comparison of means (Orlando and Guillette, 2001). In other words, variance should not be viewed as an undesired, unfortunate attribute of a dataset. Rather, variance can be used to identify, predict, and explain the responses of organisms to environmental change.

In many studies, variance increases in populations exposed to environmental disturbance, even when measures of central tendency are similar among groups. For example, in mosquitofish (*Gambusia holbrooki*) exposed to pulp mill effluent, which contains a mixture of androgenic chemicals, mean hormone concentrations within a sex do not differ significantly between the exposed and reference mosquitofish populations. However, males and females exposed to pulp mill effluent demonstrate more variable testosterone (T) and 17β-estradiol (E_2) concentrations, respectively, indicating altered hormone regulation in a subset of individuals within the exposed population (Toft et al., 2004). Similarly, a small percentage of females in the contaminated population also develop markedly enlarged gonopodia (anal fin rays that function as an intromittent organ in males). However, the frequency of these masculinized females is small enough that mean fin size does not differ significantly between exposed and reference populations (Orlando and Guillette, 2001). Dr. Guillette realized that such differences in variance induced by a subset of the population could be biologically significant. Indeed, when a low potentially non-statistically significant percentage (e.g., ~1%) of human children display morphological abnormalities (e.g., congenital heart defects and hypospadias), we consider them devastatingly common birth defects.

Exposure to environmental contaminants is also associated with increased variance (without concomitant changes in mean) of body and organ sizes in gar (*Lepisosteus platyrhincus*) and fathead minnows (*Pimephales promelas*) (Orlando and Guillette, 2001). Similarly, female mosquitofish from a contaminated lake demonstrate greater seasonal variation in muscular E_2 than females from a reference lake (Edwards et al., 2010). In white ibises (*Eudocimus albus*), fecal corticosterone are more variable as MeHg exposure increases (Adams et al., 2009). Increased variance is thought to result from spatial or temporal heterogeneity of the environment (Maurer and Holt, 1996), as can be seen in environments impacted by pulsatile release of effluent or in habitats characterized by pollution gradients. Increased variance may also result from heterogeneity in xenobiotic metabolism among individuals (Silva et al., 2003). For example, organisms with diminished capacity for hepatic detoxification (the "responders") can be severely impacted by environmental toxicants even if the same toxicants have no discernable effects on the majority of individuals in a population (reviewed in Dorn, 2010). These negative outcomes can be innately difficult to detect via measures of central tendency if data from a sufficiently large number of "non-responders" are included in a dataset.

In other cases, contaminant exposure is associated with decreased variation in measured endpoints. For example, female mosquitofish from a contaminated lake demonstrate decreased variation in the timing of ovarian recrudescence, leading to increased synchronicity of this process (Edwards et al., 2010). Similarly, male phallus size and female hormone concentrations are less variable in American alligators (*Alligator mississippiensis*) from a highly contaminated lake than in their counterparts from a less contaminated site (Gunderson et al., 2004), and juvenile alligators from a contaminated lake demonstrate less seasonal variability in circulating sex steroids (Rooney et al., 2004). Less variation in T concentrations is also seen in male giant toads (*Bufo marinus*, now *Rhinella marina*) from highly agricultural areas in South Florida (McCoy et al., 2008). In summary, increases and decreases in the variance of measured outcomes within a population occur in response to contaminant exposure, indicating that variance is a parameter that should be explored rather than ignored in studies of environmental disturbance. Dr. Guillette believed that to truly understand how the environment affects wildlife and human health we must change our perspective from focusing on determining *if* an environmental insult can change the mean response, to understanding how the occurrence and severity of abnormalities scale along environmental gradients.

3. Contributions to endocrinology

Variation in hormone signaling is known to be essential for proper endocrine function, and abnormal hormone fluctuation can be an indication of disruption. Hormones drive much of reproduction and development, thus hormone assessments remain a key tool in studies of endocrine function. Understanding the particular hormones produced, the diel or seasonal cyclicity of these hormones, and the ways in which hormones are regulated, is often the first objective addressed when studying the reproductive biology of a species. It is not surprising that altered hormone regulation and signaling are hallmarks of studies of endocrine disruption, and forms the cornerstone of many assessments of reproductive dysregulation. The Guillette laboratory has employed hormone evaluations to greatly increase our understanding of reproductive biology as well as the ways in which environmental contaminants contribute to reproductive impairment.

Establishing seasonal variation in reproductive hormone profiles allows us to identify the breeding season for most species studied to date and lays the foundation for many studies of reproductive function. Dr. Guillette and colleagues have identified seasonal reproductive hormone profiles for a variety of species including mosquitofish, Florida gar, loggerhead turtle, white ibis and every life stage of the American alligator from a number of locales (Bermudez et al., 2005; Edwards et al., 2006b, 2013, 2010; Guillette et al., 1991, 1997; Hamlin et al., 2011, 2014; Heath et al., 2003; Kristensen et al., 2007; Orlando et al., 2007, 2003; Rooney et al., 2004). His laboratory has used hormone assessments to answer questions fundamental to reproductive physiology, including the endocrine control of parturition and oviposition, gonadotropin regulation of hormone synthesis, gene