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Neuroendocrine Disruption of Organizational and Activational Hormone Programming in Poikilothermic Vertebrates

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Abstract

In vertebrates, sexual differentiation of the reproductive system and brain is tightly orchestrated by organizational and activational effects of endogenous hormones. In mammals and birds, the organizational period is typified by a surge of sex hormones during differentiation of specific neural circuits; whereas activational effects are dependent upon later increases in these same hormones at sexual maturation. Depending on the reproductive organ or brain region, initial programming events may be modulated by androgens or require conversion of androgens to estrogens. The prevailing notion based upon findings in mammalian models is that male brain is sculpted to undergo masculinization and defeminization. In absence of these responses, the female brain develops. While timing of organizational and activational events vary across taxa, there are shared features. Further, exposure of different animal models to environmental chemicals such as xenoestrogens such as bisphenol A- BPA and ethinylestradiol- EE2, gestagens, and thyroid hormone disruptors, broadly classified as neuroendocrine disrupting chemicals (NED), during these critical periods may result in similar alterations in brain structure, function, and consequently, behaviors. Organizational effects of neuroendocrine systems in mammals and birds appear to be permanent, whereas teleost fish neuroendocrine systems exhibit plasticity. While there are fewer NED studies in amphibians and reptiles, data suggest that NED disrupt normal organizational-activational effects of endogenous hormones, although it remains to be determined if these disturbances are reversible. The aim of this review is to examine how various environmental chemicals may interrupt normal organizational and activational events in poikilothermic vertebrates. By altering such processes, these chemicals may affect reproductive health of an animal and result in compromised populations and ecosystem-level effects.

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Keywords

Amphibians; Brain; Endocrine Disrupting Chemicals; EDCs; Fishes; Neural Circuits Neuroendocrine Disrupting Chemicals; NEDs; Reptiles

Introduction

Sexual differentiation across taxa involves programming the gonad and brain. The factors that guide gonadal sex differentiation differ from those that regulate brain sexual differentiation. Thus, there may be discordance between the sex of the gonads versus the brain. This phenomenon was recognized back in the 19th century when the scientist Karl Heinrich Ulrichs proposed the term Uranian to describe an individual with the mind of a female but trapped in a male's body. This is based on the Greek goddess Aphrodite Urania, being created from the testicles of the god Uranus as reviewed in Money (1990).

It is now recognized that brain sexual differentiation is dependent upon tight orchestration of steroid hormones during embryonic development and also later in adulthood. This concept is now considered the organization-activational programming of later adult behaviors in a sex-specific manner (Morris et al. 2004; Phoenix et al. 1959; Arnold and Breedlove 1985). The organizational-activational hypothesis was originally developed based upon observations with guinea pigs (*Cavia porcellus*). Prenatal exposure to testosterone propionate on female brains at a specific time during development, a period during which sex-specific neural networks form, resulted in permanent effects (Phoenix et al. 1959). These findings were extended to other mammals including rats and other animals (Morris et al. 2004; Phoenix et al. 1959; Arnold and Breedlove 1985). This embryonic brain programming is considered the organizational period and is generally typified by spikes in androgens and/or estrogens to sculpt the brain to be "male" (Watson and Adkins-Regan 1989a; 1989b; Bowers et al. 2010; Konkle and McCarthy 2011).

Full manifestation of most sex-dependent behaviors, especially in males, requires a later resurgence of such steroid hormones ("activational period") to stimulate sex-specific neuronal pathways. Steroid-induced epigenetic changes in various brain regions may be the primary drivers of these two critical periods (Chung and Auger 2013; Tsai et al. 2009; Jasarevic et al. 2012). While there is ample evidence and reviews of organizational-activational effects in mammals and birds (Adkins-Regan 1999; Balthazart et al. 1996; 2009; Morris, et al. 2004; Arnold and Breedlove 1985; Phoenix et al. 1959; Ottinger and Dean 2011), this concept has not been fully considered in poikilothermic species that may exhibit a wide range of developmental and reproductive strategies. Most of these species also lack sex chromosomes, yet still have genotypic sex determination (GSD) and some possess environmental sex determination (ESD) dependent upon temperature (TSD) or behavior to determine whether the brain and gonads differentiate along male or female pathways (Blazquez and Somoza 2010; Kohno et al. 2014; Merchant-Larios and Diaz-Hernandez 2013; Devlin and Nagahama 2002; Martinez et al. 2014; Godwin 2010). Some examples of poikilothermic species with TSD include Atlantic silverside fish (*Menidia menidia*), painted turtles, (*Chrysemys picta*), and all crocodilians (Blazquez and Somoza 2010; Kohno et al.

2014; Merchant-Larios and Diaz-Hernandez 2013). It is not clear the extent to which TSD influences organizational-activational programming of the brain or whether such effects are predominantly mediated through its actions on gonads. For these reasons, GSD versus TSD will only be considered briefly in the context of how it might influence brain sexual differentiation in poikilothermic species. While brain sexual differentiation in rodents and birds is considered permanent (Arnold and Breedlove 1985), this might not be the case in fish, frogs, and reptiles. For instance, false clown anemonefish (*Amphiprion ocellaris*) are considered to exhibit protandrous sex changes with a monogamous mating system where a single fish can start out life as a male and then, possibly due to transcriptomic changes in the brain, undergoes sexual differentiation into a female (Iwata et al. 2012). An exploration of organizational-activational programming of the brain in poikilothermic species that exhibit this extreme variation might enable a better understanding of these processes across taxa. With this goal in mind, it is important to consider what is currently known regarding organizational-activational effects of steroid and non-steroid hormones in fish, amphibians, and reptiles on neuroendocrine function. In addition, how neuroendocrine disrupting chemicals (NED) and other environmental changes, in particular climate change, might disrupt normal brain programming affecting neuroendocrine function in these species will be considered.

Neuroendocrine effects include changes in production and release of neurotransmitters, neuropeptides and neurohormones, and alterations in their respective receptors in the brain, often impacting specific behaviors. Several reviews provide insight into the growing body of evidence reporting exposure effects attributed to NED (Gore and Patisaul 2010; Waye and Trudeau 2011; LePage et al. 2011; Ottinger and Dean 2011; León-Olea et al. 2014). Some NED were characterized first and more generally as endocrine disrupting chemicals (EDC) (Frye et al. 2012; Söffker and Tyler 2012), and others emerged from investigations examining effects of neuroactive pharmaceuticals and other personal care products measured in wastewater treatment plant effluent and surface water (Overturf et al. 2015; Waye and Trudeau 2011; Mennigen et al. 2011). Many studies in adults determined effects on reproductive behavior, but other key behaviors may be affected and they need to be studied in greater detail.

It is understood that early life stages are the most sensitive periods in vertebrates to exposure effects from NED and EDC (Bern et al. 1992; Guillette et al. 1995). The reasoning given for this is that when organs are developing and when the ability to metabolize and excrete toxins is not as efficient, organisms are more susceptible. It is during this period that organizational effects from endogenous steroid hormones occur, helping to shape brain neural circuitry in a sex-specific manner to control reproduction, growth, and behavior. It is also the time when NED and EDC might potentially exert organizational effects (Bern et al. 1992; Guillette et al. 1995), sometimes with consequences that permanently alter the ability of the organism to efficiently reproduce. If organizational effects occur as tissues, organs, and behaviors are differentiating during initial development from embryo to larva, juvenile, and sub-adult life stages, it seems reasonable that seasonally recrudescing tissues, organs, and behaviors in adults might also be sensitive to exposure to a NED, yet no apparent studies were found that directly addressed this question in fish (Figure 1).

Extrinsic factors, including NED, encountered during key developmental periods might result in permanent health effects in various vertebrates (Chow and Lee 1964; Roeder and Chow 1972; Buklijas 2014). The late Sir David Barker recognized that many non-communicable diseases (NCDs) may trace their origins to the embryonic/perinatal period, and thus, this concept was initially coined the “Barker Hypothesis” (Barker 1999; 2007). The term then changed to fetal origin of adult disease (FOAD), and the currently acceptable classification is developmental origins of health and disease (DOHaD). The broader terminology recognizes that environmental factors may promote or be detrimental to later health. In this review, we will thus consider how exposure to NED during critical developmental periods might disrupt the delicate balance of steroid hormone programming of the brain that may result in long-term consequences.

Organizational effects of NEDs in teleost fishes

Several studies are beginning to analyze the influence of NED on neuroendocrine function in early fish life stages. Organizational effects are difficult to examine in fish because their brains demonstrate high levels of plasticity and neurogenesis through adulthood, and thus organizational effects may be more challenging to quantify than in mammals and birds (Zupanc 2006). However, there are two cases where early exposure to NEDs during windows of susceptibility appears to result in permanent changes in some teleost fish. The first example is olfactory imprinting in salmon. The second example is in sex steroid modulation of neurogenesis for neurons involved in reproduction. In the case of olfactory imprinting in salmon, the window of susceptibility is during smolting (the period during which fish accustomed to fresh water change their physiology to be able to live in seawater) where imprinting has been linked to surges in thyroid hormones (Nevitt and Dittman 1998). Olfactory imprinting appears to be a permanent change. In the second example, sex hormones and their mimics may alter brain neuronal circuits in fish, masculinizing or feminizing subsequent events (Piferrer 2001). Feminization and masculinization might be reversed in some circumstances and thus a permanent alteration may require constant exposure to a given NED. Both of these examples are discussed in more detail below.

Olfactory imprinting in salmon—Salmon are able to find their natal spawning grounds years after they have left the region. In studies with Coho salmon, it was determined that this ability was due to olfaction. It is widely regarded that this olfactory imprinting occurs with the parr (juvenile salmon acclimated to fresh water) to smolt (juvenile fish that live in seawater) transition. The main effectors for the transition are likely to involve thyroid hormones (TH), which surge during this time and are required for the parr-smolt transformation to occur (Lema and Nevitt 2004). The process of smolting results in many physiological changes in fish that allow them to traverse from the freshwater environment to reside in the sea. The importance of olfactory imprinting was shown by Scholz et al. (1976) in an experiment where parr were exposed to artificial odorants (e.g., morpholine) while undergoing this process and the investigators were able to demonstrate that these chemicals directed salmon migration to a different location.

The olfactory epithelium in Coho salmon proliferates markedly in the presence of triiodothyronine (T3), the most active TH (Lema and Nevitt 2004). This region of the brain

is rich with progenitor cells for olfactory receptor neurons. The importance of TH in development of olfactory capabilities was also noted in experiments that exposed parr for 30 days to brominated flame retardants (BFR), some of which are TH antagonists (Lower and Moore 2007). Exposure decreased olfactory responses to urine in fish treated with BFR compared to controls after placing them in saltwater, but did not impair osmoregulatory capacity in smolts. Plasma levels of TH were reduced when fish were transferred to saltwater but hormone levels were not markedly different during the exposures, bringing into question the direct regulating link between TH and olfaction (Lower and Moore 2007). Other contaminant exposures, such as cadmium (Cd), may damage olfactory sensory neurons through oxidative stress (Wang et al. 2012) and these also affect critical behaviors for salmon. Salmon phospholipid hydroperoxide glutathione metabolizing peroxidase (*gpx4*) plays an important antioxidant role in olfactory and liver tissues with exposures to Cd. At low Cd concentrations (3.7 ppb), *gpx4* expression is not altered, suggesting that it is expressed and able to counter oxidative stress. At increased Cd exposure (347 ppb) gpx4a mRNA is significantly decreased by 50% in olfactory tissues at 24 and 48 hr post exposure, suggesting that its ability to counter oxidative stress has been exceeded (Wang et al. 2012). This mechanism of olfactory dysfunction is distinct from changes in neuronal circuits observed in parr.

Sex hormone modulation of neuronal circuits in teleost fishes—Sexual plasticity, the ability of fish to change their phenotypic sex, is pronounced in the teleost lineage. There are many studies demonstrating that early exposure to exogenous estrogens or androgens during the period of sexual differentiation alters the sex of certain fish (Devlin and Nagahama 2002). This practice is routinely used in fish farming to obtain monosex populations. In addition to changes in gonadal phenotype, exposures to sex hormones also alter behavioral traits in fish, such as aggression or nest-holding, suggesting a direct effect on the brain. While most of the investigations examining sexual plasticity examine only the gonads, there are some studies that suggest involvement of several neuropeptides including gonadotropin-releasing hormone (GnRH), gonadotropin inhibitory hormone (GnIH), arginine vasotocin (AVT) and isotocin, among others (Panzica et al. 2011; Le Page et al, 2011). For a comprehensive review of this subject and an intriguing model of how these neuropeptides may be involved, please see Liu et al (2017). Cortisol was also found to be involved in stress- or temperature-induced change in sex determination (Yamaguchi et al. 2010), apparently by elevating production of 11-ketotestosterone (11-KT) through increased activation of 11β−hydroxysteroid dehydrogenase (HSD11B) (Fernandino et al. 2012). Interestingly, in the hermaphroditic fish, *Lythrypnus dalli*, where males exhibit parenting behavior, increased activation of 11β-HSD elevates 11-KT, as expected, but also appears to decrease plasma cortisol levels (Pradhan et al. 2014). There are still many gaps in understanding the precise involvement of neuropeptides and cortisol in sexual plasticity in fish, but it appears that there is relevant crosstalk between the hypothalamic–pituitary–gonadal (HPG) and hypothalamic-pituitary-interrenal (HPI) axes and neuropeptides may play a larger role than previously anticipated.

In an effort to identify differences between male and female brains in medaka (*Oryzias latipes*), Hiraki et al. (2012) mapped the distribution of estrogen and androgen receptors.

These investigators found the distributions to be different in the ventral telencephalon and preoptic areas, where only in females these areas were rich in both estrogen (*esr1* and *esr2b*) and androgen receptors (*ara* and *arb*), as evidenced from *in situ* hybridization. Males expressed both estrogen and androgen receptors, but in different brain regions. Further, results showed that expression of *esr1*, *esr2b*, *ara*, and *arb* in ovariectomized females was elevated by E2 but no marked effects were observed on expression levels of *esr2a*, indicating differential regulation. It is of interest that administration of 11-KT to ovariectomized females significantly diminished expression of *esr1*, *esr2b* and *arb* in the ventral striatum and ventral pallidum promoting male-like expression patterns. These experiments exemplify the sexual plasticity of fish brains and suggest that plasticity depends upon expression of sex hormone receptors in specific nuclei in the brain, which subsequently rely on the presence of E2 and 11-KT (Hiraki et al. 2012). Both systemic and site-specific synthesis of sex hormones may underlie changes in expression of sex hormone receptors and explain activational effects noted for behavior in adult fish.

Aromatase is the enzyme responsible for the conversion of androgens to estrogens. Fish possess two distinct genes for aromatase, one that is particularly expressed in the gonad (*cyp19a1a*), which is similar to the aromatase gene in mammals and a second that is more highly expressed in the brain (*cyp19a1b*) (Diotel et al. 2010). Considerable attention has been devoted to understanding how the gonadal aromatase gene is controlled at the level of transcription, as this gene would be immediately involved in gonadal sex differentiation (Guiguen et al. 2010). Yet, it is now becoming evident that brain aromatase may play an important role in sexual plasticity of the brain and because it may be involved in neurogenesis, it may contribute to both activational and organizational events. Brain aromatase activity is high in the pituitary, hypothalamus and preoptic area of fish brains (Pasmanik and Callard 1985). In adult fish, such as goldfish, there are seasonal variations in aromatase expression and activity, corresponding to the peak reproductive period (Pasmanik and Callard 1988, Zhang et al. 2009a). In medaka, site-specific brain aromatase activity is sexually dimorphic, and the female expression patterns are induced by estradiol (E2) treatment of males (Melo and Ramsdell 2001). In European sea bass *(Dicentrarchus labrax)*, males generally display higher brain aromatase activity than females (Gonzalez and Piferrer 2003). In teleost, radial glial cells (RGC), are the exclusive site of *cyp19a1b* expression (Pellegrini et al. 2016), which raises the intriguing question of how RGC signal to adjacent neurons via estrogens. Both estrogens and androgens (aromatizable and non-aromatizable) increase GnRH (Breton and Sambroni 1996, Dubois et al. 1998), but it is unclear if this is direct or indirect, since the expression of ER in GnRH neurons remains controversial. Numerous other neurons express nuclear and membrane ER, so both local estrogen from RGC, and peripheral estrogens could differentially affect central neuronendocrine systems (Pellegrini et al. 2016; Xing et al. 2014). Chronic deprivation of estrogens via aromatase inhibition with fadrozole in adult female goldfish revealed that numerous hypothalamic genes were affected: these included calmodulin, activin-βA, and ornithine decarboxylase 1 (Zhang et al. 2009b). By using immunohistochemistry in developing zebrafish, it was shown that EE2 exposure induces an increase in the number of GnRH-immunoreactive neurons. The weak estrogenic chemical nonylphenol was able to produce similar effects in a concentration-and receptor dependent manner (Vosges et al. 2012).

In mammals and birds, sex steroids are important for organizational and activational effects on the brain (Diotel et al. 2011b; Pellegrini et al. 2016; do Rego and Vaudry 2016). In fish, there are many examples of activational effects by sex steroids, but organizational events due to these hormones are more difficult to ascertain due to their high brain plasticity. Studies showed that femaleness in a variety of adult fish, including medaka, tilapia and zebrafish, depends upon the presence of E2, as inhibiting its biosynthesis with the use of aromatase inhibitors results in full masculinization (Paul-Prasanth et al. 2013; Takatsu et al. 2013). Pasmanik and Callard (1985) demonstrated steroidogenic activity in the brain of goldfish (*Carassius auratus*) with the discovery of aromatase and 5α-reductase activities. Since this original report, complete steroidogenesis pathways were identified in the brain of various fish species. Several reviews illustrate the currently identified steroidogenic pathways and speculate on their importance for establishing and maintaining neural networks (Diotel et al. 2011a; Pasmanik and Callard 1985). Expression of steroid hormone receptors and steroidogenesis in the brain begins in early fish development (Dong and Willett 2008; Gorelick et al. 2008; Mouriec et al. 2009; Shi et al. 2013). Using the mummichog (*Fundulus heteroclitus)* model, Dong and Willett (2008) visualized *cyp19a1b* mRNA as early as 3 days post-fertilization (pfd) in the midbrain using *in situ* hybridization. As mummichog embryos grow, the expression is detected progressively in the hypothalamus and telencephalon by 4 pfd. Estrogen receptors including both the nuclear and membrane receptors are expressed in embryos between 24 and 48 hr post-fertilization (Pikulkaew et al. 2010).

Notably, most of the steroidogenic enzymes have been localized by *in situ* hybridization and co-localize with each other in the preoptic area, telencephalon, and hypothalamus, among other areas of the brain (Diotel et al. 2010), suggesting that these enzymes function in concert. Neurosteroid biosynthesis occurs in RGC (Xing et al. 2014; Pellegrini et al. 2016); the exclusive site for brain aromatase activity in teleosts. The mRNA for steroidogenic acute regulatory protein (*star)*, responsible for shuttling cholesterol into the mitochondria), as well as mRNA for steroidogenic enzymes *cyp17a1* and *hsd3b*, involved in key biosynthetic steps of neurosteroids, were sequenced from cultured RGCs (Xing et al. 2014). Radial glial cells are stem-like neuronal precursors (Kriegstein and Alvarez-Buylla 2009; Pellegrini et al. 2016), which are highly abundant in teleost brains, persisting at high levels in adults compared to other vertebrates. This is probably the fundamental reason underlying the remarkable plasticity and regenerative activities in adult teleost brain (Xing et al. 2014; Hiraki et al. 2012; Kuhl et al. 2005).

Radial glial cells may respond to estrogens, androgens, and gestagens (progesterone receptor ligands), suggesting a close and direct relationship among these signaling pathways that might be important for neurogenesis. These pathways may be involved in organizational effects in fish if new neurons are generated in response to the steroids or possibly also in activational effects in fish (Xing et al. 2014; 2015; 016). Recently, Xing et al. (2015) demonstrated that E2 concentrations in the 100 nM range plus activation of the dopamine D1 receptor (D1R) with a specific dopamine agonist up-regulated brain aromatase in RGCs. At higher concentrations (1 μM E2), this effect was inhibited. Exposure of RGC to ESR1 and ESR2 antagonist 1,3-*Bis*(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1*H*-pyrazole dihydrochloride (MPP) blocked D1R-mediated effects, illustrating a dependence on ER for aromatase up-regulation. In the model proposed

to explain these results, it is clear that D1R functions through cAMP signaling to phosphorylate cAMP Responsive Element Binding Protein (CREB), which then acts in concert with the ER to upregulate brain aromatase. This dopaminergic regulation of RGC is thus modulated by E2 (Xing et al. 2015).

Activational effects of NEDs in adult teleost fish

Most teleost fish are iteroparous, which indicates they alternate between reproductive and non-reproductive periods and reproduce multiple times during their life span. During the non-reproductive season, the reproductive neuroendocrine axis is in a quiescent state, where the concentration of brain, pituitary, and gonadal hormones are relatively low, and in the quiescent gonad, reproductive ducts decrease in size and gametogenesis is suspended (Whittier and Crews 1987; Sumpter 1990; Flett et al. 1993). Reproductive seasonality is regulated by multiple environmental factors and in many temperate species, photoperiod and water temperature are critical cues. Seasonal changes in circulating sex steroids and other hormones and multiple neurohormonal receptors occur in the brain (Zhang et al. 2009a; Migaud et al. 2012; Moussavi et al. 2012; Forlano et al. 2014), pituitary (Moussavi et al. 2013; Nakane et al. 2013), and gonad (Orlando et al. 2007; Geraudie et al. 2010; Elisio et al. 2014), as a fish transitions from a reproductively quiescent to a reproductively active state through a process called recrudescence. Further, there are differences in neuroestrogen synthesis as glial cell aromatase is not only 1–2 orders of magnitude greater in fish brains compared to that in mammals, but also varies significantly with reproductive season (Aggarwal et al. 2014; Pasmanik and Callard 1988). Regulation of aromatase activity may in part explain the sexual plasticity of the adult fish brain (Le Page et al. 2010; Pellegrini et al. 2016). Thus, in a similar manner to development, another potential vulnerable window of susceptibility to NED may occur during seasonal recrudescence (Figure 1). It is known that pituitary sensitivity to steroid (T, E2) positive feedback on GnRH-stimulated luteinizing hormone release varies seasonally in goldfish (Trudeau et al. 1991). Therefore, it is tempting to speculate that disruption of steroid action by a NED might also vary with seasonal reproductive cyclicity.

The effects of NED on neuroendocrine function of fish, amphibians, birds, and mammals were reviewed (León-Olea et al. 2014). Additional data show that traditional neurotransmitter systems are also disrupted by numerous environmental contaminants (Basu 2015). These reviews provide detailed examples of how contaminants interfere with brain function resulting in altered behavior and physiology in fish. Contaminants that behave as NED may display activational effects on the neuroendocrine system, possibly interfering with normal sex hormone pathways that control sexual behavior and possibly even reproduction. Investigators found that exogenous estrogens, estrogen mimics and antiandrogens are able to change significantly the function of *cyp19a1b* in the brain, suggesting that these NED may alter important homeostatic functions of this enzyme. Generally, estrogens increase expression of brain aromatase while antiandrogens, such as flutamide (Schiller et al. 2014) or the aromatase inhibitor fadrozole (Zhang et al. 2009b) diminish expression. As previously indicated,*cyp19a1b* expression in the RGC is important for neurogenesis, and also possibly for other brain functions. Gene profiling experiments in goldfish and zebrafish treated with either fadrozole or ethinylestradiol (EE2), respectively,

uncovered several groups of genes that were either up- or down-regulated (Zhang et al. 2009b; Martyniuk et al. 2007). In addition, sex steroids are important in controlling sex-specific behaviors in fish. While most studies focused on estrogens and androgens, a recent study with progestins suggests that this type of NED might also alter RGC function (Cano-Nicolau et al. 2016).

Synthetic progestins derived from 19-nortestosterone, such as levonorgestrel found in birth control pills, induce expression of brain aromatase in RGCs (Cano-Nicolau et al. 2016) in the low nM range, probably after metabolic activation. Sources of the progestins are likely human wastewater effluents, but these substances are also found in runoff from concentrated animal feeding operations at ng to ug/L concentrations (Creusot et al. 2014; Orlando and Ellestad 2014). Aqueous exposure to levonorgestrel and other progestins, including gestodene and desogestrel, produce rapid and marked reduction in fathead minnow egg deposition on breeding tunnels (Runnalls et al. 2013). In another study, gestodene also markedly lowered egg deposition and adversely affected reproductive behavior in adult fathead minnows exposed to 10 or 100 ng/L for 8 days. Frankel et al. (2016) observed that female fathead minnows' reproductive behaviors were masculinized and gestodene exposed males were more aggressive and spent less time tending the nest or courting females compared to control male fathead minnows.

León-Olea et al. (2014) reviewed in detail the neuroendocrine effects attributed to different pesticide classes, and thus these will not be covered here except to emphasize that many pesticides may act as agonists or antagonists of endogenous sex hormones and thus influence the neuroendocrine system directly. An example is vinclozolin (VZ), a widely used fungicide, which functions as an antiandrogen and may target the neuroendocrine system differentially depending on dose and exposure period. At 100 μg/L VZ, two to three year old mature male goldfish treated for 7, 15, and 30 d increased their circulating levels of 11-KT after 30 days, perhaps to compensate for the antiandrogenic activity of this fungicide. Golshan et al. (2014) suggested that at relatively higher levels, exposure to VZ at 800 μg/L for 7 and 30 but not 15 days, 11-KT levels were lowered suggesting feedback inhibition to the brain and or pituitary gland. Golshan et al. (2014) found circulating LH concentrations were also significantly elevated compared to controls at 100 μg/L VZ for 30 days, but LH returned to control levels at 800 μg/L VZ. Further, *gnrh3* mRNA expression paralleled the expression pattern of circulating LH after 30 day exposure. Golshan et al (2014) also measured circulating E2 and reported E2/11KT ratio and reported no marked effects of VZ on circulating E2 and interpreted the observed rise in E2/11KT at 800 μg/L VZ, as being due to changes in 11KT. Overall, these findings demonstrate effects of VZ on both neuroendocrine and gonadal endocrine systems and may impair male goldfish fertility. The combination of EDCs and stress, as shown in zebrafish exposed to methyl-parathion and stressed by being chased with a net, which dramatically decreased neuronal *star* while at the same time enhanced expression of mRNA for glucocorticoid receptor (da Rosa et al., 2015), further illustrates the convolution of neural effects from complex environmental stressors.

Human neuroactive pharmaceuticals released into the environment might also act as NED in exposed fish. Many of these have been formulated to be active in humans at low doses and to have long half-lives. One such pharmaceutical is Prozac, which was found in some rivers at

approximately 1 μg/L concentration (Brooks et al. 2003). Prozac is the trade name for fluoxetine (FLX), a potent selective serotonin (5-HT) reuptake inhibitor that acts to block the presynaptic membrane 5-HT transporter (*slc6a4*). It is now known that 5-HT plays key roles in the regulation of several neuroendocrine pathways including reproduction, stress, appetite and behavioral functions (Mennigen et al. 2011), which are disrupted by FLX. One unintended consequence in fish is the disturbance of steroidogenesis in female and male fish (Mennigen et al. 2011). Serotonin stimulates LH release in fish (Somoza et al. 1988) and alters plasma levels of E2. Perhaps the most important finding is that exposure to FLX modulates estrogen receptor expression in the brain and may alter steroid sensitivity (Mennigen et al. 2011). In a separate study, exposure of goldfish to a mixture of 0.54 μg FLX and 5 ng EE2/L was more estrogenic than either chemical alone. The mixture produced a significant rise in hepatic mRNA expression for *esr1* and vitellogenin (*vtg*) as well as fall in hepatic mRNA expression for *esr2* (Silva de Assis et al. 2013). An interesting finding is that FLX altered the expression of two miRNAs in liver of zebrafish, *dre-let-7d* and *dre-miR-140–5p* (Craig et al. 2014) that are predicted to regulate transcript abundance of adenosine monophosphate-activated protein kinase (AMPK). This is an important metabolic hepatic pathway and its disruption leads to loss of weight, as reported by Mennigen et al (2011) for goldfish. AMPK also plays a key role in the brain, and remains to be tested whether FLX similarly influences metabolism in this tissue.

Developmental sensitivity to EDC/NED in amphibia

In contrast to mammals, birds, and some fishes, there is little information regarding organizational or activational effects in the brain either naturally or from NED in amphibians. There have been few attempts at the systematic testing of amphibian brain sensitivity to exogenous TH, sex steroids, or environmental contaminants binding to nuclear or membrane-bound receptors. This is a major gap in our knowledge compared to mammalian model systems.

Nevertheless, amphibians possess certain features that make them especially sensitive to pollutants and therefore useful for the study of EDC and NED. In amphibians embryogenesis is *ex vivo,* their skin is permeable and they go through metamorphosis. For these reasons, amphibians are considered highly sensitive test organisms for hormone-dependent development (Tata 2006) and employed in ecotoxicological assessments (Santos et al. 2015; Correia et al. 2014; Maselli et al. 2010; da Rosa, 2016; Gardner et al., 2017), including standard methods such as the Amphibian Metamorphosis Assay (OECD), and the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) (ASTM). Anuran metamorphosis, and in particular the neuroendocrine control of the process, may represent a reliable model system to examine NED; since it is a process dependent upon TH consisting of thyroxine (T4) and triiodothyronine (T3) (Ishizuya-Oka 2011; Sachs et al. 2000), and considered equivalent to postembryonic organogenesis in mammals (Tata 2006). Amphibian metamorphosis is controlled primarily by the hypothalamic-pituitary-thyroid (HPT) axis, whereas corticosterone from the interrenal gland plays a modulatory role (Ishizuya-Oka 2011; Galton 1990; Bonett et al. 2010; Denver 2013) (Figure 2a). This hormone-dependent transformation from larval body to a juvenile with adult features is depicted in Figure 2-b. TH controls the regression and transformation of larval tissues to the adult forms.

Environmental cues trigger synthesis and release of hypothalamic corticotropin-releasing hormone (CRH) (Galas et al. 2009; Okada et al. 2009; Denver 2013) into the hypophyseal portal system to enhance pituitary synthesis and release of thyroid stimulating hormone (TSH) (Denver 2013) and adrenocorticotropic hormone (ACTH) (Denver 2013). In contrast to mammalian systems where thyrotropin-releasing hormone (TRH) is the principle stimulator of TSH, CRH controls TSH release in amphibians. Once released from the pituitary TSH binds to its receptor in the thyroid gland to signal the synthesis of TH. The thyroid gland releases mainly T3 or T4 that is locally converted by deiodinases (Bianco and Larsen 2005) to its more active form T3. Thyroid hormones are transported inside the cell through the activation of organic anion transporter polypeptides (OATP) (Pizzagalli et al. 2002). Once T4 has been transported across the membrane, T4 is deiodinated to T3 by type II deiodinase (D2) in the cytoplasm. T3 binds to nuclear thyroid hormone receptors TRα and TRβ, inducing the heterodimerization with 9-cis retinoic acid receptor to modulate expression of key genes involved in metamorphosis (Buchholz et al. 2006, Sachs et al. 2000). Corticosterone from the interrenals acts as a synergist of TH, to accelerate metamorphosis (Bonett et al. 2010). TRH plays a role in tadpole growth through its actions to stimulate both growth hormone (GH) and prolactin (PRL) (Denver 2013). The expression of the TR is developmentally regulated in the tadpole brain with expression of TRα notably increasing as a frog progresses through metamorphosis (Hogan et al. 2007; Duarte-Guterman and Trudeau 2010). This implies that the sensitivity of neural tissues to T3 may also be changing accordingly. Exogenous T3 treatment increased proliferation of specific neural stem cell populations in post-metamorphic juvenile *Xenopus laevis*, exerting no apparent effect on proliferation in pre-metamorphic tadpoles (Preau et al. 2015). In addition, negative feedback effects of T3 on TSH synthesis and secretion develops in the prometamorphic tadpole, and pituitary sensitivity to this feedback decreases at the metamorphic climax (Denver 2013; Sternberg et al. 2011). Data indicate sensitivity to a neuroactive pollutant capable of binding to TR in brain and/or pituitary is present, or that TH dynamics also change during metamorphosis. This has not been systematically studied, but might be important to assess fully the neuroendocrine disruption hypothesis, and has major implications for understanding the detrimental effects of EDC on development in Amphibia.

During early stages of development, anuran gonads are undifferentiated. Previously it was postulated that TH played little if any role in sexual development (Buchholz et al. 2006; Hayes 1998). However, several observations following exposure to known TH disruptors suggested otherwise (Carr and Patino 2011). Exposure to environmentally relevant doses of ammonium perchlorate resulted in a female-biased sex ratio in *X. laevis* (Goleman et al. 2002). Data demonstrated that indeed tadpole gonads are sensitive to TH as indicated by the observations that T3 induced genes associated with androgens, or suppressed those associated with estrogens, depending upon anuran species (Duarte-Guterman et al. 2014; Flood et al. 2013). This has important implications for our understanding of sexual development and its disruption by environmental pollutants. The concept of thyroid-gonad cross talk is covered in detail elsewhere (Castaneda Cortes et al. 2014; Flood et al. 2013, Flood and Langlois 2014; Hogan et al. 2007, Duarte-Guterman et al. 2014).

The period of sexual differentiation is highly variable between anuran species (Hayes 1998) but in many occurs in the pre- and pro-metamorphic stages before TH peak (Figure 2b). The

first GnRH-immunoreactive neurons appear at some point in this period (D'Aniello et al. 1995), as found for LH- and FSH-immunoreactive gonadotrophs in the anterior pituitary (Pinelli et al. 1996). Data obtained from field-collected recently metamorphosed green frogs *(Lithobates clamitans)* and bullfrogs *(L. catesbeinaus)* provide some evidence for a link between developmental upsets, limb malformations and suppression of GnRH and androgen levels (Sower et al. 2000). Intriguingly, hypophysectomy experiments indicate that the pituitary is not required for early gonadal differentiation, but may be involved in gonadal growth after differentiation occurs (Hayes 1998). However, Suda et al. (2011) in *Rana rugosa* provided data in disagreement with this proposal, which suggest a role for FSH in sex determination and early ovarian steroidogenesis. Regardless, it is clear that gonadal steroids play a major role in gonadal differentiation (Hayes 1998). Most of our knowledge regarding sexual differentiation arises from the influence of exogenous steroids, aromatase and 5α-reductase inhibitors (Hayes 1998, Duarte-Guterman et al. 2009). These data indicate that estrogens are ovary-promoting and androgens are testes-promoting steroids. Emerging from this, and relevant here, is that there are steroid-sensitive and steroid-insensitive periods for sex-reversal. Early exposure to EE2 induced sex-reversal and a female bias at metamorphosis. On the other hand, exposure in the later metamorphic period exerted no marked effects on sex ratio. Neither of these treatments affected time to metamorphic climax, yet, EE2 exposure in a mid-metamorphic period delayed metamorphosis but exerted no significant effects on sex ratios (Hogan et al. 2008). Data thus indicate major shifts in estrogen sensitivity of gonads with development. In marked contrast, little is known regarding the developmental sensitivity of brain and pituitary to sex steroids in Anura. Expression of androgen and estrogen receptors in tadpole brains increases significantly at the metamorphic climax concomitant with aromatase activity (Duarte-Guterman and Trudeau 2010). Brain expression of 5α-reductases (SRD5A), enzymes responsible for the synthesis of 5α-dihydrotestosterone (5αDHT or DHT) from testosterone (T) falls at metamorphosis. Data suggest major changes in steroid sensitivity of the neuroendocrine brain of tadpoles as they develop (Duarte-Guterman and Trudeau 2010). Documented organizational and activational effects of exogenous sex steroids on courtship vocalization in *Xenopus laevis* support this phenomenon (Zornik and Kelley 2011; Hoffmann and Kloas 2012b). Moreover, acute and reversible effects of EDC, such as EE2 (Hoffmann and Kloas 2012a), diclofenac (Hoffmann and Kloas 2012a), dichlordiphenyldichloroethylene (p,p'-DDE) (Hoffmann and Kloas 2016), and VZ (Hoffmann and Kloas 2010) on vocalizations and mating behavior in *Xenopus laevis* indicate neuroendocrine disruption of these processes in frogs.

Organization and Activational Effects in Reptiles and Susceptibility to NEDs

Temperature Sex Determination vs. Genetic Sex Determination in Reptiles—

Among amniotes, reptiles exhibit a wide range of reproductive and sex-determining mechanisms. First, there are those with separate sexes (gonochorism) but others are unisexual (parthenogenesis). Reptile reproduction can also be categorized as oviparity (egg laying without embryonic development within the female), viviparity (live birth), ovoviviparity (embryos within eggs develop within the female's body until they are ready to hatch but only receive nutrients from the yolk sac). Finally, reptiles may be classified as possessing GSD with male (XX/XY) and female (ZZ/ZW) heterogamety or TSD (Ezaz et al.

2009; Wapstra and Warner 2010). Of the 1000 karyotyped lizard species, less than 200 possess sex chromosomes. In some species, such as the leopard gecko (*Eublepharis macularius*), incubation temperature during a critical period of embryonic development determines gonadal sex, along with sex-differences in body growth, adult morphology, aggressiveness, reproductive physiology, and neurobehavioral organization and programming (Crews et al. 1998). By altering steroid hormone synthesis, especially for T and E2, and increasing expression of either male or female temperature-sensitive transcripts, incubation temperature alone may sculpt early brain programming and later behavioral responses.

Effects of Temperature Sensitive Period (TSP) on Gonad and Organizational/ Activational Actions in Brain—There has been interest in identifying transcripts in the gonad and brain that are altered due to GSD or TSD in contrasting reptilian species. Recently, several genes were identified in the gonad to be elevated in response to the male-producing temperature (MPT) (33.5ºC) in American alligators (*Alligator mississippiensis*), including Wnt signaling factor *wnt11*, histone demethylase *kdm6b*, and transcription factor *cebpa* (Yatsu et al. 2016). Another gene associated with the MPT and male sexual differentiation is the alligator ortholog of the transient receptor potential cation channel subfamily V member 4 (*trpv4*) (Yatsu et al. 2015). In painted turtles (*Chrysemys picta*), steroidogenic factor 1 (*sf1*) and Wilm's tumor 1 (*wt1*) are activated in the gonad during the thermosensitive period and dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (*dax1*) may contribute to male development (Valenzuela et al. 2013). Collectively, the findings suggest that these genes result in organizational changes within the brain of these reptile species.

TSP-Induced Aromatase Expression in Developing Brain of Various Reptilian Species—At the beginning of the thermo-sensitive period (TSP), aromatase activity increases in the brain of developing red-eared slider turtles (*Trachemys scripta elegans*), incubated at the higher temperature, presumably destined to be females, relative to those incubated at the lower temperature, predestined to be males (Willingham et al. 2000). After this period, aromatase activity declines in brain of males and females. In Olive Ridley sea turtles (*Lepidochelys olivacea*), the female-producing (higher) temperature results in greater efficiency of aromatase conversion of T to E2 within the diencephalon region (Salame-Mendez et al. 1998). Analysis of aromatase activity in the gonad and brain of alligators around the TSP indicates this enzyme is expressed in low amounts in the gonad during the early stages of TSP for both sexes but increases in the gonad of potential females later in the TSP (Milnes et al. 2002), whereas brain aromatase activity rises early in the TSP for both sexes, suggesting that neural aromatase activity does not modulate gonadal differentiation. E2 treatment of eggs incubated at the MPT results in sex-reversed females that possess intermediate gonad function with gonad aromatase activity less in sex-reversed females compared to control females at the same stage but by stage 22, E2-derived females had greater gonad aromatase activity than control males. E2-derived females did not show any change in brain aromatase activity from stage 22 to 24, whereas, by stage 24, brain aromatase activity was lower in sex-reversed females compared to control females but not different than in control males.

Organizational and Activational Effects of Neurosteroids in Select Reptilian Species Green anoles (Anolis carolinensis)—Green anoles possess an XX/XY system of sex chromosomes and are seasonal breeders with male sexual behavior induced by an annual elevation in T levels. Several studies examined sex-dependent gene expression in anole brains. The enzyme 5α-reductase (SRD5A) converts T into 5α-DHT, and the SRD5A1 isoform may stimulate development of the forebrain and other brain regions that modulate later male reproductive behaviors in green anoles (Cohen and Wade 2012). In these males, T stimulates steroid receptor coactivator-1 (*src1*) expression in the preoptic area and amygdala (Kerver and Wade 2015). Conversely, females show greater expression of estrogen receptor alpha (*esr1*) and CREB binding protein (*cbp*) in the ventromedial hypothalamus and *cbp* in the preoptic area than males (Beck and Wade 2009; Kerver and Wade 2016). Testosterone treatment in males of this species also produces enhanced SRD5A activity regardless of the season but only up-regulates aromatase activity during the breeding season. Interestingly, comparable responses are absent in testosterone-treated females (Cohen and Wade 2010). This latter response might be considered an activational effect of testosterone as it occurs during the adult period. However, it is not clear if such adult-treatment effects are reversible.

Whiptail Lizards (Cnemidophorus uniparens and C. inornatus)—Whiptail lizards (*C. uniparens*) are a triploid unisexual species consisting of females alternating between male- and female-like pseudosexual behaviors at varying points throughout the estrous cycle. In contrast, the diploid ancestral whiptail lizard species (*C. inornatus)* exhibits sexual reproduction. *C. uniparens* may have originated from the inter-species hybridization between two living gonochoristic species: *C. inornatus* and *C. gularis*. TSD appears to be absent in *C. uniparens* and *C. inornatus* (Crews 1989). Parallel studies with parthenogenetic ancestral and sexually reproducing descendent species are useful in determining the evolutionary origins of organizational and activational effects of sex steroid hormones on neurobehavioral responses (Woolley et al 2004). Female *C. uniparens* demonstrate male-like pseudosexual behaviors resembling those of male *C. inornatus* (Crews et al. 1986). When a conspecific male is present, a greater number of female *C. inornatus* ovulate and latency to ovulation is shortened. Analogous findings are found when a male-like *C. uniparens* individual is placed with female conspecifics.

Neuroendocrine Effects of Androgens in Whiptail Lizards—Androgen-implanted, ovariectomized (OVX) *C. uniparens* exhibit male-like mounting behaviors, whereas, estrogen-implanted, OVX animals express receptive-like behaviors (Dias and Crews 2008). In *C. uniparens* and *C. inornatus,* an intraperitoneal (i.p.) T implant into gonadectomized individuals resulted in greater induction of male-like courtship behaviors relative to controls and those implanted with progesterone (Sakata et al. 2003). *In ovo* treatment of *C. uniparens* with aromatase inhibitors produced sex-reversed male parthenogens (*virago C. uniparens*) that acted morphologically and behaviorally similar to males of *C. inornatus* (Wennstrom and Crews 1995). Administration of T to gonadectomized control and virago *C. uniparens*, along with gonadectomized male and female *C. inornatus* enhanced mounting behaviors by both sexes and species that was accompanied by increased arginine vasotocin (AVT) immunoreactivity in the brain except in lab-generated parthenoforms (Hillsman et al. 2007). 5α-DHT implanted into the anterior hypothalamus-preoptic area (AH-POA), but not in the

ventromedial hypothalamus, induced courtship and copulatory behaviors in castrated male *C. inornatus* (Rozendaal and Crews 1989). These latter effects are assumingly due to activational effects of these exogenous androgens in *C. uniparens* and *C. inornatus*.

Neuroendocrine Effects of Estrogens in Whiptail Lizards—E2 concentrations are elevated during vitellogenesis and decline after ovulation in *C. uniparens* and *C. inornatus.* However, reproductively active female *C. uniparens* exhibit greater circulating E2 concentrations, as typified by follicles with yolk formation, female *C. Inornatus* (~900 pg/ml compared to ~98 pg/ml, respectively*)* (Young et al. 1995b; Moore et al. 1985; Moore and Crews 1986). Injection of estradiol benzoate (EB) into 1 and 30 day old individuals of both species increased expression of progesterone receptor (*pgr)* in the ventro-medial hypothalamus (VMH) but expression was greater in female *C. inornatus* than in conspecific males (Wennstrom et al. 2003). No marked expression differences in *pgr* were observed in female *C. uniparens vs.* male due to aromatase inhibitor treatment. The expression of *esr* (presumably *esr1*) was up-regulated in the VMH of EB-treated one day old male and female *C. inornatus* and 30 day old male-induced *C. uniparens* but not in EB-treated female *C. uniparens* at either age. EB treatment of OVX *C. uniparens* elevated receptive behavior, upregulated *esr1* expression within the ventromedial nucleus of the hypothalamus (VMN) and *torus semicircularis* but down regulated this transcript in the lateral septum (Young et al. 1995a). The EB-treated females also exhibited increased *pgr* expression in VMN and periventricular nucleus of the preoptic area. Exogenous estrogen increased *pgr* in the periventricular preoptic area in *C. inornatus* and *C. uniparens* (Godwin and Crews 1999). The above effects might be ascribed to activational effects of EB, but it is not clear if these can be reversed.

Neuroendocrine Effects of Progesterone in Whiptail Lizards—Progesterone administration to OVX *C. inornatus* and *C. uniparens* results in suppression of receptivity behavior in both EB-primed species and down regulation of expression of *esr* (presumably e*sr1*) and *pgr* in the VMN (Godwin et al. 1996). Progesterone-treated female *C. uniparens* displayed a masculinized behavioral response and mounted receptive estrogen-treated female *C. uniparens* (Grassman and Crews 1986). Progesterone treatment of castrated *C. inornatus* stimulated androgen-dependent sex behaviors but did not replicate seasonal-dependent effects of androgens on accessory sex structures (Lindzey and Crews 1993). Implantation of progesterone into the anterior hypothalamus of castrated *C. inornatus* resulted in some *C. uniparens* being sensitive to this hormone with complete restoration of sexual behaviors, whereas, others appeared insensitive with the hormonal implant failing to elicit sexual activities (Crews et al. 1996). The progesterone-sensitive males displayed elevated expression of *ar* in the preoptic area, amygdala, and lateral septum relative to the insensitive group. Conversely, *pgr* expression was reduced in the preoptic area in the sensitive compared to insensitive males. Taken together, the results suggest that progesterone administration to select adult reptiles may induce mixed organizational/activational effects, and there might be some plasticity in brain programming in certain reptile species.

Neuroendocrine Effects of Serotonin and Dopamine in Whiptail Lizards—Injection of 5-HT into the POA of OVX and T treated *C. uniparens* suppressed mounting

(Dias and Crews 2008). In contrast, administration of 5-HT into the VMH of OVX + E2 treated *C. uniparens* inhibited receptivity. *In ovo* treatment of *C uniparens* with aromatase inhibitors resulted in sex-reversed male parthenogens (*virago C. uniparens*) that act morphologically and behaviorally like males of *C. inornatus* (Wennstrom and Crews 1995). Both castrated male *C. inornatus* and OVX *C. uniparens* treated with a dopamine D1 agonist exhibited greater mounting behaviors and shorter latency to mount with *C. uniparens* being vulnerable to lower doses than *C. inornatus* (Woolley et al. 2001). This enhanced sensitivity in the triploid parthenogenetic lizard might be due to existing elevations in D1R in the limbic brain areas modulating courtship behavior. In male castrated *C. inornatus*, androgen implantation increased neuronal nitric oxide synthase (*Nos*) gene expression in the nucleus accumbens (NAc), POA, and VMH (O'Connell et al. 2012). However, this treatment decreased the NMDA receptor- *glun2* (previously termed *nr1*) gene expression in the medial amygdala region and NAc as well as *d1r* and *d2r* in the NAc.

Neurosteroids in other Reptilian Species—The broad-snouted caiman (*Caiman latirostris*) presents TSD. Similar to green anoles, *esr1* expression is greater in the medial cortex of TSD-females and females derived from E2 administration (hormone-dependent sex determination) during the critical developmental period (Varayoud et al. 2012). Male leopard geckos (*Eublepharis macularius*) also exhibit TSD with embryonic temperature governing sex determination and polymorphisms in each sex (Huang and Crews 2012). Males generated at varying incubation temperatures show differing patterns of behavior even when gonadectomized, suggestive that incubation temperature might influence brain programming. However, treatment of this species with 5α-DHT stimulates territorial scent-marking behavior. Thus, both egg incubation temperature and exogenous hormones alter neurobehavioral programming, including sociosexual behaviors. In the species studied above, steroid hormones might override potential temperature effects on brain programming. However, it is not clear if such effects might be generalized across reptilian species. It is also not clear in reptiles, whether the steroid hormone production may occur via both the gonad and brain as reported in fish (Diotel et al. 2011b).

Effects of Exposure to Environmental Chemicals on Organizational and Activational Programming of the Brains in Reptiles—Although there are extensive reports on neuroendocrine disruptors in fish, less information is available on direct effects of such chemicals on reptilian brain development. However, there are data on how EDC affect gonadal sexual differentiation and steroidogenesis. Disturbances in these physiological responses may be another means by which EDC alter neurobehavioral programming in reptiles. Thus, the effects of EDC and other environmental chemicals on gonadal sexual differentiation, hormone production, and current available reports on neurobehavioral programming are considered within the context of neuroendocrine disruption.

Gonadal Effects—Developmental exposure of broad-snouted caiman, a species with TSD, to bisphenol A (BPA) or E2 results in sex-reversal with individuals at the MPT of 33°C developing ovaries (Stoker et al. 2003). A follow-up study incubated caiman eggs either at the male- (33°C) or female- (30°C) producing temperature, and they were then exposed to E2, BPA, endosulfan, or atrazine (Stoker et al. 2008). Neonatal females generated

through hormonal sex determination (HSD) or “sex reversion”- i.e. exposure to one of these EDC, as opposed to TSD, lack type III follicles. In contrast, eggs incubated at the FPT and prenatally treated with the lowest doses of E2 (0.014 ppm), BPA (1.4 ppm), or atrazine (0.2 ppm) gave rise to females that possess elevated number of type III follicles. Prenatal exposure to BPA or E2 stimulated greater number of poly-ovular follicles in juvenile animals. Increased circulating estrogen levels were noted in neonatal females exposed to BPA or E2, and E2, BPA, atrazine, and endosulfan decreased T concentrations, but the mechanisms for these steroid hormone changes were not examined. Although atrazine and endosulfan exposure of caiman eggs incubated at the MPT failed to induce sex reversal, these chemicals produced several histopathological changes in testes involving the germ and somatic (peritubular cells) and reduced T concentrations (Rey et al. 2009). Treatment of caiman eggs incubated at the MPT with endosulfan (20 ppm) or BPA (1.4 ppm) at stage 20 of embryonic development produced all male hatchlings with altered germ cell proliferation (decreased in endosulfan-individuals, increased in BPA-individuals) (Durando et al. 2016). Caiman males derived from temperature exposures showed greater expression of anti-Mullerian hormone (*Amh*) and *Sox9* than TSD-induced females (Durando et al. 2013). However, females derived from hormonal treatment (BPA or E2- hormonal-dependent sexual determination [HSD]) displayed elevated expression of *Sox9* compared to TSD-derived females. Thus, females derived due to exposure to exogenous hormones (HSD) might exhibit different signature gene expression patterns in the gonad and brain than those derived due to incubation temperature (TSD, Figure 3). Endosulfan exposure induced elevated expression of sex-determining genes in males. Gene expression changes were also found in the ovary of red-eared slider turtles incubated at the MPT but treated with two potent polychlorinated biphenyls (PCB, 4-hydroxy-2d PCBi or 3′,4′,5′-tetrachlorobiphenyl [PCB-G]) (Matsumoto et al. 2014). This treatment altered hatchling sex ratio to females with activation of ovarian expression markers, including *foxl2*, *rspo1* but suppression of testicular markers: *dmrt1* and *sox9*. Such gene expression differences might be due to hyper- or hypomethylation of the promoter regions. However, DNA methylation pattern in the aromatase promoter continued to resemble that of control males rather than TSD-females, indicative that even in absence of this epigenetic change, these chemicals still induce feminization of the gene expression profile within the resulting ovary. Incubation of painted turtles at the MPT (25°C) but treated with BPA or EE2 during the critical window of sexual differentiation produced partial to full gonadal sex reversal to females (Jandegian et al. 2015). Male Italian wall lizards (*Podarcis sicula*) are seasonal breeders, but exposure to a diet contaminated with nonylphenol (NP) during this time suppressed spermatogenesis, inhibited expression of *ar*, *esr1*, and *esr2* in spermatogonia and primary spermatocytes, and reduced secretory activity of the corpus epididymis, presumably by enhancing expression of *esr1* in this region (Verderame and Limatola 2015).

Environmental Chemical Disruption of Neurosteroids and Other Hormones— Gold mining has triggered an increase in mercury (Hg) exposures of Western pond turtles (*Emys marmorata*), a long-lived species that are already in jeopardy in California and through the Pacific Northwest. Greater concentrations of Hg in this species are associated with elevated levels of T4 but declining concentrations of biologically active T3 (Meyer et al. 2014). Suppression of T3 concentrations may lead to a wide-range of pathological

effects, especially on the reproductive and central nervous system (CNS). Increased exposure to chlorinated and brominated hydrocarbon contaminants and metabolites in the Laurentian Great Lakes regions are associated with altered T4 and vitamin A concentrations in snapping turtles (*Chelydra serpentina*) (Letcher et al. 2015).

Embryonic exposure of male red-eared slider turtles to the polychlorinated biphenyl (PCB) mixture, Aroclor1242, or chlordane inhibited T production at hatching, and females exposed to these same chemicals displayed decreased progesterone, T, and 5α-DHT concentrations (Willingham et al. 2000). BPA-exposure of this same turtle species during the first 9 days of embryonic development inhibited estrogen metabolism, especially estrone sulfate formation, which was lower, compared to control-treated eggs, which resulted in a rise of estrone, and E2 (Clairardin et al. 2013). A follow-up study with this same species tested this idea by applying estrone at the time of oviposition that is rapidly metabolized to estrone sulfate (Paitz and Bowden 2015). However, this metabolism was reduced with co-exposure to BPA, facilitating an excess in estrone concentrations within the egg.

Endocrine disruptors might also modulate brain programming by stimulating the HPG axis, resulting in an increase in T production. Although exposure of alligator (*Alligator mississippiensis*) eggs to toxaphene (10 or 0.01 μg/kg) did not markedly affect gonadal development, circulating T concentrations were higher in animals treated with either dose, which may reflect enhanced hypothalamic-pituitary stimulation or reduced hepatic steroid metabolism (Milnes et al. 2004). Greater internal concentrations of PCB were positively correlated with elevated T concentrations in Mexican crocodiles (*Crocodylus moreletii*) sampled in Campeche, Mexico (Gonzalez-Jauregui et al. 2012). Thus, one mechanism by which environmental chemicals may alter brain sexual differentiation is by increasing T synthesis and release.

EDC-induced Changes in Gene Expression in Reptile Brain—Exposure of hatchling and year-old male snapping turtles to octylphenol (OP) increases hypothalamic expression of *aplp2* and *app* transcripts (both are associated with amyloid protein), whereas E2 exposure only upregulated *aplp2* in yearling males (Trudeau et al. 2002). Italian wall lizards treated with NP demonstrated disruptions in HPA axis, as evidenced by elevated hypothalamic corticotrophin-releasing factor (*crf*), alterations in plasma catecholamine levels, adrenal cortical hyperplasia, but reduced number of catecholamine-producing chromaffin cells in the adrenal medulla (De Falco et al. 2014). Brain aromatase concentrations, as determined by the tritiated water assay to assess the pattern of E2 biosynthesis, are different during TSP in females compared to male red-eared slider turtles with females showing greater aromatase activity than potential males at the beginning of the TSP (Willingham et al. 2000). However, after this period, both males and females show reduced brain aromatase activity, and this enzyme is undetectable in females just prior to hatching. Gomez-Picos et al. (2014) demonstrated in Olive Ridley sea turtles (*Lepidochelys olivacea*) that BPA exposure during TSP only affected brain aromatase activity in the female, but not male-producing temperature. Recently, *in ovo* exposure of painted turtles to BPA and to a lesser extent EE2, was found to affect the brain transcriptome, especially for genes associated with oxidative phosphorylation, mitochondrial activity, and ribosomal function (Manshack et al. 2017). Any differences between BPA and E2/EE2 might be due to the

ability of BPA to bind and activate other steroid receptors besides ESR such as AR and PPARs (Vandenberg et al. 2009).

EDC-induced Behavioral Disruptions—Red-eared slider turtles subjected to one of three pre-hatching manipulations: estrone sulfate or corticosterone exposure or thermal fluctuations exhibited no significant alterations in hatchling behaviors, including righting response and exploration (Carter et al. 2016). However, developmental exposure following BPA or EE2 improved spatial navigational learning and memory in painted turtles relative to control males (Manshack et al. 2016). This behavioral difference suggests that early contact with BPA or EE2 might induce feminization of the brain in individuals who would otherwise possess testes and demonstrate male-programmed behaviors. These studies suggest that developmental exposure to NED might alter neurobehavioral development. However, it remains to be determined whether females derived from exposure to xenoestrogens (HSD) exhibit similar neural programming and behavioral responses as control females derived from TSD. In alligators, there are differences in gonadal and brain aromatase activity in E2-derived females compared to control females. Thus, there are likely other gene expression and possibly epigenetic differences between these two groups of females.

Conclusions and Future Perspectives

Examination of organizational and activational effects in poikilothermic species is essential to understand the normal biological mechanisms that control these processes and whether there are commonalities across the taxa. Studies are needed to understand how NED might dysregulate these processes, which might lead to both individual and even population effects. In this review fish, amphibians, and reptiles were considered.

Activational and organizational effects in poikilothermic species—In teleost fish, it is clear that NED exert activational effects on the functions of the neuroendocrine system and on behaviors. The evidence for organizational effects of endogenous hormones or NED in fish is emerging. Brain sexuality and reproductive behavior, and perhaps other neuroendocrine endpoints are relatively plastic compared to mammals and birds. It is postulated that E2 regulates neurogenesis through its action on RGC in fish brains. One might argue that steroid-dependent regulation of neurogenesis in fish is an organizational event, but this remains to be fully investigated.

While early exposures to exogenous androgens and estrogens are known to change the sexual phenotype of fish, the continued presence of E2 is required to maintain the female phenotype. It is debatable whether this is an organizational effect since fish retain their sexual plasticity even into the adult stage as demonstrated by complete sex reversal of adult female tilapia (1 year old) and medaka (5 months old) induced by treating with an aromatase inhibitor, fadrozole for tilapia and exemestane for medaka (Paul-Prasanth et al. 2013). Similar sex reversal was observed in adult female zebrafish treated with fadrozole (Takatsu et al. 2013). In both of these experiments, sexual behavior followed the phenotypic sex, suggesting that the responses occurred not only in gonads but also in brain. These experiments emphasize the importance of phenotypic maintenance by continued presence of the sex steroid.

Migratory behavior facilitated by olfactory imprinting appears to be an organizational effect that is established in Coho salmon. Whether this organizational effect also occurs in other diadromous fish needs to be investigated to gain a more comprehensive understanding of the process. It may be that TH is important. One testable hypothesis is that TH disrupters such as flame-retardants, PCB, and perchlorate, alter olfactory system development and sensitivities and disrupt migration. Another question to address in future studies is potential plasticity in amphibians and reptiles. As discussed above, this is clearly the case in fish, especially in sex-changing species (Iwata et al. 2012). This potential sexual plasticity has not been clearly established for amphibians and reptiles, especially those with TSD.

In mammals, sex chromosomes and SRY have been implicated in brain sexual differentiation (Arnold et al. 2004; Burgoyne and Arnold 2016; Davies and Wilkinson 2006; Maekawa et al. 2014; Majdic and Tobet 2011; Xu and Disteche 2006). However, several poikilothermic species lack sex chromosomes and possibly also SRY. Such species might thus help elucidate whether other genes encoded for on autosomal chromosomes may compensate for the absence of SRY and possibly other sex-chromosome-associated genes and underpin organizational-activational effects in the brain (Crews 1993). Such putative genes might be influenced by incubation temperature and/or steroid hormones.

Future studies need to consider whether there are commonalities in terms of epigenetic changes, including DNA methylation, histone protein modifications, and miRNA that may guide organizational/activational effects across taxa. In mice, the organizational effects of T results in striking and delayed brain methylome and transcriptomic effects (Ghahramani et al. 2014), suggesting that critical windows of exposure to NED might be even more prolonged that originally envisioned. Recently, Mosley et al (2017) reported in mice that neonatal treatment with a DNA methyltransferase (DNMT) inhibitor, zebularine, increased the number of calbindin-D28K (CALB) cell number in the medial preoptic area (mPOA) in both sexes and resulted in greater ESR1 cell density in the ventrolateral portion of the ventromedial nucleus of the hypothalamus (VMHvl) and mPOA. This latter zebularine affect thus abolished sex differences in ESR1 expression observed otherwise in control individuals.

Potential interactions between NEDs and climate change effects in poikilothermic species—A related and under-investigated research area is the potential interaction between rising water temperature as a result of climate change and NED on neuroendocrine functions and behavior of fish. There are conceptual papers that discuss hypotheses, use modeling to suggest outcomes and effects of these potential interactions, and provide guidance on how to design future studies (Hooper et al. 2013; Moe et al. 2013). It is clear that behavior and reproduction depend upon exogenous environmental factors such as temperature and photoperiod. How global climate change confounds biological rhythms for fish, especially how it might alter neuroendocrine signaling in the brain is not well understood and more research is required. While fish exhibit wide tolerances for variation in temperature for development of embryos (Devlin 2002), it is clear that for many fish species temperature may affect sex determination, even if sex is primarily controlled by GSD (Devlin and Nagahama 2002; Conover and Heins 1987; D'Cotta et al. 2001). A recent study by White et al (2017) modeled the effects of sex ratio changes on populations for *Menidia beryllina*, the inland silverside. In their model, "mating function," the relationship between

sex ratio and reproductive success, was estimated for *M. beryllia*. Evidence indicated that skewing the sex ratio might be detrimental to populations with the most marked effects seen with masculinization of the population as might occur with higher temperature and exposure to contaminants that modulate brain aromatase. Higher order effects on populations need to be understood and studies such as White et al. (2017) add to our understanding of the potential consequences of increased global temperatures.

In one of the first climate change experimental studies with fish, yellow perch (*Perca flavescens*) exhibited decreased reproductive success of a wild population and in lab experiments in response to shorter and warmer than average winter seasons (Farmer et al. 2015). Short winter, female perch produced smaller eggs that had lower hatching rates, which yielded smaller larvae compared to females exposed to longer winter seasons. In this study, only the effects of climate change were investigated.

To our knowledge, the first study to investigate the potential interaction between climate change and a NED or EDC is a study where investigators exposed two zebrafish strains (one outbred and one inbred- which may reflect an already genetically-bottlenecked species) to both increased temperature (normal mean lab temperature of 28°C and projected temperature for the year 2100 of 33°C) and clotrimazole (2 and 10 μg/L) (Brown et al. 2015). Clotrimazole is an antifungal pharmaceutical detected in the environment and a known disruptor of both steroidogenesis and spermatogenesis in fish. Inbred zebrafish lab populations developed skewed sex ratio toward males at both higher temperature and with clotrimazole exposure and projected population growth rates were markedly reduced especially in inbred zebrafish. Brown et al (2015) concluded that interactive effects of climate change and this pollutant might reasonably affect smaller isolated freshwater fish populations, particularly those at the brink of being extinct. This is a provocative study that stimulates others to rigorously test their hypothesis. Although, there are no *in vivo* studies investigating the potential for clotrimazole as a NED, given the steroidogenic (neuroestrogen synthesis) capacity of the fish brain and *in vitro* data showing that clotrimazole modulates cytochrome P450 steroidogenic enzymes, it is reasonable to expect that such exposure may result in altered neuroestrogen production. It is not clear whether interactive effects might be greater on embryos, juveniles, and recrudescing adults versus reproductively active adults.

Studies of NED in amphibia are in their infancy. While much is known regarding control and disruption of gonadal development and metamorphosis in tadpoles, little is actually known about influence of EDC on the brain. Developmental changes in sex-steroid-synthesis enzymes and steroid or TH receptors suggest important alterations in steroid sensitivity in the developing amphibian brain. Further, analysis of steroid action and effects of various pollutants on adult male courtship vocalization lay the foundation for specific testing of the neuroendocrine disruption hypothesis in frogs.

Long-term changes in global temperatures may be an important stimulus in those piscine and reptilian species that transitioned from GSD to TSD and corresponding chromosome number increased rapidly in phylogenic branches that shifted to TSD (Valenzuela and Adams 2011). Although this response may have evolved as an adaptation to marked fluctuations in temperature, it resulted in such species being vulnerable to climate change

(Mitchell and Janzen 2010; Hulin et al. 2009; Neuwald and Valenzuela 2011; Refsnider and Janzen 2016; Santidrian Tomillo et al. 2015; Schwanz and Janzen 2008) and environmental chemicals designed to mimic naturally occurring sex steroid hormones, such as endocrine disruptors, as discussed below (Crews 1996; Crews et al. 1995). It has been projected that for some sea turtle populations currently under study that global warming might result in their extirpation in the next 50 years (Santidrian Tomillo et al. 2015). On the other hand, those species demonstrating a greater transitional range of temperature that yields both sexes are predicted to be somewhat buffered against the effects of climate change (Hulin et al. 2009).

It is uncertain in poikilothermic species demonstrating HSD whether those females generated by HSD fully mirror programming of the gonad and brain compared to females derived due to TSD. As illustrated in Figure 3, it is possible that such females may retain male-persistent traits. If such is the case, data suggest that organizational/activational pathways might be influenced both by temperature-regulated genes within the gonad and possibly by the brain and steroidogenic hormones.

Future Directions—While the collective studies that examine an individual vertebrate species provide evidence that NED might alter organizational-activational effects in poikilothermic vertebrates, many questions remain. The critical issue is whether there are certain neural transcripts and epigenetic markers more vulnerable to such chemicals across taxa. For direct comparisons to be made, a single study needs to examine transcriptomic and epigenetic (DNA methylation, histone protein modifications, and miRNA) patterns in several vertebrate classes exposed to the same NED and approximate dose. For instance, vitellogenin is a yolk-precursor protein considered as a biomarker of EDC exposure in fish, amphibians, and reptiles (Porte et al. 2006). Direct biomarkers for neuroendocrine disruption are awaiting discovery. There are some caveats in designing such cross-vertebrate experiments, as it may be necessary to adjust the timing of exposure to cover the critical period of brain development for a given species and dose, since each species may have varying ability to metabolize different classes of NED. While it is clear that brain plasticity exists in fish, especially those that undergo sex reversal, it is uncertain whether there is similar malleability in amphibians and reptiles. Some of the above studies in reptiles that showed behavioral and gene expression changes in adults treated with exogenous steroid hormones indicate that this might be the case. If so, evidence suggests that adult exposure to NED might alter neuronal circuitry and induce potential brain masculinization or feminization in these species.

In summary, organizational and activational paradigm described originally in mammals is also important in poikilothermic species. However, there are clear species differences, especially as it relates to malleability of the neural circuitry to the male or female pathway. While in mammals it appears to be permanent, there might be fluctuations throughout the lifespan in fish and also possibly in amphibians and reptiles. Further investigations in this area are needed. The organizational-activational concept also provides a framework to understand how exposure to NED at various time points might influence brain sexual differentiation. In those species where females are generated due to either TSD or HSD (including exposure to NED), it is important to determine whether females generated

through each of these pathways exhibits identical molecular profiles in the gonad and brain. With even greater global production of such chemicals, there is a clear urgency to understand the ramifications of such exposures at the individual and population levels. Climate change and NED might also interact to affect organizational-activational programming in these species. Finally, by understanding organizational-activational effects in a variety of vertebrate classes and whether certain neural transcripts and epigenetic marks are susceptible across taxa, it might reveal important shared features that render diverse animals vulnerable to NED.

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References

Adkins-Regan E 1999 Testosterone increases singing and aggression but not male-typical sexual partner preference in early estrogen treated female zebra finches. Horm Behav 35: 63–70. [PubMed: 10049604]

Aggarwal N, Goswami SV, Khandewal p, and Sehgal N 2014 Aromatase activity in the brain and ovary: Seasonal variation correlated with circannual gonadal cycle in the catfish, *Heteropneustes fossilis*. Indian J. Exp. Biol. 52: 527–537. [PubMed: 24851417]

Arnold AP, and Breedlove SM. 1985 Organizational and activational effects of sex steroids on brain and behavior: A reanalysis. Horm Behav 19: 469–498. [PubMed: 3910535]

Arnold AP, Xu J, Grisham W, Chen X, Kim YH, and Itoh Y. 2004 Minireview: Sex chromosomes and brain sexual differentiation. Endocrinology 145: 1057–1062. [PubMed: 14670983]

ASTM. *Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX)*: ASTM INTERNATIONAL.

Balthazart J, Cornil CA, Charlier TD, Taziaux M, and Ball GF. 2009 Estradiol, a key endocrine signal in the sexual differentiation and activation of reproductive behavior in quail. J Exp Zool A Ecol Genet Physiol 311: 323–345. [PubMed: 18481266]

Balthazart J, Tlemcani O, and Ball GF. 1996 Do sex differences in the brain explain sex differences in the hormonal induction of reproductive behavior? What 25 years of research on the Japanese quail tells us. Horm Behav 30: 627–661. [PubMed: 9047287]

Barker DJ 1999 Fetal origins of cardiovascular disease. Ann Med 31 Suppl 1: 3–6.

Barker DJ 2007 The origins of the developmental origins theory. J Intern Med 261: 412–417. [PubMed: 17444880]

Basu N 2015 Applications and implications of neurochemical biomarkers in environmental toxicology. Environ Toxicol Chem 34: 22–29. [PubMed: 25331165]

Beck LA, and Wade J. 2009 Effects of estradiol, sex, and season on estrogen receptor alpha mRNA expression and forebrain morphology in adult green anole lizards. Neuroscience 160: 577–586. [PubMed: 19272420]

Bern HA, Mills KT, Hatch DL, Ostrander PL, and Iguchi T. 1992 Altered mammary responsiveness to estradiol and progesterone in mice exposed neonatally to diethylstilbestrol. Cancer Lett 63: 117–24. [PubMed: 1562988]

Bianco AC, and Larsen PR. 2005 Cellular and structural biology of the deiodinases. Thyroid 15: 777–7786. [PubMed: 16131321]

Blazquez M, and Somoza GM. 2010 Fish with thermolabile sex determination (TSD) as models to study brain sex differentiation. Gen Comp Endocrinol 166: 470–477. [PubMed: 19854192]

Bonett RM, Hoopfer ED, and Denver RJ. 2010 Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. Gen Comp Endocrinol 168: 209–219. [PubMed: 20338173]

Bowers JM, Waddell J, and McCarthy MM. 2010 A developmental sex difference in hippocampal neurogenesis is mediated by endogenous oestradiol. Biol Sex Differ 1: 8. [PubMed: 21208470]

Breton B, and Sambroni E. 1996 Steroid activation of the brain-pituitary complex gonadotropic function in the triploid rainbow trout *Oncorhynchus mykiss*. Gen Comp Endocrinol 101: 155–164. [PubMed: 8812359]

Brooks BW, Turner PK, Stanley JK, Weston JJ, Glidewell EA, Foran CM, Slattery M, La Point TW, and Huggett DB. 2003 Waterborne and sediment toxicity of fluoxetine to select organisms. Chemosphere 52: 135–142. [PubMed: 12729696]

Brown AR, Owen SF, Peters J, Zhang Y, Soffker M, Paull GC, Hosken DJ, Wahab MA, and Tyler CR. 2015 Climate change and pollution speed declines in zebrafish populations. Proc Natl Acad Sci U S A 112: E1237–46. [PubMed: 25733876]

Buchholz DR, Paul BD, Fu L, and Shi YB. 2006 Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog. Gen Comp Endocrinol 145: 1–19. [PubMed: 16266705]

Buklijas T 2014 Food, growth and time: Elsie Widdowson's and Robert McCance's research into prenatal and early postnatal growth. Stud Hist Philos Biol Biomed Sci 47: 267–277. [PubMed: 24378592]

Burgoyne PS, and Arnold AP. 2016 A primer on the use of mouse models for identifying direct sex chromosome effects that cause sex differences in non-gonadal tissues. Biol Sex Differ 7: 68. [PubMed: 27999654]

Cano-Nicolau J, Vaillant C, Pellegrini E, Charlier TD, Kah O, and Coumailleau P. 2016 Estrogenic effects of several BPA analogs in the developing zebrafish brain. Front Neurosci 10: 112. [PubMed: 27047331]

Carr JA, and Patino R. 2011 The hypothalamus-pituitary-thyroid axis in teleosts and amphibians: Endocrine disruption and its consequences to natural populations. Gen Comp Endocrinol 170: 299–312. [PubMed: 20566362]

Carter AW, Paitz RT, McGhee KE, and Bowden RM. 2016 Turtle hatchlings show behavioral types that are robust to developmental manipulations. Physiol Behav 155: 46–55. [PubMed: 26657026]

Castaneda Cortes DC, Langlois VS, and Fernandino JI. 2014 Crossover of the hypothalamic pituitary-adrenal/interrenal, -thyroid, and -gonadal axes in testicular development. Front Endocrinol 5: 139.

Chow BF, and Lee CJ. 1964 Effect of dietary restriction of pregnant rats on body weight gain of the offspring. J Nutr 82: 10–18. [PubMed: 14110926]

Chung WC, and Auger AP. 2013 Gender differences in neurodevelopment and epigenetics. Pflugers Arch 465: 573–584. [PubMed: 23503727]

Clairardin SG, Paitz RT, and Bowden RM. 2013 In ovo inhibition of steroid metabolism by bisphenol-A as a potential mechanism of endocrine disruption. Proc Biol Sci 280: 20131773. [PubMed: 24004940]

Cohen RE, and Wade J. 2010 Testosterone selectively affects aromatase and 5alpha-reductase activities in the green anole lizard brain. Gen Comp Endocrinol 166: 128–133. [PubMed: 19917285]

Cohen RE, and Wade J. 2012 Expression of aromatase and two isozymes of 5alpha-reductase in the developing green anole forebrain. J Neuroendocrinol 24: 1213–1221. [PubMed: 22487458]

Conover DO, and Heins SW. 1987 Adaptive variation in environmental and genetic sex determination in a fish. Nature 326: 496–498. [PubMed: 3561487]

Correia LO, Siqueira Junior S, Carneiro PL, and Bezerra MA. 2014 Evaluation of the use of *Leptodactylus ocellatus* (Anura: Leptodactylidae) frog tissues as bioindicator of metal contamination in Contas River, Northeastern Brazil. Ann Acad Bras Cienc 86: 1549–1561.

Craig PM, Trudeau VL, and Moon TW. 2014 Profiling hepatic microRNAs in zebrafish: Fluoxetine exposure mimics a fasting response that targets AMP-activated protein kinase (AMPK). PLoS One 9: e95351. [PubMed: 24751937]

Creusot N, Ait-Aissa S, Tapie N, Pardon P, Brion F, Sanchez W, Thybaud E, Porcher JM, and Budzinski H. 2014 Identification of synthetic steroids in river water downstream from pharmaceutical manufacture discharges based on a bioanalytical approach and passive sampling. Environ Sci Technol 48: 3649–57. [PubMed: 24579728]

Crews D 1989 Absence of temperature-dependent sex determination in congeneric sexual and parthenogenetic Cnemidophorus lizards. J Exp Zool 252: 318–320. [PubMed: 2607330]

Crews D 1993 The organizational concept and vertebrates without sex chromosomes. Brain Behav Evol 42: 202–214. [PubMed: 8252373]

Crews D 1996 Temperature-dependent sex determination: The interplay of steroid hormones and temperature. Zool Sci 13: 1–13. [PubMed: 8688803]

Crews D, Bergeron JM, and McLachlan JA. 1995 The role of estrogen in turtle sex determination and the effect of PCBs. *Environ Health Persp* 103 Suppl 7: 73–77.

Crews D, Godwin J, Hartman V, Grammer M, Prediger EA, and Sheppherd R. 1996 Intrahypothalamic implantation of progesterone in castrated male whiptail lizards (*Cnemidophorus inornatus*) elicits courtship and copulatory behavior and affects androgen receptor- and progesterone receptor-mRNA expression in the brain. J Neurosci 16: 7347–7352. [PubMed: 8929441]

Crews D, Grassman M, and Lindzey J. 1986 Behavioral facilitation of reproduction in sexual and unisexual whiptail lizards. Proc Natl Acad Sci U S A 83: 9547–9550. [PubMed: 3467325]

Crews D, Sakata J, and Rhen T. 1998 Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 119: 229–241. [PubMed: 9826996]

D'Aniello B, Pinelli C, Di Fiore MM, Tela L, King JA, and Rastogi RK. 1995 Development and distribution of gonadotropin-releasing hormone neuronal systems in the frog (*Rana esculenta*) brain: Immunohistochemical analysis. Brain Res Dev Brain Res 89: 281–288. [PubMed: 8612331]

D'Cotta H, Fostier A, Guiguen Y, Govoroun M, and Baroiller JF. 2001 Aromatase plays a key role during normal and temperature-induced sex differentiation of tilapia Oreochromis niloticus. Mol Reprod Dev 59: 265–276. [PubMed: 11424212]

da Rosa JG, Koakoski G, Piato AL, Bogo MR, Bonan CD, Barcellos LJ. 2016 Impaired brain StAR and HSP 70 gene expression in zebrafish exposed to methyl-parathion based insecticide. J Toxicol Environ Health A 79: 1–7. [PubMed: 26699931]

Davies W, and Wilkinson LS. 2006 It is not all hormones: alternative explanations for sexual differentiation of the brain. Brain Res 1126: 36–45. [PubMed: 17101121]

De Falco M, Sellitti A, Sciarrillo R, Capaldo A, Valiante S, Iachetta G, Forte M, and Laforgia V. 2014 Nonylphenol effects on the HPA axis of the bioindicator vertebrate, *Podarcis sicula* lizard. Chemosphere 104: 190–196. [PubMed: 24290296]

Denver RJ 2013 Neuroendocrinology of amphibian metamorphosis. Curr Top Dev Biol 103: 195–227. [PubMed: 23347520]

Devlin RH, Nagahama Y 2002 Sex determination and sex differentiation in fish: An overview of the genetic, physiological, and environmental influences. Aquaculture 208: 191–364.

Dias BG, and Crews D. 2008 Regulation of pseudosexual behavior in the parthenogenetic whiptail lizard, *Cnemidophorus uniparens*. Endocrinology 149: 4622–31. [PubMed: 18483155]

Diotel N, Do Rego JL, Anglade I, Vaillant C, Pellegrini E, Gueguen MM, Mironov S, Vaudry H, and Kah O. 2011a Activity and expression of steroidogenic enzymes in the brain of adult zebrafish. Eur J Neurosci 34: 45–56. [PubMed: 21692878]

Diotel N, Do Rego JL, Anglade I, Vaillant C, Pellegrini E, Vaudry H, and Kah O. 2011b The brain of teleost fish, a source, and a target of sexual steroids. Front Neurosci 5:137. [PubMed: 22194715]

Diotel N, Le Page Y, Mouriec K, Tong SK, Pellegrini E, Vaillant C, Anglade I, Brion F, Pakdel F, Chung BC, and Kah O. 2010 Aromatase in the brain of teleost fish: Expression, regulation and putative functions. Front Neuroendocrinol 31: 172–192. [PubMed: 20116395]

do Rego JL, and Vaudry H. 2016 Comparative aspects of neurosteroidogenesis: From fish to mammals. Gen Comp Endocrinol 227: 120–129. [PubMed: 26079790]

Dong W, and Willett KL. 2008 Local expression of CYP19A1 and CYP19A2 in developing and adult killifish (Fundulus heteroclitus). Gen Comp Endocrinol 155: 307–317. [PubMed: 17582409]

Duarte-Guterman P, Langlois VS, Hodgkinson K, Pauli BD, Cooke GM, Wade MG, and Trudeau VL. 2009 The aromatase inhibitor fadrozole and the 5-reductase inhibitor finasteride affect gonadal differentiation and gene expression in the frog *Silurana tropicalis*. Sex Dev 3: 333–341. [PubMed: 20130388]

Duarte-Guterman P, Navarro-Martin L, and Trudeau VL. 2014 Mechanisms of crosstalk between endocrine systems: Regulation of sex steroid hormone synthesis and action by thyroid hormones. Gen Comp Endocrinol 203: 69–85. [PubMed: 24685768]

Duarte-Guterman P, and Trudeau VL. 2010 Regulation of thyroid hormone-, oestrogen- and androgen-related genes by triiodothyronine in the brain of *Silurana tropicalis*. J Neuroendocrinol 22: 1023–1031. [PubMed: 20626568]

Dubois EA, Florijn MA, Zandbergen MA, Peute J, and Goos HJ. 1998 Testosterone accelerates the development of the catfish GnRH system in the brain of immature African catfish (Clarias gariepinus). Gen Comp Endocrinol 112: 383–393. [PubMed: 9843644]

Durando M, Canesini G, Cocito LL, Galoppo GH, Zayas MA, Luque EH, and Munoz-de-Toro M. 2016 Histomorphological changes in testes of broad-snouted caimans (*Caiman latirostris*) associated with in ovo exposure to endocrine-disrupting chemicals. J Exp Zool A Ecol Genet Physiol 325: 84–96. [PubMed: 26639912]

Durando M, Cocito L, Rodriguez HA, Varayoud J, Ramos JG, Luque EH, and Munoz-de-Toro M. 2013 Neonatal expression of amh, sox9 and sf-1 mRNA in *Caiman latirostris* and effects of in ovo exposure to endocrine disrupting chemicals. Gen Comp Endocrinol 191: 31–38. [PubMed: 23747749]

Elisio M, Chalde T, and Miranda LA. 2014 Seasonal changes and endocrine regulation of pejerrey (*Odontesthes bonariensis*) oogenesis in the wild. Comp Biochem Physiol A Mol Integr Physiol 175: 102–109. [PubMed: 24892730]

Ezaz T, Sarre SD, O'Meally D, Graves JA, and Georges A. 2009 Sex chromosome evolution in lizards: Independent origins and rapid transitions. Cytogenet Genome Res 127: 249–260. [PubMed: 20332599]

Farmer TM, Marschall EA, Dabrowski K, and Ludsin SA. 2015 Short winters threaten temperate fish populations. Nat Commun 6: 7724. [PubMed: 26173734]

Fernandino JI, Hattori RS, Kishii A, Strussmann CA, and Somoza GM. 2012 The cortisol and androgen pathways cross talk in high temperature-induced masculinization: The 11beta-hydroxysteroid dehydrogenase as a key enzyme. Endocrinology 153: 6003–6011. [PubMed: 23041673]

Flett P A, Leatherland JF, and Van Der Kraak GJ. 1993 Endocrine correlates of seasonal reproduction in fish: Thyroid hormones and gonadal growth, steroidogenesis, and energy partitioning in Great Lakes salmon. XII International Congress of Comparative Endocrinology, Toronto, Ontario, Canada, 5 16–21, 1993.

Flood DE, Fernandino JI, and Langlois VS. 2013 Thyroid hormones in male reproductive development: Evidence for direct crosstalk between the androgen and thyroid hormone axes. Gen Comp Endocrinol 192: 2–14. 10.1016/j.ygcen.2013.02.038. [PubMed: 23524004]

Flood DE, and Langlois VS. 2014 Crosstalk between the thyroid hormone and androgen axes during reproductive development in *Silurana tropicalis*. Gen Comp Endocrinol 203: 232–240. [PubMed: 24713448]

Forlano PM, Sisneros JA, Rohmann KN, and Bass AH. 2014 Neuroendocrine control of seasonal plasticity in the auditory and vocal systems of fish. Front Neuroendocrinol. 37: 129–145. [PubMed: 25168757]

Frankel TE, Meyer MT, Kolpin DW, Gillis AB, Alvarez DA, and Orlando EF. 2016 Exposure to the contraceptive progestin, gestodene, alters reproductive behavior, arrests egg deposition, and masculinizes development in the fathead minnow (*Pimephales promelas*). Environ Sci Technol 50: 5991–5999. [PubMed: 27129041]

Frye C, Bo E, Calamandrei G, Calzà L, Dessì-Fulgheri F, Fernández M, Fusani L, Kah O, Kajta M, Le Page Y, Patisaul HB, Venerosi A, Wojtowicz AK, and Panzica GC. 2012 Endocrine disrupters: A review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. J Neuroendocrinol 24: 144–159. [PubMed: 21951193]

Galas L, Raoult E, Tonon MC, Okada R, Jenks BG, Castano JP, Kikuyama S, Malagon M, Roubos EW, and Vaudry H. 2009 TRH acts as a multifunctional hypophysiotropic factor in vertebrates. Gen Comp Endocrinol 164: 40–50. [PubMed: 19435597]

Galton VA 1990 Mechanisms underlying the acceleration of thyroid hormone-induced tadpole metamorphosis by corticosterone. Endocrinology 127: 2997–3002. [PubMed: 2249638]

Gardner S Cline G, Mwebi N, and Rayburn J 2017 Developmental and interactive effects of arsenic and chromium to developing *Ambystoma maculatum* embryos: Toxicity, teratogenecity, and whole body concentrations. J Toxicol Environ Health A 80: 91–104. [PubMed: 28085642]

Geraudie P, Gerbron M, and Minier C 2010 Seasonal variations and alterations of sex steroid levels during the reproductive cycle of male roach (Rutilus rutilus). Mar Environ Res 69: S53–S55. [PubMed: 20006903]

Ghahramani NM, Ngun TC, Chen PY, Tian Y, Krishnan S, Muir S, Rubbi L, Arnold AP, de Vries GJ, Forger NG, Pellegrini M, and Vilain E. 2014 The effects of perinatal testosterone exposure on the DNA methylome of the mouse brain are late-emerging. Biol Sex Differ 5: 8. [PubMed: 24976947]

Godwin J 2010 Neuroendocrinology of sexual plasticity in teleost fishes. Front Neuroendocrinol 31: 203–216. [PubMed: 20176046]

Godwin J, and Crews D. 1999 Hormonal regulation of progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: Regional and species differences. J Neurobiol 39: 287–293. [PubMed: 10235682]

Godwin J, Hartman V, Grammer M, and Crews D. 1996 Progesterone inhibits female-typical receptive behavior and decreases hypothalamic estrogen and progesterone receptor messenger ribonucleic acid levels in whiptail lizards (genus Cnemidophorus). Horm Behav 30:138–144. [PubMed: 8797022]

Goleman WL, Carr JA, and Anderson TA. 2002 Environmentally relevant concentrations of ammonium perchlorate inhibit thyroid function and alter sex ratios in developing *Xenopus laevis*. Environ Toxicol Chem 21: 590–597. [PubMed: 11878472]

Golshan M, Hatef A, Zare A, Socha M, Milla S, Gosiewski G, Fontaine P, Sokolowska-Mikolajczyk M, Habibi HR, and Alavi SM. 2014 Alternations in neuroendocrine and endocrine regulation of reproduction in male goldfish (*Carassius auratus*) following an acute and chronic exposure to vinclozolin, in vivo. Aquat Toxicol 155:73–83. [PubMed: 24995616]

Gomez-Picos P, Sifuentes-Romero I, Merchant-Larios H, Hernandez-Cornejo R, Diaz-Hernandez V, and Garcia-Gasca A. 2014 Expression of aromatase in the embryonic brain of the olive ridley sea turtle (*Lepidochelys olivacea*), and the effect of bisphenol-A in sexually differentiated embryos. Int J Dev Biol 58 :733–741. [PubMed: 26154314]

Gonzalez-Jauregui M, Valdespino C, Salame-Mendez A, Aguirre-Leon G, and Rendon-Vonosten J. 2012 Persistent organic contaminants and steroid hormones levels in Morelet's crocodiles from the Southern Gulf of Mexico. Arch Environ Contam Toxicol 62: 445–454. [PubMed: 22002785]

Gonzalez A, and Piferrer F. 2003 Aromatase activity in the European sea bass (*Dicentrarchus labrax* L.) brain. Distribution and changes in relation to age, sex, and the annual reproductive cycle. Gen Comp Endocrinol 132: 223–230. [PubMed: 12812769]

Gore AC, and Patisaul HB. 2010 Neuroendocrine disruption: Historical roots, current progress, questions for the future. Front Neuroendocrinol 31: 395–399. [PubMed: 20638407]

Gorelick DA, Watson W, and Halpern ME. 2008 Androgen receptor gene expression in the developing and adult zebrafish brain. Dev Dyn 237: 2987–2995. [PubMed: 18816841]

Grassman M, and Crews D. 1986 Progesterone induction of pseudocopulatory behavior and stimulus-response complementarity in an all-female lizard species. Horm Behav 20: 327–335. [PubMed: 3770655]

Guiguen Y, Fostier A, Piferrer F, and Chang CF. 2010 Ovarian aromatase and estrogens: A pivotal role for gonadal sex differentiation and sex change in fish. Gen Comp Endocrinol 165:352–366. [PubMed: 19289125]

Guillette LJ, Jr., Crain DA, Rooney AA, and Pickford DB. 1995 Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environ Health Persp 103 Suppl 7: 157–164.

Hayes TB 1998 Sex determination and primary sex differentiation in amphibians: Genetic and developmental mechanisms. J Exp Zool 281: 373–399. [PubMed: 9662826]

Hillsman KD, Sanderson NS, and Crews D. 2007 Testosterone stimulates mounting behavior and arginine vasotocin expression in the brain of both sexual and unisexual whiptail lizards. Sex Dev 1: 77–84. [PubMed: 18391518]

Hiraki T, Takeuchi A, Tsumaki T, Zempo B, Kanda S, Oka Y, Nagahama Y, and Okubo K. 2012 Female-specific target sites for both oestrogen and androgen in the teleost brain. Proc Biol Sci 279: 5014–5023. [PubMed: 23075834]

Hoffmann F, and Kloas W. 2010 An environmentally relevant endocrine-disrupting antiandrogen, vinclozolin, affects calling behavior of male Xenopus laevis. Horm Behav 58: 653–659. [PubMed: 20600051]

Hoffmann F, and Kloas W. 2012a Estrogens can disrupt amphibian mating behavior. PLoS One 7: e32097. [PubMed: 22355410]

Hoffmann F, and Kloas W. 2012b The synthetic progestogen, levonorgestrel, but not natural progesterone, affects male mate calling behavior of *Xenopus laevis*. Gen Comp Endocrinol 176: 385–390. [PubMed: 22391239]

Hoffmann F, and Kloas W. 2016 p,p'-Dichlordiphenyldichloroethylene (p,p'-DDE) can elicit antiandrogenic and estrogenic modes of action in the amphibian *Xenopus laevis*. Physiol Behav 167: 172–178. [PubMed: 27640133]

Hogan NS, Crump KL, Duarte P, Lean DR, and Trudeau VL. 2007 Hormone cross-regulation in the tadpole brain: Developmental expression profiles and effect of T3 exposure on thyroid hormone- and estrogen-responsive genes in *Rana pipiens*. Gen Comp Endocrinol 154: 5–15. [PubMed: 17400220]

Hogan NS, Duarte P, Wade MG, Lean DR, and Trudeau VL. 2008 Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): Identifying critically vulnerable periods of development. Gen Comp Endocrinol 156: 515–523. [PubMed: 18430423]

Hooper MJ, Ankley GT, Cristol DA, Maryoung LA, Noyes PD, and Pinkerton KE. 2013 Interactions between chemical and climate stressors: A role for mechanistic toxicology in assessing climate change risks. EnvironToxicol Chem 32: 32–48.

Huang V, and Crews D. 2012 Differences induced by incubation temperature, versus androgen manipulation, in male leopard geckos (*Eublepharis macularius*). Physiol Behav 107: 121–124. [PubMed: 22750461]

Hulin V, Delmas V, Girondot M, Godfrey MH, and Guillon JM. 2009 Temperature-dependent sex determination and global change: are some species at greater risk? Oecologia 160: 493–506. [PubMed: 19277719]

Ishizuya-Oka A 2011 Amphibian organ remodeling during metamorphosis: Insight into thyroid hormone-induced apoptosis. Dev Growth Differ 53: 202–212. [PubMed: 21338346]

Iwata E, Mikami K, Manbo J, Moriya-Ito K, and Sasaki H. 2012 Social interaction influences blood cortisol values and brain aromatase genes in the protandrous false clown anemonefish, *Amphiprion ocellaris*. Zool Sci 29: 849–855. [PubMed: 23215977]

Jandegian CM, Deem SL, Bhandari RK, Holliday CM, Nicks D, Rosenfeld CS, Selcer KW, Tillitt DE, Vom Saal FS, Velez-Rivera V, Yang Y, and Holliday DK. 2015 Developmental exposure to bisphenol A (BPA) alters sexual differentiation in painted turtles (*Chrysemys picta*). Gen Comp Endocrinol 216: 77–85. [PubMed: 25863134]

Jasarevic E, Geary DC, and Rosenfeld CS. 2012 Sexually selected traits: A fundamental framework for studies on behavioral epigenetics. Inst. Lab Animal Res J 53: 253–269.

Kerver HN, and Wade J. 2015 Hormonal regulation of steroid receptor coactivator-1 mRNA in the male and female green anole brain. J Neuroendocrinol 27: 223–233. [PubMed: 25557947]

Kerver HN, and Wade J. 2016 Sexually dimorphic expression of CREB binding protein in the green anole brain. Gen Comp Endocrinol 225: 55–60. [PubMed: 26363452]

Kohno S, Parrott BB, Yatsu R, Miyagawa S, Moore BC, Iguchi T, and Guillette L, Jr. 2014 Gonadal differentiation in reptiles exhibiting environmental sex determination. Sex Dev 8: 208–226. [PubMed: 24642710]

Konkle AT, and McCarthy MM. 2011 Developmental time course of estradiol, testosterone, and dihydrotestosterone levels in discrete regions of male and female rat brain. Endocrinology 152: 223–235. [PubMed: 21068160]

Kriegstein A, and Alvarez-Buylla A. 2009 The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32: 149–184. [PubMed: 19555289]

Kuhl AJ, Manning S, and Brouwer M. 2005 Brain aromatase in Japanese medaka (*Oryzias latipes*): Molecular characterization and role in xenoestrogen-induced sex reversal. J Steroid Biochem Mol Biol 96:67–77. [PubMed: 15876529]

Yann Le Page, Diotel N, Vaillant C, Pellegrini E, Anglade I, Merot Y, and Kah O 2010 Aromatase, brain sexualization and plasticity: the fish paradigm. Eur J Neurosci 32: 2105–2115. [PubMed: 21143665]

Le Page Y, Vosges M, Servili A, Brion F and Kah O. 2011 Neuroendocrine effects of endocrine disruptors in teleost fish. J Toxicol Environ Health B 14: 370–386

Lema SC, and Nevitt GA. 2004 Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. J Exp Biol 207: 3317–3327. [PubMed: 15326208]

León-Olea M, Martyniuk C, Orlando EF, Ottinger MA, Rosenfeld CS, Wolstenholme J, and Trudeau VL. 2014 Current concepts in neuroendocrine disruption. Gen Comp Endocrinol 203: 158–173. [PubMed: 24530523]

Letcher RJ, Lu Z, de Solla SR, Sandau CD, and Fernie KJ. 2015 Snapping turtles *(Chelydra serpentina*) from Canadian areas of concern across the southern Laurentian Great Lakes: Chlorinated and brominated hydrocarbon contaminants and metabolites in relation to circulating concentrations of thyroxine and vitamin A. Environ Res 143: 266–278. [PubMed: 26519832]

Lindzey J, and Crews D. 1993 Effects of progesterone and dihydrotestosterone on stimulation of androgen-dependent sex behavior, accessory sex structures, and in vitro binding characteristics of cytosolic androgen receptors in male whiptail lizards (*Cnemidophorus inornatus*). Horm Behav 27: 269–281. [PubMed: 8349284]

Liu H, Todd EV, Lokman PM, Lamm MS, Godwin JR, and Gemmell NJ. 2017 Sexual plasticity: A fishy tale. Mol Reprod Dev 84: 171–194. [PubMed: 27543780]

Lower N, and Moore A 2007 The impact of a brominated flame retardant on smoltification and olfactory function in Atlantic salmon (Salmo salar L.) smolts. Mar Freshwater Behav Physiol 40: 267–284.

Maekawa F, Tsukahara S, Kawashima T, Nohara K, and Ohki-Hamazaki H. 2014 The mechanisms underlying sexual differentiation of behavior and physiology in mammals and birds: Relative contributions of sex steroids and sex chromosomes. Front Neurosci 8: 242. [PubMed: 25177264]

Majdic G, and Tobet S. 2011 Cooperation of sex chromosomal genes and endocrine influences for hypothalamic sexual differentiation. Front Neuroendocrinol 32: 137–145. [PubMed: 21338619]

Manshack LK, Conard CM, Bryan SJ, Deem SL, Holliday DK, Bivens NJ, Givan SA, and Rosenfeld CS. 2017 Transcriptomic alterations in the brain of painted turtles (*Chrysemys picta*) developmentally exposed to bisphenol A or ethinyl estradiol. Physiol Genom 49: 201–215.

Manshack LK, Conard CM, Johnson SA, Alex JM, Bryan SJ, Deem SL, Holliday DK, Ellersieck MR, and Rosenfeld CS. 2016 Effects of developmental exposure to bisphenol A and ethinyl estradiol on ppatial navigational learning and memory in painted turtles (*Chrysemys picta*). Horm Behav 85: 48–55. [PubMed: 27476434]

Martinez P, Vinas AM, Sanchez L, Diaz N, Ribas L, and Piferrer F. 2014 Genetic architecture of sex determination in fish: Applications to sex ratio control in aquaculture. Front Genet 5: 340. [PubMed: 25324858]

Martyniuk CJ, Gerrie ER, Popesku JT, Ekker M, and Trudeau VL. 2007 Microarray analysis in the zebrafish (*Danio rerio*) liver and telencephalon after exposure to low concentration of 17alpha-ethinylestradiol. Aquat Toxicol 84: 38–49. [PubMed: 17606305]

Maselli V, Polese G, Rippa D, Ligrone R, Kumar Rastogi R, and Fulgione D. 2010 Frogs, sentinels of DNA damage induced by pollution in Naples and the neighbouring Provinces. Ecotoxicol Environ Saf 73: 1525–1529. [PubMed: 20684845]

Matsumoto Y, Hannigan B, and Crews D. 2014 Embryonic PCB exposure alters phenotypic, genetic, and epigenetic profiles in turtle sex determination, a biomarker of environmental contamination. Endocrinology 155: 4168–4177. [PubMed: 25105783]

Melo AC, and Ramsdell JS. 2001 Sexual dimorphism of brain aromatase activity in medaka: induction of a female phenotype by estradiol. Environ Health Persp 109: 257–64.

Mennigen JA, Stroud P, Zamora JM, Moon TW, and Trudeau VL. 2011 Pharmaceuticals as neuroendocrine disruptors: Lessons learned from fish on Prozac. J Toxicol Environ Health B 14: 387–412.

Merchant-Larios H, and Diaz-Hernandez V. 2013 Environmental sex determination mechanisms in reptiles. Sex Dev 7: 95–103. [PubMed: 22948613]

Meyer E, Eagles-Smith CA, Sparling D, and Blumenshine S. 2014 Mercury exposure associated with altered plasma thyroid hormones in the declining western pond turtle (*Emys marmorata*) from California mountain streams. Environ Sci Technol 48: 2989–2996. [PubMed: 24559486]

Migaud H, Ismail R, Cowan M, and Davie A. 2012 Kisspeptin and seasonal control of reproduction in male European sea bass (*Dicentrarchus labrax*). Gen Comp Endocrinol 179: 384–399. [PubMed: 23036731]

Milnes MR, Allen D, Bryan TA, Sedacca CD, and Guillette LJ, Jr. 2004 Developmental effects of embryonic exposure to toxaphene in the American alligator (*Alligator mississippiensis*). Comp Biochem Physiol C Toxicol Pharmacol 138: 81–87. [PubMed: 15313450]

Milnes MR, Jr., Roberts RN, and Guillette LJ, Jr. 2002 Effects of incubation temperature and estrogen exposure on aromatase activity in the brain and gonads of embryonic alligators. Environ Health Persp 110 Suppl 3: 393–396.

Mitchell NJ, and Janzen FJ. 2010 Temperature-dependent sex determination and contemporary climate change. Sex Dev 4:129–140. [PubMed: 20145383]

Moe SJ, De Schamphelaere K, Clements WH, Sorensen MT, Van den Brink PJ, and Liess M. 2013 Combined and interactive effects of global climate change and toxicants on populations and communities. Environ Toxicol Chem 32: 49–61. [PubMed: 23147390]

Money J 1990 Androgyne becomes bisexual in sexological theory: Plato to Freud and neuroscience. J Am Acad Psychoanal 18: 392–413. [PubMed: 2258314]

Moore MC, and Crews D. 1986 Sex steroid hormones in natural populations of a sexual whiptail lizard Cnemidophorus inornatus, a direct evolutionary ancestor of a unisexual parthenogen. Gen Comp Endocrinol 63: 424–430. [PubMed: 3557067]

Moore MC, Whittier JM, and Crews D. 1985 Sex steroid hormones during the ovarian cycle of an all-female, parthenogenetic lizard and their correlation with pseudosexual behavior. Gen Comp Endocrinol 60: 144–153. [PubMed: 4065527]

Morris JA, Jordan CL, and Breedlove SM. 2004 Sexual differentiation of the vertebrate nervous system. Nat Neurosci 7: 1034–1039. [PubMed: 15452574]

Mosley M, Weathington J, Cortes LR, Bruggeman E, Castillo-Ruiz A, Xue B, and Forger NG. 2017 Neonatal inhibition of DNA methylation alters cell phenotype in sexually dimorphic regions of the mouse brain. Endocrinology 158: 1838–1848. [PubMed: 28398586]

Mouriec K, Lareyre JJ, Tong SK, Le Page Y, Vaillant C, Pellegrini E, Pakdel F, Chung BC, Kah O, and Anglade I. 2009 Early regulation of brain aromatase (cyp19a1b) by estrogen receptors during zebrafish development. Dev Dyn 238: 2641–2651. [PubMed: 19718764]

Moussavi M, Wlasichuk M, Chang JP, and Habibi HR. 2012 Seasonal effect of GnIH on gonadotrope functions in the pituitary of goldfish. Mol CellEndocrinol 350: 53–60.

Moussavi M, Wlasichuk M, Chang JP, and Habibi HR. 2013 Seasonal effect of GnIH on GnRH-induced gonadotroph functions in the goldfish pituitary. J Neuroendocrinol 223: 191–202.

Nakane Y, Ikegami K, Iigo M, Ono H, Takeda K, Takahashi D, Uesaka M, Kimijima M, Hashimoto R, Arai N, Suga T, Kosuge K, Abe T, Maeda R, Senga T, Amiya N, Azuma T, Amano M, i Abe H, Yamamoto N, and Yoshimura T. 2013 The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nat Commun 4.

Neuwald JL, and Valenzuela N. 2011 The lesser known challenge of climate change: thermal variance and sex-reversal in vertebrates with temperature-dependent sex determination. PLoS One 6: e18117. [PubMed: 21448294]

Nevitt G, and Dittman A 1998 A new model for olfactory imprinting in salmon. Integr Biol Issues News Rev 1: 215–223.

O'Connell LA, Mitchell MM, Hofmann HA, and Crews D. 2012 Androgens coordinate neurotransmitter-related gene expression in male whiptail lizards. Genes Brain Behav 11: 813–818. [PubMed: 22862958]

OECD. *Test No. 231: Amphibian Metamorphosis Assay*: OECD Publishing.

Okada R, Kobayashi T, Yamamoto K, Nakakura T, Tanaka S, Vaudry H, and Kikuyama S. 2009 Neuroendocrine regulation of thyroid-stimulating hormone secretion in amphibians. Ann N Y Acad Sci 1163: 262–270. [PubMed: 19456347]

Ottinger MA and Dean KM. 2011 Neuroendocrine impacts of endocrine-disrupting chemicals in birds: Life stage and species sensitivities. J Toxicol Environ Health B 14: 413–422.

Orlando EF, and Ellestad LE. 2014 Sources, concentrations, and exposure effects of environmental gestagens on fish and other aquatic wildlife, with an emphasis on reproduction. Gen Comp Endocrinol 203: 241–249. [PubMed: 24759355]

Orlando EF, Binczik GA, Denslow ND, and Guillette LJ, Jr. 2003 Reproductive seasonality of the female Florida gar, *Lepisosteus platyrhincus*. Gen Comp Endocrinol 131: 365–371. [PubMed: 12714019]

Overturf MD, Anderson JC, Pandelides Z, Beyger L, and Holdway DA. 2015 Pharmaceuticals and personal care products: A critical review of the impacts on fish reproduction. Crit Rev Toxicol 45: 469–491. [PubMed: 25945515]

Paitz RT, and Bowden RM. 2015 The in ovo conversion of oestrone to oestrone sulfate is rapid and subject to inhibition by bisphenol A. Biol Lett 11: 20140946. [PubMed: 25904318]

Panzica GC, Bo E, Martini MA, Miceli D, Mura E, Viglietti-Panzica C, and Gotti S. 2011 Neuropeptides and enzymes are targets for the action of endocrine disrupting chemicals in the vertebrate brain. J Toxicol Environ Health B 14: 449–472.

Pasmanik M, and Callard GV. 1985 Aromatase and 5 alpha-reductase in the teleost brain, spinal cord, and pituitary gland. Gen Comp Endocrinol 60: 244–251. [PubMed: 4065533]

Pasmanik M, and Callard GV. 1988 Changes in brain aromatase and 5 alpha-reductase activities correlate significantly with seasonal reproductive cycles in goldfish (*Carassius auratus*). Endocrinology 122: 1349–1356. [PubMed: 3345716]

Paul-Prasanth B, Bhandari RK, Kobayashi T, Horiguchi R, Kobayashi Y, Nakamoto M, Shibata Y, Sakai F, Nakamura M, and Nagahama Y. 2013 Estrogen oversees the maintenance of the female genetic program in terminally differentiated gonochorists. Sci Rep 3: 2862. [PubMed: 24096556]

Pellegrini E, Diotel N, Vaillant-Capitaine C, Perez Maria R, Gueguen MM, Nasri A, Cano Nicolau J, and Kah O. 2016 Steroid modulation of neurogenesis: Focus on radial glial cells in zebrafish. J Steroid Biochem Mol Biol 160: 27–36. [PubMed: 26151741]

Phoenix CH, Goy RW, Gerall AA, and Young WC. 1959 Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65:369–382. [PubMed: 14432658]

Piferrer F 2001 Endocrine sex control strategies for the feminization of teleost fish. Aquaculture 197: 229–281.

Pikulkaew S, De Nadai A, Belvedere P, Colombo L, and Dalla Valle L. 2010 Expression analysis of steroid hormone receptor mRNAs during zebrafish embryogenesis. Gen Comp Endocrinol 165: 215–220. [PubMed: 19576895]

Pinelli C, Fiorentino M, D'Aniello B, Tanaka S, and Rastogi RK. 1996 Immunohistochemical demonstration of FSH and LH in the pituitary of the developing frog, *Rana esculenta*. Gen Comp Endocrinol 104: 189–196. [PubMed: 8930609]

Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, and Meier PJ. 2002 Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. Mol Endocrinol 16: 2283–2296. [PubMed: 12351693]

Porte C, Janer G, Lorusso LC, Ortiz-Zarragoitia M, Cajaraville MP, Fossi MC, and Canesi L. 2006 Endocrine disruptors in marine organisms: Approaches and perspectives. Comp Biochem Physiol C Toxicol Pharmacol 143: 303–15. [PubMed: 16723279]

Pradhan DS, Solomon-Lane TK, Willis MC, and Grober MS. 2014 A mechanism for rapid neurosteroidal regulation of parenting behaviour. Proc Biol Sci 281: 1786.

Preau L, Le Blay K, Saint Paul E, Morvan-Dubois G, and Demeneix BA. 2015 Differential thyroid hormone sensitivity of fast cycling progenitors in the neurogenic niches of tadpoles and juvenile frogs. Mol Cell Endocrinol 420: 138–151. [PubMed: 26628040]

Refsnider JM, and Janzen FJ. 2016 Temperature-dependent sex determination under rapid anthropogenic environmental change: Evolution at a turtle's pace? J Hered 107: 61–70. [PubMed: 26245920]

Rey F, Gonzalez M, Zayas MA, Stoker C, Durando M, Luque EH, and Munoz-de-Toro M. 2009 Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male *Caiman latirostris*. Gen Comp Endocrinol 162:286–292. [PubMed: 19364509]

Roeder LM, and Chow BF. 1972 Maternal undernutrition and its long-term effects on the offspring. Am J Clin Nutr 25: 812–821. [PubMed: 4558370]

Rozendaal JC, and Crews D. 1989 Effects of intracranial implantation of dihydrotestosterone on sexual behavior in male *Cnemidophorus inornatus*, a direct sexual ancestor of a parthenogenetic lizard. Horm Behav 23: 194–202. [PubMed: 2744738]

Runnalls TJ, Beresford N, Losty E, Scott AP, and Sumpter JP. 2013 Several synthetic progestins with different potencies adversely affect reproduction of fish. Environ Sci Technol 47: 2077–2084. [PubMed: 23360115]

Sachs LM, Damjanovski S, Jones PL, Li Q, Amano T, Ueda S, Shi YB, and Ishizuya-Oka A. 2000 Dual functions of thyroid hormone receptors during Xenopus development. Comp Biochem Physiol B Biochem Mol Biol 126: 199–211. [PubMed: 10874167]

Sakata JT, Woolley SC, Gupta A, and Crews D. 2003 Differential effects of testosterone and progesterone on the activation and retention of courtship behavior in sexual and parthenogenetic whiptail lizards. Horm Behav 43: 523–530. [PubMed: 12799168]

Salame-Mendez A, Herrera-Munoz J, Moreno-Mendoza N, and Merchant-Larios H. 1998 Response of diencephalon but not the gonad to female-promoting temperature with elevated estradiol levels in the sea turtle *Lepidochelys olivacea*. J Exp Zool 280: 304–313. [PubMed: 9493349]

Santidrian Tomillo P, Genovart M, Paladino FV, Spotila JR, and Oro D. 2015 Climate change overruns resilience conferred by temperature-dependent sex determination in sea turtles and threatens their survival. Glob Chang Biol 21: 2980–2988. [PubMed: 25929883]

Santos TG, Melo R, Costa-Silva DG, Nunes M, Rodrigues NR, and Franco JL. 2015 Assessment of water pollution in the Brazilian Pampa biome by means of stress biomarkers in tadpoles of the leaf frog *Phyllomedusa iheringii* (Anura: Hylidae). Peer J 3: e1016. [PubMed: 26056614]

Schiller V, Zhang X, Hecker M, Schafers C, Fischer R, and Fenske M. 2014 Species-specific considerations in using the fish embryo test as an alternative to identify endocrine disruption. Aquat Toxicol 155: 62–72. [PubMed: 24992288]

Scholz AT, Horrall RM, Cooper JC, and Hasler AD. 1976 Imprinting to chemical cues: The basis for home stream selection in salmon. Science 192: 1247–1249. [PubMed: 1273590]

Schwanz LE, and Janzen FJ. 2008 Climate change and temperature-dependent sex determination: Can individual plasticity in nesting phenology prevent extreme sex ratios? Physiol Biochem Zool 81: 826–834. [PubMed: 18831689]

Shi Y, Liu X, Zhu P, Li J, Sham KW, Cheng SH, Li S, Zhang Y, Cheng CH, and Lin H. 2013 G-protein-coupled estrogen receptor 1 is involved in brain development during zebrafish (*Danio rerio*) embryogenesis. Biochem Biophys Res Commun 435: 21–27. [PubMed: 23583372]

Silva de Assis HC, Simmons DB, Zamora JM, Lado WE, Al-Ansari AM, Sherry JP, Blais JM, Metcalfe CD, and Trudeau VL. 2013 Estrogen-like effects in male goldfish co-exposed to fluoxetine and 17 alpha-ethinylestradiol. Environ Sci Technol 47: 5372–5382. [PubMed: 23590834]

Söffker M, and Tyler CR. 2012 Endocrine disrupting chemicals and sexual behaviors in fish – A critical review on effects and possible consequences. Crit Rev Toxicol 42:653–668. [PubMed: 22697575]

Somoza GM, Yu KL, and Peter RE. 1988 Serotonin stimulates gonadotropin release in female and male goldfish, *Carassius auratus* L. Gen Comp Endocrinol 72: 374–382. [PubMed: 3266487]

Sower SA, Reed KL, and Babbitt KJ. 2000 Limb malformations and abnormal sex hormone concentrations in frogs. Environ Health Persp 108: 1085–1090.

Sternberg RM, Thoemke KR, Korte JJ, Moen SM, Olson JM, Korte L, Tietge JE, and Degitz SJ, Jr. 2011 Control of pituitary thyroid-stimulating hormone synthesis and secretion by thyroid hormones during Xenopus metamorphosis. Gen Comp Endocrinol 173: 428–437. [PubMed: 21803044]

Stoker C, Beldomenico PM, Bosquiazzo VL, Zayas MA, Rey F, Rodriguez H, Munoz-de-Toro M, and Luque EH. 2008 Developmental exposure to endocrine disruptor chemicals alters follicular dynamics and steroid levels in *Caiman latirostris*. Gen Comp Endocrinol 156: 603–612. [PubMed: 18384790]

Stoker C, Rey F, Rodriguez H, Ramos JG, Sirosky P, Larriera A, Luque EH, and Munoz-de-Toro M. 2003 Sex reversal effects on *Caiman latirostris* exposed to environmentally relevant doses of the xenoestrogen bisphenol A. Gen Comp Endocrinol 133: 287–296. [PubMed: 12957472]

Suda M, Kodama M, Oshima Y, Yamamoto K, Nakamura Y, Tanaka S, Kikuyama S, and Nakamura M. 2011 Up-regulation of FSHR expression during gonadal sex determination in the frog *Rana rugosa*. Gen Comp Endocrinol 172 : 475–486. [PubMed: 21521644]

Sumpter JP 1990 General concepts of seasonal reproduction In Reproductive Seasonality in Teleosts: Environmental Influences, edited by Munro AD, Scott AP and Lam TJ, Boca Raton: CRC, Press, Inc pp 13–31.

Takatsu K, Miyaoku K, Roy SR, Murono Y, Sago T, Itagaki H, Nakamura M, and Tokumoto T. 2013 Induction of female-to-male sex change in adult zebrafish by aromatase inhibitor treatment. Sci Rep 3: 3400. [PubMed: 24292399]

Tata JR 2006 Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. Mol Cell Endocrinol 246: 10–20. [PubMed: 16413959]

Trudeau VL, Chiu S, Kennedy SW, and Brooks RJ. 2002 Octylphenol (OP) alters the expression of members of the amyloid protein family in the hypothalamus of the snapping turtle, Chelydra serpentina serpentina. Environ Health Persp 110: 269–275.

Trudeau VL, Peter RE, and Sloley BD. 1991 Testosterone and estradiol potentiate the serum gonadotropin response to gonadotropin-releasing hormone in goldfish. Biol Reprod 44: 951–960. [PubMed: 1873395]

Tsai HW, Grant PA, and Rissman EF. 2009 Sex differences in histone modifications in the neonatal mouse brain. Epigenetics 4: 47–53. [PubMed: 19029819]

Valenzuela N, and Adams DC. 2011 Chromosome number and sex determination coevolve in turtles. Evolution 65:1808–1813. [PubMed: 21644965]

Valenzuela N, Neuwald JL, and Literman R. 2013 Transcriptional evolution underlying vertebrate sexual development. Dev Dyn 242: 307–319. [PubMed: 23108853]

Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, and Soto AM. 2009 Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. Endocr Rev 30: 75–95. [PubMed: 19074586]

Varayoud J, Monje L, Moreno-Piovano GS, Galoppo GH, Luque EH, Munoz-de-Toro M, and Ramos JG. 2012 Sexually dimorphic expression of receptor-alpha in the cerebral cortex of neonatal *Caiman latirostris* (Crocodylia: Alligatoridae). Gen Comp Endocrinol 179: 205–213. [PubMed: 22964531]

Verderame M, and Limatola E. 2015 Interferences of an environmental pollutant with estrogen-like action in the male reproductive system of the terrestrial vertebrate *Podarcis sicula*. Gen Comp Endocrinol 213: 9–15. [PubMed: 25680815]

Vosges M, Kah O, Hinfray N, Chadili E, Le Page Y, Combarnous Y, Porcher JM, and Brion F. 2012 17alpha-Ethinylestradiol and nonylphenol affect the development of forebrain GnRH neurons through an estrogen receptors-dependent pathway. Reprod Toxicol 33: 198–204. [PubMed: 21549831]

Wang L, Harris SM, Espinoza HM, McClain V, and Gallagher EP. 2012 Characterization of phospholipid hydroperoxide glutathione metabolizing peroxidase (gpx4) isoforms in Coho salmon olfactory and liver tissues and their modulation by cadmium. Aquat Toxicol 114-115: 134–141. [PubMed: 22446825]

Wapstra E, and Warner DA. 2010 Sex allocation and sex determination in squamate reptiles. Sex Dev 4: 110–118. [PubMed: 20051672]

Watson JT, and Adkins-Regan E. 1989a Activation of sexual behavior by implantation of testosterone propionate and estradiol benzoate into the preoptic area of the male Japanese quail (*Coturnix japonica*). Horm Behav 23: 251–268. [PubMed: 2744741]

Watson JT, and Adkins-Regan E. 1989b Testosterone implanted in the preoptic area of male Japanese quail must be aromatized to activate copulation. Horm Behav 23: 432–447. [PubMed: 2793083]

Waye A, and Trudeau VL. 2011 Neuroendocrine disruption: More than hormones are upset. J Toxicol Environ Health B 14: 270–291.

Wennstrom KL, and Crews D. 1995 Making males from females: The effects of aromatase inhibitors on a parthenogenetic species of whiptail lizard. Gen Comp Endocrinol 99: 316–322. [PubMed: 8536943]

Wennstrom KL, Gill CJ, and Crews D. 2003 Sex differences in estrogen-induced progesterone and estrogen receptor mRNA in the ventromedial hypothalamus of hatchling whiptail lizards. Brain Res Dev Brain Res 145:151–157. [PubMed: 14519501]

White JW, Cole BJ, Cherr GN, Connon RE, and Brander SM. 2017 Scaling up endocrine disruption effects from individuals to populations: Outcomes depend on how many males a population needs. Environ Sci Technol 51: 1802–1810. [PubMed: 28064479]

Whittier JM, and Crews D. 1987 Seasonal reproduction: Patterns and controls In Hormones and Reproduction in Fishes, Amphibians, and Reptiles, edited by Norris DO and Jones RE, New York: Plenum Press pp 385–409.

Willingham E, Baldwin R, Skipper JK, and Crews D. 2000a Aromatase activity during embryogenesis in the brain and adrenal-kidney-gonad of the red-eared slider turtle, a species with temperature-dependent sex determination. Gen Comp Endocrinol 119: 202–227. [PubMed: 10936040]

Willingham E, Rhen T, Sakata JT, and Crews D. 2000b Embryonic treatment with xenobiotics disrupts steroid hormone profiles in hatchling red-eared slider turtles (*Trachemys scripta elegans*). Environ Health Persp 108: 329–332.

Woolley SC, Sakata JT, and Crews D. 2004 Tracing the evolution of brain and behavior using two related species of whiptail lizards: *Cnemidophorus uniparens* and *Cnemidophorus inornatus*. Inst Lab Animal Res J 45: 46–53.

Woolley SC, Sakata JT, Gupta A, and Crews D. 2001 Evolutionary changes in dopaminergic modulation of courtship behavior in Cnemidophorus whiptail lizards. Horm Behav 40: 483–489. [PubMed: 11716577]

Xing L, Esau C, and Trudeau VL. 2015 Direct regulation of aromatase B expression by 17beta-estradiol and dopamine D1 receptor agonist in adult radial glial cells. Front Neurosci 9: 504. [PubMed: 26793050]

Xing L, Goswami M, and Trudeau VL. 2014 Radial glial cell: Critical functions and new perspective as a steroid synthetic cell. Gen Comp Endocrinol 203: 181–185. [PubMed: 24675515]

Xing L, Venables MJ, and Trudeau VL. 2016 Role of aromatase and radial glial cells in neurotoxin-induced dopamine neuron degeneration and regeneration. *Gen Comp* Endocrinol 241: 69–79. [PubMed: 26873632]

Xu J, and Disteche CM. 2006 Sex differences in brain expression of X- and Y-linked genes. Brain Res 1126: 50–55. [PubMed: 16962077]

Yamaguchi T, Yoshinaga N, Yazawa T, Gen K, and Kitano T. 2010 Cortisol is involved in temperature-dependent sex determination in the Japanese flounder. Endocrinology 151: 3900–3908. [PubMed: 20534725]

Yatsu R, Miyagawa S, Kohno S, Parrott BB, Yamaguchi K, Ogino Y, Miyakawa H, Lowers RH, Shigenobu S, Guillette LJ, Jr., and Iguchi T. 2016 RNA-seq analysis of the gonadal transcriptome during *Alligator mississippiensis* temperature-dependent sex determination and differentiation. BMC Genomics 17: 77. [PubMed: 26810479]

Yatsu R, Miyagawa S, Kohno S, Saito S, Lowers RH, Ogino Y, Fukuta N, Katsu Y, Ohta Y, Tominaga M, Guillette LJ, Jr., and Iguchi T. 2015 TRPV4 associates environmental temperature and sex determination in the American alligator. Sci Rep 5: 18581. [PubMed: 26677944]

Young LJ, Nag PK, and Crews D. 1995a Regulation of estrogen receptor and progesterone receptor messenger ribonucleic acid by estrogen in the brain of the whiptail lizard (*Cnemidophorus uniparens*). J Neuroendocrinol 7: 119–125. [PubMed: 7767324]

Young LJ, Nag PK, and Crews D. 1995b Species differences in estrogen receptor and progesterone receptor-mRNA expression in the brain of sexual and unisexual whiptail lizards. J Neuroendocrinol 7: 567–76. [PubMed: 7496397]

Zhang D, Xiong H, Mennigen JA, Popesku JT, i L V. Marlatt CJ Martyniuk K Crump A Cossins R, Xia X, and Trudeau VL. 2009a Defining global neuroendocrine gene expression patterns associated with reproductive seasonality in fish. PLoS ONE 4: e5816. [PubMed: 19503831]

Zhang D, Popesku JT, Martyniuk CJ, Xiong H, Duarte-Guterman P, Yao L, Xia X, and Trudeau VL. 2009b Profiling neuroendocrine gene expression changes following fadrozole-induced estrogen decline in the female goldfish. Physiol Genom 38: 351–361.

Zornik E, and Kelley DB. 2011 A neuroendocrine basis for the hierarchical control of frog courtship vocalizations. Front Neuroendocrinol 32:353–366. [PubMed: 21192966]

Zupanc GK 2006 Neurogenesis and neuronal regeneration in the adult fish brain. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 192: 649–670. [PubMed: 16463148]

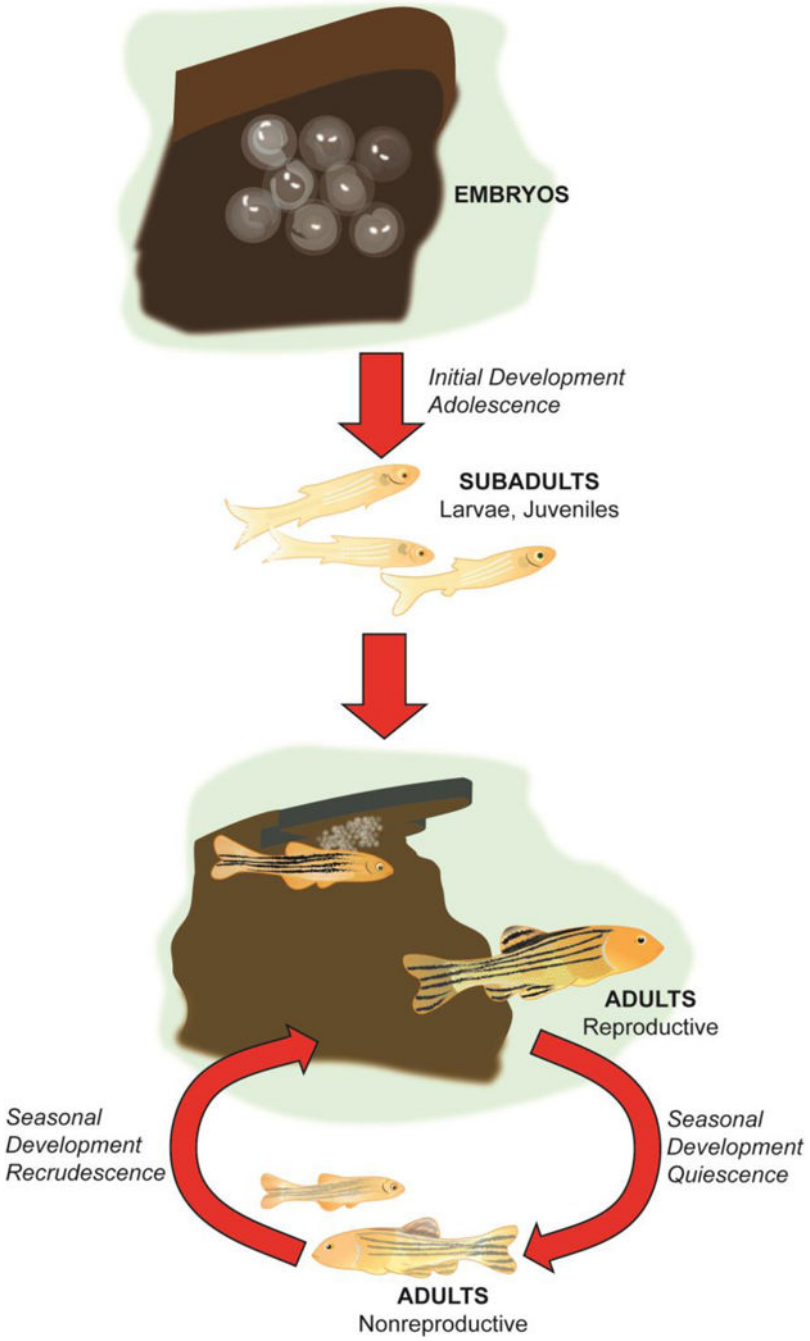

Figure 1.

Neuroendocrine disrupters may exert activational and organizational effects on health, physiology, and behavior of multiple life history stages of fish. This figure illustrates an example fish developing from embryo to subadult to adult stages. Iteroparous fish, species that reproduce in multiple years, undergo seasonal development as the neuroendocrine system alternates between reproductive and non-reproductive periods of a year. Examples of organizational effects appear to occur during initial development of embryo and sub-adult stages Activational effects are found in fish exposed as adults as in other vertebrates, yet

fishes demonstrate remarkable sexual plasticity in behavior, morphology, and physiology as adults. It is hypothesized that iteroparous fish may also exhibit organizational effects in their neuroendocrine systems during seasonal development as they transition from non-reproductive to reproductive adult stages.

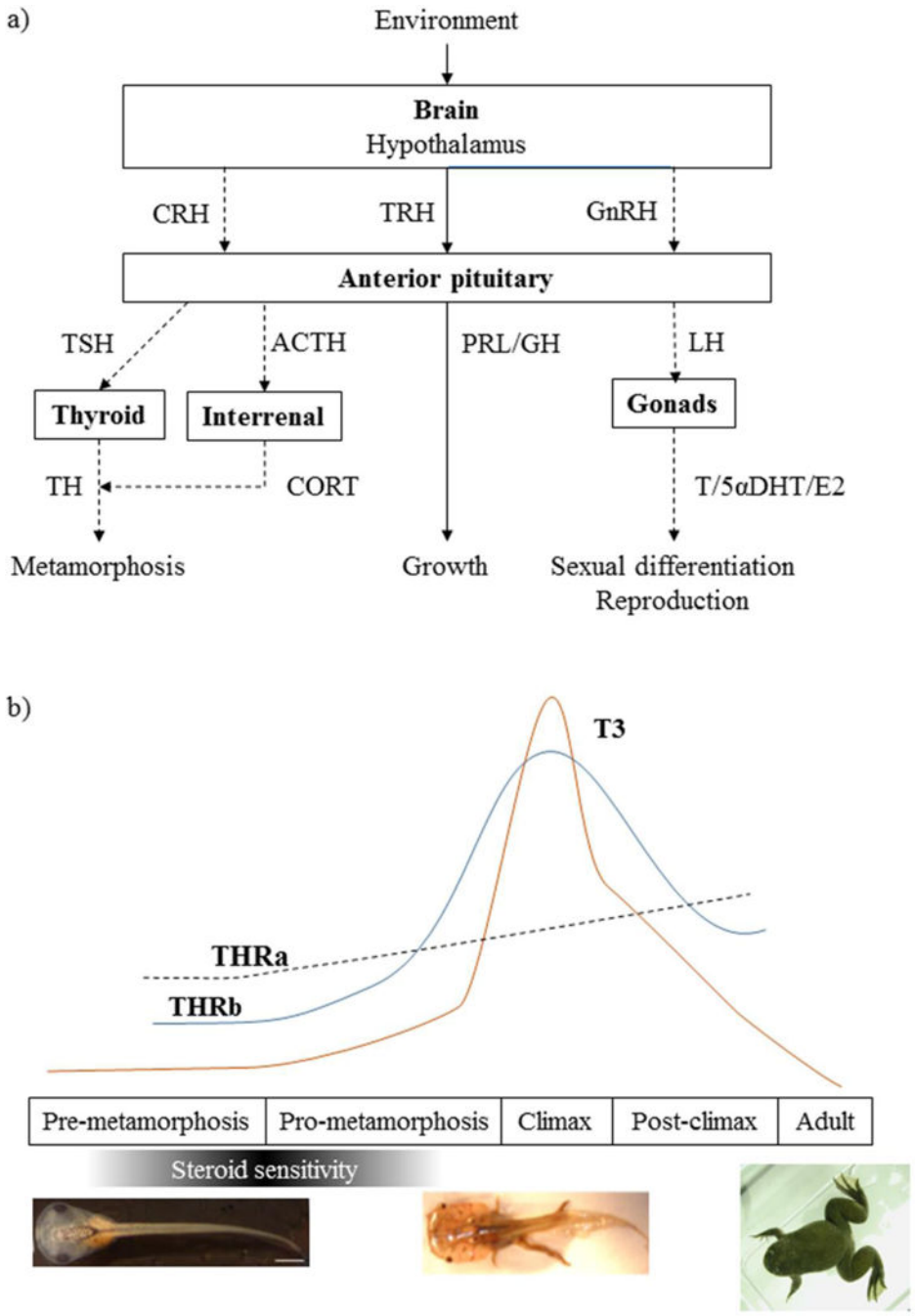

Figure 2.

a) General schematic representation of the hypothalamic-pituitary-thyroid (HPT), hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes. Abbreviations CRH, corticotropin-releasing hormone; TRH, thyrotropin-releasing hormone; GnRH, gonadotropin-releasing hormone; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone; PRL, prolactin; GH, growth hormone; LH, luteinizing hormone; TH, thyroid hormone; CORT, corticosterone; T, testosterone; 5α-DHT, 5α-dihydrotestosterone; E2, estradiol. b) Representation of the expression of thyroid hormone

and its receptors (TRα and TRβ) through late stages of tadpole development. Abbreviations, T3, triiodothyronine; TRα, thyroid receptor alpha; TRβ, thyroid receptor beta.

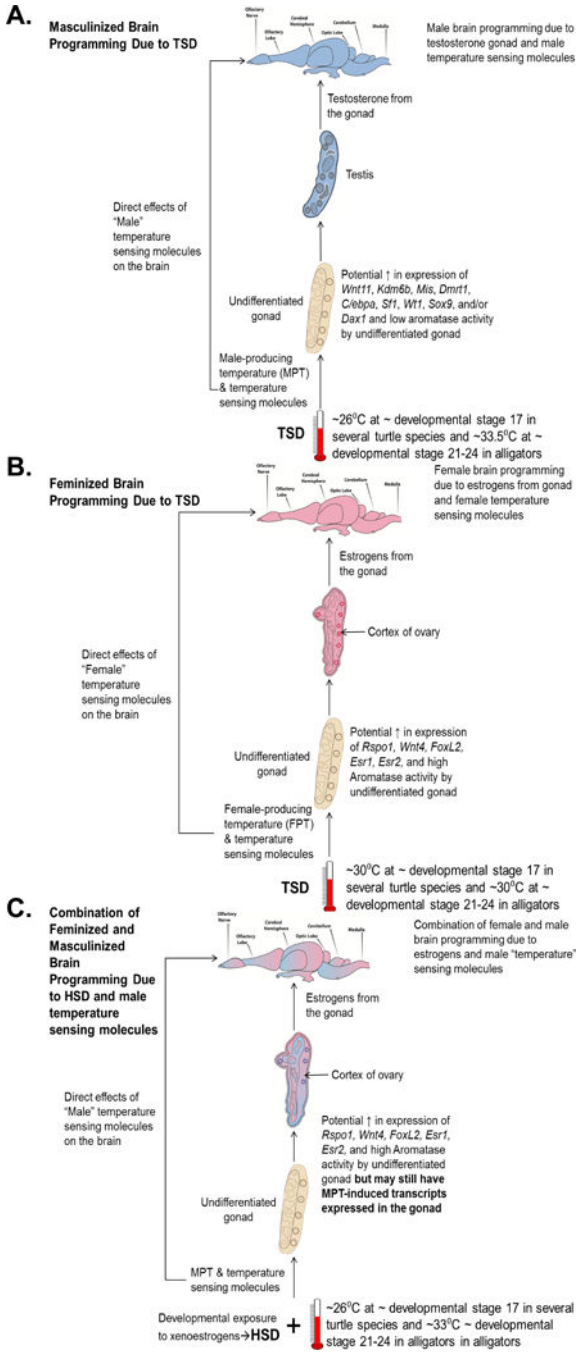

Figure 3.

Neural programming in reptiles due to temperature sex determination (TSD) and/or hormonal sexual determination (HSD). A) Panel A illustrates sexual differentiation and neural programming to the male pathway due to TSD. B) Panel B illustrates sexual differentiation and neural programming to the female pathway due to TSD. C) Panel C illustrates a combination of female and male sexual differentiation and neural programming due to TSD and exogenous estrogenic treatment during a critical window of embryonic development, otherwise considered HSD. In each panel, the color represents known sex

differences in anatomy, gene expression, and receptor density in the turtle brain and is not meant to indicate that the brains are completely different. Gonad and brain colors in the MPTplus xenoestrogen treatment convey a mixture of male and female neuroanatomy, gene expression, and receptor density. In essence, although these individuals may be considered “female”, evidence indicates that these HSD-females are not molecularly or structurally equivalent to those derived due to TSD, and they retain masculine aspects.