Published in final edited form as:

Acta Ethol. 2013 June 1; 16(2): . doi:10.1007/s10211-012-0137-x.

Cryptic confounding compounds: A brief consideration of the influences of anthropogenic contaminants on courtship and mating behavior

Tomica D. Blocker and Alexander G. Ophir

Department of Zoology, Oklahoma State University, Stillwater, OK 74078

Abstract

Contaminants, like pesticides, polychlorinated biphenyls (PCBs), dioxins and metals, are persistent and ubiquitous and are known to threaten the environment. Traditionally, scientists have considered the direct physiological risks that these contaminants pose. However, scientists have just begun to integrate ethology and toxicology to investigate the effects that contaminants have on behavior. This review considers the potential for contaminant effects on mating behavior. Here we assess the growing body of research concerning disruptions in sexual differentiation, courtship, sexual receptivity, arousal, and mating. We discuss the implications of these disruptions on conservation efforts and highlight the importance of recognizing the potential for environmental stressors to affect behavioral experimentation. More specifically, we consider the negative implications for anthropogenic contaminants to affect the immediate behavior of animals, and their potential to have cascading and/or long-term effects on the behavioral ecology and evolution of populations. Overall, we aim to raise awareness of the confounding influence that contaminants can have, and promote caution when interpreting results where the potential for cryptic effects are possible.

Keywords

Contaminants; Courtship; Evolution; Mating; Sexual Behavior; Toxicants

Introduction

As early as the sixteenth century, scientists have been drawing associations between human health and toxicants in their environment. In 1567, Paracelsus wrote one of the first major works on this subject with a focus on the toxicity of metals to miners (Casarett and Doull 1975). Occupational toxicology dominated the next few centuries as the industrial revolution gave rise to increased workplace-related diseases, often caused by metals, smoke and soot. War prompted the creation of organic compounds used as gases, industries profited by developing chemical and synthetic pesticides, and scientists began synthesizing hormones for medical treatments. It was not until the twentieth century that awareness of the effects of these contaminants was broadened from human health to the health of non-human animals and the health of the environment (Casarett and Doull 1975). In the beginning, much of the attention on non-human animals was focused on adverse effects of contaminants on the outward physiology of organisms. More recently, scientists have begun to consider the potential impact such agents could have on behavior. This shift in focus toward sub-lethal contaminant effects on behavior is crucial if we are to understand the anthropogenic

influence on the evolution of species and the cascading effects this can have at the individual, population, community, and ecosystem levels.

Unfortunately, contaminants in the environment are becoming ubiquitous, resulting from farming, industrialization, mining, burning of organics, and the production and use of pharmaceuticals (Casarett and Doull 1975). Sub-lethal effects of contaminants have great potential to shape the evolution of populations, leading to behavioral outcomes that are not readily explained by theory or intuitive observation. A profound body of literature has accumulated, indicating that anthropogenic contaminants disrupt behavior. Existing reviews focus on these contaminants, the mechanisms by which they disrupt general behavior, and the consequences of the developmental and physiological outcomes of exposure to contaminants on behavior (Clotfelter et al. 2004; Zala and Penn 2004; Patisaul and Adewale 2009; Schug et al. 2011; Tuomainen and Candolin 2011; Frye et al. 2012).

The aims of our review are two-fold. First we provide a discussion of anthropogenic pressures and their effects on the various stages of mating behavior. Specifically we review the effects toxicants can have on courtship, sexual behaviors and the act of mating. Finally, we aim to provide an accessible synthesis of this information to those with expertise in animal behavior that may be less familiar with toxicology or the numerous influences that contaminants may have on behavioral ecology. We begin by introducing some broad categories of chemical compounds that we discuss throughout the review, including metals, pesticides, pharmaceuticals, industrial compounds, and by-products. However, our review emphasizes classes of behaviors and provides a broad view of the various contaminants that may influence them, rather than focusing on specific classes of contaminants and the influence each has on behavior. To this end, we discuss the influences of contaminants on several components of mating behavior, beginning with courtship, where we discuss three main sensory modalities (olfactory, auditory, and visual) used to attract mates. This is followed by a discussion of how contaminants disrupt sexual behavior (sexual receptivity and motivation) and briefly discuss their ability to reverse traditional sex-roles. Our discussion then turns to actual mating behavior (ways contaminants disrupt sexual performance and decrease mating). We then briefly shift our focus to how contaminants affect sexual differentiation and development, covering genital malformation and intersex, sex ratio, and multi-generational influences. We conclude with a discussion of how contaminant effects can be conflated with natural variation, how non-lethal exposure to contaminants may affect sexual selection and fitness in the field, and the potential to observe artifacts in the lab. As ethologists, it is imperative that we consider anthropogenic selective pressures and recognize that in some cases human influence may best explain 'natural' behavior. We hope to leave the impression that human influence should be viewed as an extension of ecological factors for other organisms.

Contaminants

A contaminant can vaguely be defined as any substance that is found in the environment at levels above which it would naturally be found. Contamination can take many forms, including radioactive, biological, or chemical. To simplify our discussion, we focus our review on chemical contamination. The broadest categorization of chemical contaminants with the potential to affect behavior is the endocrine disrupting compounds (EDC). EDCs disrupt homeostasis by interfering with the endocrine system, and more specifically with the reproductive system, metabolism and growth (Klassen 2001). Reproductive and sexually selected behaviors are primarily affected by EDCs through their influences on mechanisms closely associated steroidal hormones and their interaction with other systems. Endocrine disruptors are introduced to the environment from many different sources (i.e., sewage effluents, metal smelters, and the breakdown of industrial materials like plastics) (Diamanti-

Kandarakis et al. 2009). Despite the attention EDCs have received, there is still considerable controversy over issues like their potential to affect humans, the doses at which they are toxic, and their persistence in the environment (for more on the controversies see Vandenberg et al. 2009).

Given the general importance of a normal functioning endocrine system on most aspects of behavior, it goes without saying that chemical compounds have the potential to have enormous impact on mating behavior (Clotfelter et al. 2004; Zala and Penn 2004; Patisaul and Adewale 2009; Schug et al. 2011; Tuomainen and Candolin 2011; Frye et al. 2012). Although we spend relatively less effort discussing the specific mechanisms by which contaminants affect behavior, it is important to consider that their effects on animal behavior can be convoluted and synergistic (e.g., Birkhoj et al. 2004; Jarfelt et al. 2005; Christiansen et al. 2009; Jacobsen et al. 2010). For example, some contaminants have both direct and indirect effects (e.g., chlorpyrifos) (see Fleege et al. 2003; Ford et al. 2007). Other contaminants may be metabolized or chemically converted into more dangerous forms of the precursor contaminant (e.g., Agent Orange). As such, the influence of a single contaminant can be much more far-reaching than its immediate effects.

Taken together, EDCs are ubiquitous and represent an enormous overarching group of contaminants that can affect behavior. Indeed few, if any, contaminants have no EDC effects. Therefore, in the current review, we breakdown contaminants based on structural and/or utilitarian function rather than explicitly addressing EDCs as a unique class of contaminants because all the contaminants discussed here have endocrine disrupting features. The major classes of contaminants considered within this review include metals, pesticides, pharmaceuticals, industrial compounds, and by-products. We refer the interested reader to (Clotfelter et al. 2004; Zala and Penn 2004; Woodruff et al. 2008) for tables categorizing contaminants and their mechanisms. The groups of chemical compounds we discuss should be viewed as broad categories, and should interest those studying behavior because they have detrimental effects on organisms both in the lab (Haeghele and Hudson 1977; Fry and Toone 1981; Gray et al. 1989; Howdeshell et al. 1999; Baatrup and Junge 2001; Nash et al. 2004; Sebire et al. 2009; Xu et al. 2010; Hued et al. 2012) and in the field (Guillette et al. 1994; Kirby et al. 2004; Gagne et al. 2006; Eeva et al. 2008; Lye et al. 2008; Hinck et al. 2009; Hallinger et al. 2010). Below we list and briefly discuss some of the major classes of contaminants that we consider here (see table 1).

Metals

Many of the most basic elements are metals - and many of these are used for industrial or manufacturing purposes. Though they are naturally produced, industrial use of metals considerably increases organismal exposure to these potentially harmful contaminants. Metal exposure can occur through inhalation, topical exposure, and ingestion. Metals can be detrimental to nearly every organ in the body (Casarett and Doull 1975). Some heavy metals have even been implicated in autistic-like behavior (Curtis et al. 2010).

Pesticides

Pesticides are chemicals that are specifically manufactured for the purpose of killing. Although they are often designed to target a specific pest or group of pests, they often negatively affect non-target organisms as well (e.g., Grue et al. 1997). Pesticides primarily include insecticides, fungicides, and herbicides.

There are several different classes of insecticides, each with their own mode of action. However, in general, insecticides act as agonists on excitatory mechanisms, which can lead to an overload on target organs and tissue. Organophosphate (OP) insecticides facilitate the

action of the neurotransmitter acetylcholine (ACh) by inhibiting the ability of acetylcholinesterase to degrade ACh, prolonging the excitatory action in the central and peripheral nervous systems. Common OP insecticides include parathion and chlorfenvinphos. Organochlorine (OC) insecticides open sodium ion channels in the brain, causing spontaneous firing of neurons. Common OC insecticides include dichlorodiphenyltrichloroethane (DDT), heptachlor, and methoxychlor (Casarett and Doull 1975).

Fungicides may have several different modes of action. They can be applied to the soil, to particular parts of a plant, or to an entire crop. Fungicides may be used to prevent fungal infection, or to eradicate fungus after development. Common fungicides include captan, vinclozolin, or pentachlorophenol.

Many herbicides are known to inhibit synthesis of amino acids, proteins, or lipids essential to plant life. They can also inhibit electron transfer or photosynthesis. Herbicides can be classified by their time of application: preplanting, preemergent, or postemergent. Common herbicides include Atrazine, diclofop-methyl, and glyphosate (Klassen 2001). Pesticides represent one of the largest groups of contaminants and incorporate diverse modes of action. Some may even have important links to human behavioral disorders (e.g., depression Aldridge et al. 2005). This variability contributes to this group's far-reaching effects that disrupt mating behavior.

Pharmaceuticals

One of the largest and most varied classes of contaminants is the Pharmaceuticals and Personal Care Products (PPCPs). This class encompasses prescription and non-prescription drugs, fragrances, and cosmetics, among others. Organisms are exposed to these contaminants via surface run-off and through sewage effluents. Although many PPCPs are not persistent, they are ubiquitous and their constant influx into the environment results in continual exposure (Daughton and Ternes 1999). For example, the parent compounds and metabolites of prescription and nonprescription drugs, such as contraceptives, antidepressants, anti-inflammatory drugs, and lipid-regulating agents are commonly introduced into coastal ecosystems, primarily via excretion from human users. These pharmaceuticals are then transported in domestic and industrial wastewater discharge and may accumulate in biota living in these niches (Brooks et al. 2005; Chu and Metcalfe 2007; Gibson et al. 2005; Henry and Black 2008; Houtman et al. 2004; Kidd et al. 2007), which could have negative effects on the behavior of aquatic organisms or higher order trophic levels.

Industrial Compounds

This class of contaminants is varied, however, considerable focus has been given to a particular group of contaminants within this larger class because of its ubiquity and toxicity. Polychlorinated Biphenyls (PCBs) are man-made organic compounds that were banned from manufacture in 1979 due to their long-lasting toxic effects. Nevertheless, PCBs persist in the environment and bio-accumulate upwards through trophic levels. PCBs were manufactured for use in many commercial applications and have been used in electrical applications, hydraulic equipment, plasticizers, paints, plastics, rubber production and as an insulating agent, among other things, because of their stable characteristics. PCBs are endocrine disruptors (ATSDR, 2000); they can both agonize and antagonize the action of estrogen and can induce liver microsomal enzymes producing negative effects on thyroid function (Safe 1994).

Perhaps more alarming, are PCBs potential for carcinogenicity. Certain PCB congeners are dioxin-like, while others produce dioxins and dibenzofurans when heated (these contaminants will be discussed below). Dioxin is a general term that describes a group of hundreds of chemicals known to cause cancer. However, dioxins can also interfere with hormonal systems. Among other things, exposure to dioxins has been linked to birth defects, decreased fertility, reduced sperm counts, learning disabilities, and lowered testosterone levels (ATSDR, 1998). Taken together PCBs can impose significant direct and indirect effects on sexual behavior.

By-Products

By-products can include many contaminants, but here we focus on dibenzo-dioxins and dibenzofurans. Polychlorinated dibenzo-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are two families of chemicals with chlorine atoms attached to their base carbon groups. There are multiple types of PCDDs and PCDFs and they are considered the original dioxins. Among the PCDDs is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most studied PCDD and thought to be the most toxic. A common case of TCDD toxicity is Agent Orange used by the U.S. military during the Vietnam War. PCDDs and PCDFs are byproducts of other manufactured chemicals such as chlorinated phenols, chlorinated biphenyl ethers and PCBs. Exposure to these chemicals is most often by ingestion of food containing them. These compounds have been studied most commonly as carcinogenic and mutagenic compounds (ATSDR, 1998). These molecules bind to the aryl hydrocarbon receptor and, upon binding, can cause changes in gene transcription that can lead to disruption in development and differentiation. It is this ability to affect gene transcription that lends to their carcinogenicity and mutagenicity (Hahn et al. 1997).

Other

Other classes of contaminants exist, and these are likely to affect both physiology and behavior. While we review only a subset of the potentially important contaminants that affect behavior, we restrict the current review to behaviors involved in mating and courtship. We note that some studies on the influence of contaminants on behavior focus on other categories of behavior, for example predator prey relationships and foraging behavior (Weis et al. 1999; Boone and Semlitsch 2003; Riddell et al. 2005; Brooks et al. 2009; Pestana et al. 2009).

Components of Mating Behavior Susceptible to Toxicants

Mating behavior refers to a large class of component behaviors. In this sense, the term mating behavior can be broadly considered a group of behaviors that leads to, or that occurs during, sexual reproduction. It is important to note that mating behaviors are controlled by complex mechanisms, some of which have not yet been discovered or described. Further, such behavior is regulated through interactions between the sensory system, neural processing, endocrine modification of behavior and reproductive systems, and motor output.

Here we categorize mating behavior into three categories: courtship, sexual behaviors, and mating *sensu stricto*. Courtship is a major component of mating behavior and can include complex behavioral patterns including chemical signaling, colorful displays, elaborate calls, or intricate dances (Andersson 1994). Sexual behavior and mating often cannot be initiated without the successful completion of such fixed action patterns (Eibl-Eibesfeldt 1970). Thus, courtship-related behaviors can therefore be considered among the most important appetitive behaviors that lead to mating. We use the term mating in the strict sense to refer to acts of intromission or copulatory behavior. For the purposes of our review, we include all behaviors outside of courtship and mating *sensu stricto*, to fall under “sexual behavior.” For

example, in most animals, the consumatory act of mating must be preceded by sexual receptivity and arousal, both of which are heavily regulated by sex hormones (Crews and Moore 1986). Because of their link to hormones, there is enormous potential for environmental contaminants to disrupt courtship, sexual behaviors, and mating.

Courtship and Mate Attraction

Courtship and mate attraction in the animal kingdom represent perhaps one of the most ancient forms of behavior since the evolution of sexual reproduction. The means to bring together animals for the purposes of offspring production have evolved complex forms of communication, but can often be simplified down to a dynamic between sender and receiver. Whereas several sensory modalities have evolved to serve the function of mate attraction, the primary forms of sexual signals typically include olfactory, auditory, and visual displays. Here we discuss some of the ways contaminants influence or disrupt the sender-receiver dyad with respect to these forms of sensory systems.

Olfactory Signal Disruption

Pheromone Production: Pheromones - chemicals secreted by one organism to influence the behavior of another - are useful social cues that can indicate the presence of food or predators, but are best known for their function as sexual attractants (Wyatt 2003). Some chemical agents can disrupt pheromone synthesis. For example, the herbicides diclofop acid and diclofop-methyl decrease pheromone production by inhibiting the pheromone biosynthesis-activating neuropeptide (PBAN), a peptide involved in the synthesis of pheromones in nocturnal moths (Eliyahu et al. 2003).

Pheromone-dependent female choice can be used to determine the extent to which pheromonal production has been disrupted. Female choice is commonly based on their evaluation of secondary sexual characters such as visual or pheromonal signals. The male palmate newt (*Lissotriton helveticus*) is a particularly good species to investigate contaminant effects on secondary sexual characteristics because breeding initiates the development of ornaments like bright coloration, a caudal crest, and webbing of the hind feet. Secondi et al. (2009) used *L. helveticus* to determine the effects of realistic nitrate concentrations on male attractiveness. Exposure to nitrates was found to reduce body weight, hind-foot web area and tail height, all visually detected secondary sexual characteristics important in mate attraction. Secondi et al. (2009) tested female choice for males by giving female newts the option between unexposed and exposed males. Here, females were exposed to water containing olfactory cues, but they were unable to see male conspecifics. Females preferred unexposed males to exposed males. However, this preference was only found in olfactory tests, not in visual tests, indicating that females may use olfaction as their primary mode of mate choice evaluation and that nitrate exposure decreased the olfactory-based sexual attraction of males. This study demonstrates a particularly compelling point: that even though contaminants may have multiple consequences on sexually-selected characters, their functional effects may be specific to less obvious sensory modalities. Ironically, in this case, these differential effects on mate attraction reveal much about the sources of sexual selection, indicating that visual displays in this species of newt may be more attributable to male-male competition, than female choice.

Pheromone Detection: More commonly, chemical contaminants tend to interfere with the perception of pheromonal signals. Female *Corophium volutator* signal sexual receptivity by releasing sex pheromones into water to attract males. Naphthalene is a polycyclic aromatic hydrocarbon found in motor fuel (Madsen et al. 1996). Krang (2007) showed that naphthalene disrupted pheromone-induced mate search in *C. volutator* males. Specifically,

male and female *C. volutator* were exposed to naphthalene-spiked sediment ranging from 0 to 50ug/g. When given the choice in a Y-maze between targets with and without female pheromones, half of the males never left the initial tank. Of the half that left the initial tank, approximately half of the males exposed to two environmentally relevant doses of naphthalene crawled into the arrival tank with no female odor and the other half crawled into the arrival tank with female odor. Krang and Dahstrom (2006) found similar results when *C. volutator* were exposed to the biocide medetomidine, an anti-fouling compound used to prevent barnacle-settlement on boats. Considering that naphthalene accumulates in sediments and can persist for years (Madsen et al. 1996) and medetomidine is commonly used in paints that can leach into marine waters, this disruption of male response to pheromone signals can have long term negative effects on mate attraction and successful reproduction.

Seuront (2011) found that the marine copepod, *Temora longicornis*, experienced decreased mating success after exposure to diesel oil. Marine animals can be exposed to hydrocarbons found in diesel oil from refineries, leaky ships and petroleum transport. This diesel oil can contain polycyclic aromatic hydrocarbons (PAHs), like naphthalene, or monoaromatic hydrocarbons, like toluene (Seuront 2011). Low concentrations of diesel oil in seawater resulted in a decrease in males' ability to detect, follow and successfully capture female conspecifics. Seuront (2011) suggests that this disruption could be attributed to changes in the males' chemosensory system or interactions between female pheromones and the diesel oil, resulting in an inability to recognize the female pheromone, or even a decrease in female pheromone production. Regardless of the mechanism, diesel oil exposure resulted in the disruption of pheromone signaling and subsequently decreased mating success.

Contaminants disrupt olfaction in vertebrates as well, particularly in fishes (Tierney et al. 2010). For example, mature male Atlantic salmon (*Salmo salar*) exposed to biologically relevant levels of cypermethrin, an anti-androgenic pyrethroid insecticide, exhibited a reduced olfactory response after exposure to female pheromones (Moore and Waring 2001). Female parr release a priming pheromone containing prostaglandin F₂ (PGF₂) and this pheromone attracts males. Moore and Waring (2001) investigated the effects of cypermethrin on detection of PGF₂ using an electro-olfactogram (EOG). EOG is used to measure voltage gradients across the olfactory epithelium of fishes. They found that after five days of exposure to cypermethrin, male parr exhibited decreased EOG response after exposure to PGF₂ in comparison to control males. In addition, cypermethrin-exposed males experienced reduced levels of expressible milt, plasma 17,20 -dihydroxy-4-pregnen-3-one, testosterone and 11-ketotestosterone - all of which are involved in male priming response.

The Effects of Evolved Resistance on Pheromone Communication: The diamond back moth (*Plutella xylostella*) is a common agricultural crop pest that has formed resistance to several insecticides (Lingappa et al. 2004). Resistance to the insecticide abamectin is associated with changes in pheromone systems in this species. For instance, to determine differences in pheromone secretion, calling behavior, and mate choice, Xu et al. (2010) compared two strains of *P. xylostella*: abamectin resistant and those susceptible to insecticides. They found that female pheromone secretion and calling behavior was reduced in the moths that were resistant to abamectin. Subsequently, abamectin-resistant females attracted fewer mates indicating a reduction in male attraction to resistant females (Xu et al. 2010). Contaminants, therefore, have the potential to disrupt pheromone production and perception, which in turn can interfere with mate attraction. More broadly, studies such as these provide evidence in support of the hypothesis that anthropogenic contamination shapes the evolution of mate attraction efficiency through at least one modality (sexually selected signals for mate attraction).

Auditory Signals

Vocal signals are among the most common forms of sexually selected signals for mate attraction. Acoustic signaling can be found in every metazoan taxonomic group, and several forms of acoustic signaling have evolved, including stridulation, muscle vibration, and air expulsion (Bradbury and Vehrencamp 1998; Gillooly and Ophir 2010). Of the forms of acoustic communication, bird song is among the most broadly studied, although anuran and insect acoustic communication is also incredibly well understood. For simplicity we briefly discuss some of the influences of contaminants on avian auditory signals. Indeed, birdsong is an integral part of avian courtship, governed by a complex brain system integrating learning, perception and song production. To properly convey an acoustic signal such as song, all of these aspects must work perfectly in concert (DeVoogd and Lauay 2001).

Anthropogenic contaminants have several opportunities to interfere with successful signaling, due to the nature of the mechanisms involved in birdsong and the complexity of this system. Two neural structures are crucial for avian song production. The robustus arcopallialis (RA) and the higher vocal center (HVA) are song control nuclei involved in the learning, production and perception of song (Brenowitz et al. 1997). The size and function of these nuclei are affected by estrogens and testosterone (T). For instance, higher levels of these steroid hormones are correlated with larger song nuclei (Ball et al. 2002; Garamszegi and Eens 2004). It follows that variance in the size of these structures should influence vocal behavior. Hoogsteijn et al. (2008) determined that zebra finches (*Taeniopygia guttata*) that were exposed to the PCB Aroclor 1248 demonstrated developmental deficits in RA volume. Strangely, the HVA was not affected by this treatment.

Similarly, Iwaniuk et al. (2006) found that the insecticide DDT influenced brain development of American robins (*Turdus migratorius*). Environmental DDT exposure to eggs produced a wide range of total DDT in eggs (from 14.95 to 174.67 µg DDT/g weight). As adults high exposures to DDT *in ovo* were associated with smaller RA volume. DDT exposure also tended to negatively affect the HVC size. Moreover, the intercollicularis, a brain structure critical to normal sexual behaviors, was negatively correlated with DDT in both sexes. Because hatchlings were reared in unpolluted conditions, these data indicate that these developmental differences were instigated by the influence of DDT during development *in ovo*.

In addition to PCBs and insecticides, other contaminants such as heavy metals have the potential to influence birdsong. For example, Gorissen et al. (2005) compared the dawn choruses of great tits (*Parus major*) from two sites in Flanders Belgium. One site was near a smelter known to emit lead, copper, cadmium, arsenic and zinc and considered the most extensively heavy metal air-emitting point source in Flanders. A reference site was 4 km east of the smelter, and considered relatively less polluted. Gorissen et al. (2005) found that great tits from the heavily polluted site sang less than those at the less-polluted site. Furthermore, the repertoire size of great tits from the heavily polluted site was considerably reduced. Similarly, Hallinger et al. (2010) found that birds captured at sites polluted with mercury produced atypical songs. The authors observed three oscines, including the Carolina wren (*Thyothorus ludovicianus*), house wren (*Troglodytes aedon*), and song sparrow (*Melospiza melodia*), and one suboscine, the eastern phoebe (*Sayornis phoebe*). Songs were recorded in both contaminated and reference sites. In the oscine species recorded at contaminated sites, the dominant frequency was lower, the songs were shorter, and there was a reduction in note diversity, when compared to birds from reference sites. Interestingly, the songs of the suboscine species did not differ between sites. The authors attributed this difference to the fact that oscine birds exhibit learned song, whereas suboscine birds do not require learning to produce song. These results suggest that mercury detrimentally affects learned song, but not song production.

The specific mechanisms whereby heavy metals such as mercury affect song are unclear. It is possible that energetic stress, which can have similar effects on song phenotype (Spencer et al. 2003), accounted for the differences in song learning. Oxidative stress may also affect song due to its potential to disrupt cell multiplication by interfering with redox signaling (Jones 2006). Elucidating the specific mechanisms that various contaminants assert to create biologically meaningful effects on behavior offers fertile ground for toxicologists and behavioral ecologists to collaborate.

Visual Displays

Dance and posturing are common and general forms of courtship displays. Such courtship behavior is augmented by the outward phenotype (e.g., coloration) of animals. Secondary sexual characteristics are commonly used in visual displays and result as the product of sexual selection (Andersson 1994). Conventionally, the more reproductively limited sex (usually females) carefully selects mates based on some assessment of mate quality. On the other hand, the sex that is able to mate more freely (usually males) competes with one another for access to members of the opposite sex. This can be done either directly through face-to-face interactions, or indirectly through displays of individual quality. In either case, sexual dimorphism often evolves, with males exhibiting elaborate ornamentation, coloration, and weapons used to signal their quality to females or fighting ability to other males.

Visual Displays: Fixed Action Patterns—Perhaps the best-known example of stereotyped courtship displays comes from the three-spined stickleback (*Gasterosteus aculeatus*). Three-spined sticklebacks perform an intricate, yet consistent, mating ritual. The male stakes out his territory, digs a hole in the sand using his nose as a shovel, then gathers algae that he forms into a mound over the hole. Once formed, the nest becomes a breeding ground. Male sticklebacks change to a bluish white color with bright red underbellies to court female conspecifics. Females are led to the nests, where they lay their eggs and the male is then able to fertilize them (Tinbergen 1952).

More than its profound influence on our understanding of sign stimuli and the production of fixed action patterns in animal behavior and ethology, the three-spined stickleback has emerged as a sensitive biomarker for detection of environmental anti-androgens (Katsiadaki et al. 2002). Sticklebacks naturally produce an androgen-dependent protein called spiggin in their kidneys that is used as glue in nest building. Similarly, their nest building and courtship behaviors are controlled by androgens (Borg and Mayer 1995). Administration of ethinyl estradiol (EE2), the synthetic estrogen found in contraceptives, causes changes in aggression that can affect territoriality (Bell 2001). Sebire et al. (2009) found that the organophosphate insecticide fenitrothion (FN) acts as an anti-androgen to alter androgen dependent physiology and behavior in male sticklebacks. Spiggin production decreased with increasing FN exposure. FN treated males built fewer nests than control males and at the highest doses of FN, males completely failed to build nests.

Subsequent courtship behavior is also significantly affected by anti-androgen contaminants. Exposing male sticklebacks to FN reduces biting, zigzagging of the zigzag dance, dorsal pricking, and leading females to the nest (Sebire et al. 2009). Similarly, male sticklebacks exposed to sewage effluent experienced delays in nest building, a cessation of leading behavior, decreased dorsal pricking and reduced zigzagging (Sebire et al. 2011). Taken together, these studies indicate that contaminants disrupt a suite of behaviors and physiological mechanisms that are associated with fundamental, instinct-based, patterns of courtship that lead to successful reproduction under normal circumstances. The dramatic ability of such chemicals to undo stereotyped patterns of evolved behavior indicates the profound reach that anthropogenic compounds may have on natural courtship behavior.

Methylmercury (MeHg) is a highly toxic and biologically available form of mercury, which broadly affects behavior and brain function and can interfere with the proper functioning of sex steroids (Jayasena et al. 2011). Mercury often becomes methylated in aquatic systems and is passed on to aquatic birds via insects and fish. White ibises (*Eudocimus albus*) perform courtship displays during which males head-bob, bow, and engage in aggressive behavior (Rudegeair 1975). MeHg delivered at environmentally relevant levels through diet significantly reduces male-female courtship behavior, and reduces the likelihood that females approach these males (Frederick and Jayasena 2011).

The guppy (*Poecilia reticulata*) is often the subject of studies involving sexual selection and courtship display. Male guppies have bright orange coloration, which attracts females (Pandey 1969). During the juvenile period, the anal fin of a guppy transforms into a gonopodium, a sexually selected copulatory organ. Males display both their orange coloration and the gonopodium by performing two distinct courtship displays: sigmoid displays and gonopodial thrusts. Females typically evaluate males based on the intensity of orange displayed in a sigmoid display, in which males orient their bodies in either a 'C' or 'S' shape while vibrating and swimming sideways. When females indicate their receptivity to a male's sigmoid display, males display their gonopodium in a thrusting motion (Baerends et al. 1955). This thrusting behavior usually leads to mating.

Like the stickleback, the guppy has been gaining increasing popularity as a model organism to study contaminant effects on behavior. The fungicide vinclozolin and the DDT metabolite dichlorodiphenyldichloroethylene (DDE) have been shown to have anti-androgenic properties that disrupt courtship displays and reproductive physiology of the male guppy (Baatrup and Junge 2001; Bayley et al. 2002). Vinclozolin, but not DDE, reduces the frequency of sigmoid displays by male guppies. However, if exposure to contaminants stopped after sexual maturity, sigmoid display behavior returned to normal; gonopodium length remains small compared to controls (Bayley et al. 2002). On the other hand, the pro-estrogen insecticide chlorpyrifos reduces gonopodial thrusts (De Silva and Samayawardhena 2005). Taken together, these studies suggest that anti-androgen or pro-estrogen contaminants produce detrimental effects on organizational aspects of courtship display.

Visual Displays: Ornaments—In addition to display, contaminants can have profound effects on ornamentation. Although brief treatment with DDE and vinclozolin does not alter the intensity of orange coloration in male guppies, the intensity of blue is significantly altered resulting in discolored males. However, longer exposure to these compounds produces changes in both blue and orange coloration, as well as decreased testes weight, and decreased courtship behavior (Baatrup and Junge (2001). Further, using the synthetic estrogen EE2 produced the same organizational effects on outward phenotype (Kristensen et al. 2005), suggesting that the feminizing effects on sexual differentiation can be achieved by contaminants that either disrupt androgens or mimic estrogens.

Coloration can also be influenced by cholinesterase inhibitors. The amarillo fish (*Girardinichthys multiradiatus*) uses its yellow fin color and ultraviolet color patterns during breeding to attract females (Burt de Perera & Macias Garcia 2003). Males with decreased ornament size and yellow coloration are less attractive to females. Male amarillo fish exposed postnatally to realistic levels of the insecticide methyl-parathion grew smaller than control males, developed smaller ornaments, and exhibited decreased yellow coloration of the fins, (Arellano-Aguilar & Macias 2008). Moreover, amarillo fish exposed to a cholinesterase inhibitor exhibited subdued courtship behavior (Arellano-Aguilar and Garcia 2008), indicating that the decrease in attraction associated with coloration and ornament size, may also be attributed to reduction in courtship displays.

Although it is much more common for contaminants to work counter to sexually selected sexual dimorphism, some cases exist in which they actually enhance these adaptations. Deposition of pigments, like melanin and carotenoids, play a major role in the intensity and size of coloration in birds. The expression of such traits can be indicative of quality because of their potential to be costly (e.g., Zahavi and Zahavi 1997). Great tits possess a color pattern containing carotenoid-dependent yellow breast feathers split by a melanin-dependent black stripe. Great tits inhabiting a site near a metal smelter emitting lead, cadmium, zinc and copper, exhibited lower carotenoid chroma and hue of yellow breast feathers in comparison to those in the site farthest from the smelter (Dauwe and Eens 2008). Oddly, the melanin-dependent black stripe in great tits nearest to the smelter was larger than those found on birds in the less contaminated site. Females use the size of the black stripe to select mates and lay larger clutches with males that display them (Norris 1990). Similarly, red-legged partridges exposed to low levels of the herbicide diquat displayed larger melanic bibs, but exhibited paler carotenoid-based bills and eye rings, all sexually selected traits (Alonso-Alvarez and Galvan 2011; Alonso-Alvarez et al. 2012; Galvan and Alonso-Alvarez 2009). These studies highlight the important point that contaminants in some cases may have positive effects on the expression of traits that are important to mate attraction. In both instances, contaminants appear to decrease the color intensity of one trait, while another key sexually selected characteristic was enhanced, which might produce a net increase in sexual attraction and ultimately reproductive fitness. The influence of contaminants on lifetime reproductive success, however, must be considered in terms of both their positive (i.e., increased potential for mate attraction) and negative (i.e., lifespan reducing oxidative stress or other consequences that can affect survival) influences. This requires an exhaustive consideration of behavioral and physiological effects when estimating lifetime fitness consequences.

Visual Displays: Other Traits—Although cues and signals used for mate choice are not ubiquitously sexually dimorphic, many species do display such dimorphism. Further sexual dimorphism can be seen in species where sexual selection is more typical (i.e., males compete for access to choosy females) or in less common ‘sex role reversed’ species. The Gulf pipefish (*Syngnathus scovelli*) is a polyandrous fish that is naturally sex-role reversed (Berglund et al. 1986). Because males are a limited resource, there is strong intra-sexual selection on females rather than males. Adult females are larger than males and possess a deeply keeled abdomen, a large dorsal fin, silvery-blue lateral stripes, and breeding coloration. It is thought that these secondary sexual characteristics are related to estrogens (Jones and Avise 1997; Jones et al. 2001). Partridge et al. (2010) found that male pipefish exposed to a synthetic estrogen experienced feminization. Males were exposed to environmentally relevant doses of EE2; body morphology, mating behavior and mating success were assessed. Exposed males exhibited increased body size and the iridescent lateral stripes typically found in females. Preference tests showed that EE2 did not affect male evaluation of females, but females did discriminate against “feminized” males, which decreased male reproductive success. This disruption of sex specific courtship displays can be detrimental to mate choice and consequently fitness. Further, it is important to note that some hormone disruption effects are consistent even in sex role reversed species.

Sexual Behavior

Proceptive and receptive behaviors—In rats, proceptive behavior, including ear wiggling, hops, and darts, ultimately leads to behaviors that facilitate mating, like lordosis and mounting. Motivation and sexual performance are modulated somewhat independently in rats, but sex steroid hormones largely govern both classes of behaviors (Barfield and Chen 1977; Edwards and Pfeifle 1983; Erskine 1989; Ogawa et al. 1994; Pleim et al. 1989). For instance, female proceptive behavior is induced by estradiol (E2) release, which, once

bound to estrogen receptors (i.e., estrogen receptor alpha), acts as a transcription factor to induce the synthesis of progesterone receptors in the brain (Truss and Beato 1993; Tsai and Omalley 1994). Proceptive behavior is primarily driven by the progressive increase in progesterone receptor binding events. E2 also induces lordosis, but in a more direct fashion; cannulation of E2 to the ventromedial hypothalamus is sufficient to induce lordosis in ovariectomized female rats (Rubin and Barfield 1983).

Bisphenol A (BPA), used in the manufacturing of plastics, is a contaminant with estrogenic properties (Dodds and Lawson 1936; Kuiper et al. 1998). Monje et al. (2009) found that BPA exposure at safe levels (i.e., levels within the range deemed acceptable by the U.S. Environmental Protection Agency, EPA) causes changes in proceptive behaviors exhibited by female rats. When females that were exposed to BPA as neonates were placed in a test arena with sexually experienced males, they exhibited no differences in sexually receptive behavior (i.e., lordosis), but demonstrated significantly decreased proceptive behavior. When treated as neonates, adult rats experienced decreased estrogen receptor alpha (ER α) expression in the medial preoptic area and the ventrolateral part of the ventromedial nucleus, two hypothalamic regions involved in mediating female sexual behavior (Hoshina et al. 1994; Musatov et al. 2006). Such ontological events are later expressed as changes in receptor expression that can be detrimental to proceptive behaviors in adulthood.

Many other contaminants with estrogenic properties can disrupt proceptive behavior due to their developmental effects. Fenvalerate is a pyrethroid insecticide that alters sexual behavior in female rats exposed during development. Prenatal and neonatal fenvalerate exposure delays sexual maturation (vaginal opening) and decreases lordosis, but does not affect estrous cycles or hormone levels (Moniz et al. 2005). Lordosis amplitude, but not lordosis-to-mount ratios (female lordosis: male mount), are decreased and rejection behavior is increased if females are exposed to methoxychlor neonatally (Bertolasio et al. 2011). Contaminant effects on receptive behaviors are not limited to females. For example, male Japanese quail (*Coturnix japonica*) exposed to DDE *in ovo* attempted to mount females less and require more time to achieve successful copulation when exposed to sexually receptive females as adults compared to control males (Quinn et al. 2008).

Estrogenic contaminants can also have activational effects. After delivery of single injections of the estrogenic insecticide chlordecone, females that have been exposed to a sexually motivated male demonstrate lower lordosis-to-mount ratios compared to controls, indicating decreased receptivity (Uphouse 1986).

Sexual motivation—Methoxychlor, an organochlorine insecticide with estrogenic properties, decreases sexual motivation. Male mice exposed to methoxychlor *in utero* spend equivalent time near cages containing females as they do near empty cages (Eroschenko et al. 2002). Not surprisingly, this lack of sexual motivation is correlated with decreased testosterone levels; rodent sexual motivation is modulated by testosterone, whereas sexual performance is associated with its metabolites, E2 and dihydrotestosterone (DHT) (see *Sexual Performance* below). Similarly, Japanese quail exposed to methoxychlor *in ovo* at environmentally relevant levels display either longer latency to mount or a complete lack of attempted copulation as adults (Ottinger et al. 2005). Like mice, these effects are likely due to decreased testosterone levels caused by early exposure to an estrogenic compound.

Sex role reversal—Sexual roles drive attraction, sexual motivation, and ultimately mating. Reversal of these roles can profoundly affect successful mating. Octylphenol is a component of rubber, pesticides and paints, which appears to masculinize female behavior. Female daughters from breeding pairs that have been exposed to octylphenol in low doses in food, exhibit longer than normal estrous phases and prefer to spend more time with females

over males (Pocock et al. 2002). On the other hand, sons demonstrate shorter mount and intromission latencies but these were not followed by increased sexual activity. Similarly, female rats become hypersexualized if they have been exposed to 5mg/kg of the organochlorine insecticide chlordane *in utero* (Laessig et al. 2007). At this dose, which is 25 times less than the LD₅₀ dose, females significantly increase lordosis and male mounting behavior. On the other hand, male siblings exhibit female-typical behavior, frequently adopting lordosis posture. Furthermore, methylmercury exposure increases homosexual pairing between male white ibises (Frederick and Jayasena 2011). Homosexual males are less aggressive and less likely to be approached by females, but more likely to be approached by males.

Estrogens during early development of rats are known to cause organizational effects that masculinize the structure and function of the brain (Arnold and Breedlove 1985; Kawata 1985; Wu et al. 2009). Chlordane is known to compete with estrogens for binding sites of estrogen receptors alpha and beta (Kuiper et al. 1998) and perinatal exposure may cause organizational changes that cause outcomes such as those reported by Laessig et al. (2007). Homosexuality among male ibises is correlated to changes in estradiol and testosterone in dosed males (Jayasena et al. 2011). Taken together, several contaminants that interfere with normal sex steroid functioning, often acting as agonists, can induce significant developmental changes that reverse traditional sex roles in terms of sexual motivation and preferences.

Mating

Sexual Performance—The heavy metal cadmium causes sexual dysfunction in male rats. Cadmium induces erectile dysfunction in a dose-dependent manner, and cadmium exposure reduces mounting, intromission and ejaculation (Clark et al. 1994). Although, high levels of cadmium are associated with low levels of circulating testosterone (Clark et al. 1994), the influence that cadmium asserts on sexual behavior is probably indirect. T acts as a precursor to both DHT and E2, and reduced availability of T results in reduced production of these sex steroids. DHT, not T, is typically associated with the male erectile reflex, and E2 is necessary for successful sexual performance, and modulates behaviors including mounting and copulation (McDonald et al. 1970; Baum and Vreeburg 1973; Gray et al. 1980; Michael et al. 1990; Meisel and Sachs 1994). Taken together, it is plausible that cadmium's influence on sexual performance is less attributable to the reduced T it causes, and more attributable to the reduced potential to produce T metabolites.

Some contaminants can have long acting effects on sexual performance. Apomorphine is a dopamine agonist, which induces an erection when administered to healthy mature male rats (Heaton and Varrin 1994). Flutamide (an androgen receptor antagonist) decreases apomorphine-induced erections for 48 hours. In comparison, the DDT metabolite p,p-DDE decreases apomorphine-induced erections for 2 weeks (Brien et al. 2000). Therefore, contaminants may have immediate or long lasting effects on sexual performance, which may be related to direct or indirect disruption of sex steroids.

Decreased mating—*Rabidosa rabida*, the wolf spider, is a natural predator of crop insects and thus important in insect control. Although the insecticide malathion is not intended to target the wolf spider, it does have important fitness-reducing effects in this species. Adult wolf spiders exposed to sublethal doses of malathion reduce locomotion, pheromone response (due to reduced locomotion), courtship behavior, and mating (Tietjen 2006). Exposed males are slow to initiate courtship behavior, and they continue courting females even if females exhibit readiness to mate. Unfortunately for males, the outcome is often premature cannibalization of males without mating. Ironically, in its attempt to reduce pests

that affect crops, malathion reduces the natural predators of the pests it is designed to eliminate.

Several other examples of contaminants reducing individual fitness as a result of disrupting mating behavior exist. For instance, spawning is reduced in fathead minnows (*Pimephales promelas*) that have consumed environmentally relevant levels of methylmercuric chloride-contaminated food (Sandheinrich and Miller 2006), although this could be due to methylmercury-induced hypoactivity since mercury treated minnows are relatively inactive. *Monoporeia affinis* is a productive bottom feeding amphipod that oxygenates the sediment (Aller 1988) and serves as a food source for fish (Arrhenius and Hansson 1993). The fungicide fenarimol, a known endocrine disruptor, decreases male mating ability in *Monoporeia affinis* (Jacobson and Sundelin 2006). Strangely, this change in mating ability is not related to ecdysteroid levels, a hormone that regulates reproduction and molting. Although the insecticide chlorfenvinphos is used to protect crops from the cabbage root fly, *Delia radicum*, it can significantly disrupt the natural mating behavior of non-target organisms. For example, at the concentration recommended for crop use, chlorfenvinphos irreversibly decreased mating in the wasp *Trybliographa rapae*, no matter which sex (male, female, or both) was exposed (Alix et al. 2001). The authors attributed this decrease in mating to disruptions in pheromone production and response.

Finally, it is worth noting that pesticides have off-target effects on mating behavior beyond insects and invertebrates; they can also affect vertebrate mating success. Roundup is a commonly used herbicide that contains the surfactant glyphosate (Cox 2004). Whether exposed acutely or chronically, doses of Roundup at recommended-use levels reduced the frequency of sexual contact and mating success of the male livebearing fish, *Jenynsia multidentata*, presented with sexually receptive females (Hued et al. 2012). Moreover, heptachlor disrupts estrus cycle patterns and increases the latency to mate in female rats chronically (i.e., 18 d) injected subcutaneously with this organochlorine insecticide (Oduma et al. 1995). The focus on effects of insecticides on invertebrate mating behavior does not reflect the far-reaching influences that these chemicals have on the natural mating behavior of other animals.

In sum, regardless of the mechanisms by which methylmercury, fenarimol, glyphosate or organochlorines such as heptachlor operate, the fitness of several animals, vertebrate and invertebrate alike, is reduced to zero or near zero simply due to conventionally used levels of these contaminants. Furthermore, considering the important role that some basal organisms such as amphipods serve (as oxygenators and food sources), decreased mating due to mild contaminant exposure can have cascading effects that reach across trophic levels. Such outcomes could result in ecosystem-level shifts that have much broader and more observable consequences on the behavior of species living within codependent niches within the larger system.

Sexual Differentiation

The developmental trajectory on which an animal is placed can have life-long effects on its courtship behavior, sexually receptive behavior, and ultimately its mating behavior. It is well known that there are several factors that assert organizational effects during development. These factors include endocrine, genetic, and epigenetic influences (Arnold and Breedlove 1985; Devlin and Nagahama 2002; Kawata 1985; Reik and Walter 2001; Wilhelm et al. 2007). Each of these factors is open to disruption or interference from environmental toxicants (e.g., Guillette et al. 1995). Because the sexual differentiation of an individual may set the stage for life-long function (or dysfunction) in behaviors that lead to and include

mating, we will briefly consider some of what is known about the influence of toxicants on sexual differentiation in the context of mating behavior.

Sexual development

Sexual Differentiation—Administration of exogenous sex steroids during development is well known to cause abnormal sexual differentiation. Perhaps the best example of an exogenous chemical influencing sexual differentiation comes from the administration of diethylstilbestrol (DES). In the 1940's, physicians began prescribing DES to pregnant women in an effort to reduce spontaneous abortions. Rather than preventing abortions, this synthetic estrogen caused reproductive dysfunction in the offspring of exposed women. Daughters of treated women experienced vaginal tumors, and both daughters and sons experienced genital dysplasia (Colborn et al. 1993). Similar organizational effects on sexual differentiation resulting from environmental toxicants have been observed in non-humans. Perhaps the most well known example of this comes from the controversial insecticide Atrazine, which causes feminization in males. For instance, Hayes et al. (2002; 2003; 2004; 2010; 2011) exposed male African clawed frogs (*Xenopus laevis*) to low, biologically relevant, levels of Atrazine beginning during development and throughout adulthood. The result is that male genitalia become highly feminized, testosterone levels are decreased, and the larynx becomes demasculinized. This demasculinization of the larynx, along with the other effects is associated with a decline in testosterone and with a subsequent inability to compete with other unexposed males for mates. These demasculinizing effects have been seen across vertebrates (Hayes et al. 2011).

Genital Malformations—Genital deformities are one of the most common forms of sexual differentiation disruption from toxicants. For example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), administered during gestation, causes genital malformation (a lack of complete vaginal opening) in developing female rats (Gray and Ostby 1995). Further, TCDD treatment produces a urethral orifice, separate from the vaginal orifice, and increased presence of cleft phallus and mild hypospadias. Although TCDD causes females to experience constant estrous, mating is inhibited because the vaginal opening is impeded by a membrane or thread (Gray and Ostby 1995). Dioxins like TCDD are among the most dangerous contaminants to both humans and non-human animals. They are highly toxic, and accumulate up trophic systems. Because these contaminants are ubiquitous and persistent, their effects can be long lasting.

Intersex—Intersex commonly results from contaminants because of the roles they often play as endocrine disruptors. Guillette et al. (1994) compared two lakes; one lake was contaminated with several contaminants including the estrogenic insecticide DDT, the other lake was comparably less contaminated. Alligator eggs were collected from 25 nests at each site and incubated at a temperature that was ideal for production of a 1:1 sex ratio. Hatchlings were allowed to grow for 6 months, at which time plasma hormone levels were determined and morphology of the gonadal tissue assessed. Female alligators from the contaminated site had higher plasma E2 levels than those from the reference site. Male alligators from the contaminated site had plasma testosterone levels that were nearly four times lower than those from the reference site. In fact, the testosterone levels of males from the contaminated site were similar to those of females. It was evident after gonadal assessment that two of the animals previously identified as females (because of their lack of a penis) were gonadally males, while two of the animals with penis-like structures had ovarian tissue and were actually females. While the alligator population decline that began in the 1970's was thought to be a result of decreased clutch viability (Woodward et al. 1993), Guillette and his colleagues have convincingly demonstrated that intersex in

alligators resulting from contamination significantly contributes to these population declines.

Since this classic series of studies conducted by Guillette and colleagues, increasing numbers of examples of contaminant-induced intersex have been described. For example, in a five-year study, Kirby et al. (2004) found consistent prevalence of intersex in male flounder (*Platichthys flesus*) found in estuaries throughout the United Kingdom. Similar results have been reported for smallmouth bass (*Micropterus dolomieu*) from the Potomac River (Blazer et al. 2007), and for black bass (*Micropterus spp.*) throughout the southeastern U.S. (Hinck et al. 2009). McCoy et al. (2008) found a direct relationship between agricultural areas and intersex, indicating that contaminants associated with agriculture, such as pesticides, may be the prime culprit for such intersex effects. As we have discussed, many of these compounds have estrogen-like or anti-androgen effects, suggesting that many of these malformations may be the result of unintended interactions with sex steroid function. This is supported by the observation that lifelong exposure to biologically relevant levels of EE2 resulted in complete population failure in zebrafish (*Danio rerio*), attributed to disturbed sexual differentiation in males; exposed males exhibited a lack of testes, or intersex gonads (Nash et al. 2004). Though we cannot discuss all the examples, there is considerable evidence that intersex is ubiquitous and often directly correlated with contamination.

Other Important Considerations of Contaminants on Successful Sexual Behavior

Male to Female Ratio—The ratio of males to females (M:F) is important to mate availability and selection (Emlen and Oring 1977). Beyond disrupting the ability to acquire a mate through attraction, contaminants with the ability to skew the sex ratio can reduce available mating opportunities. Many contaminants have the capability to skew sex ratios in organisms (e.g., Deng et al. 2010; Flaherty and Dodson 2005; Fort et al. 2004; Hogan et al. 2008; Huang et al. 2006; Ishihara et al. 2007; Lopes et al. 2009; Sanchez et al. 2011). For example, male juvenile guppies exposed to environmentally relevant sublethal concentrations of vinclozolin and DDE until they reached sexual maturity, showed both organizational and activational deficits in sex-specific phenotype (Bayley et al. 2002). Exposure to the highest doses of both contaminants produces a skew in the sex ratios toward females; in other words the population becomes feminized. At a variety of concentrations of DDE and vinclozolin, the development of male secondary sexual characteristics is retarded and ultimately males exhibit shorter gonopodium (Bayley et al. 2002).

Other contaminants have similar female biasing effects on sex ratio, including the herbicide Atrazine in *Xenopus laevis* (Oka et al. 2008). Likewise, the synthetic estrogen EE2 dramatically skews the sex ratio of fathead minnows (*Pimephales promelas*) to a female bias (Lange et al. 2001). A constant exposure of EE2, at nominal concentrations, through development and adulthood results in sex ratios skewed as high as 5:84 (M:F), where the remaining percentage of exposed fish had testes containing ovarian tissue. Operational sex ratio (OSR) and theories addressing reproductive skew have been well developed (Cant 1998; Emlen and Oring 1977; Johnstone and Cant 1999b, a; Keller and Reeve 1994; Kokko and Johnstone 1999; Vehrencamp 1979) and despite their differences, all agree that altering the sex ratio will have cascading effects on the resulting behavior of both male-male competition and female choice. Indeed OSR is a major driving force behind the natural mating decisions and tactics that individual animals adopt and which contribute to the larger mating system observed at the population level (Shuster and Wade 2003). If low levels of contaminants can influence the OSR, then the potential to have broad effects on life history-based decisions is also possible.

Transgenerational Influences of Contaminants—More recently, considerable attention has been given to the effects that early experience can have on an organism, and more specifically how contaminant effects can pass from parent to offspring (Anway and Skinner 2008; Csoka and Szyf 2009; Zama and Uzumcu 2010). We are only now beginning to understand the full scope of the profound impact epigenetic effects have on behavior (Crews 2010). This field is growing rapidly, but some of its earliest studies concerned transgenerational effects on sex ratio. For example, female rats exposed to the dioxin TCDD from sexual maturity throughout pregnancy produce more granddaughters than grandsons, despite the fact that the direct offspring of these females were not exposed to TCDD directly (Ikeda et al. 2005). Other studies demonstrated transgenerational effects on mate choice. Crews et al. (2007) showed that females three generations removed from exposure to vinclozolin preferred unexposed males over males who had similar histories of exposure. Though many of the early studies originally focused on maternal transmission to offspring, there is ample evidence of paternal transmission of epigenetic effects as well (Cordier 2008). Considering the ability of many contaminants to cross the placenta to be absorbed by offspring and the ability of contaminants to modify gene expression of offspring, contaminants probably have more far reaching consequences than previously appreciated.

Concluding Remarks

Rachel Carson's fears that one day, the voices of birds would no longer be heard because of pesticide abuse by humans is only fifty years past. Her book, *Silent Spring* (1962), served to launch the "environmental movement." Since then, government agencies have been created worldwide, charged with maintaining the safety of humans and the environment. Contaminant use is regulated, concentrations in the environment are screened, and scientists are constantly investigating contaminant effects on human and non-human life. It is a pressing issue to understand how human activity affects other organisms and tilts the delicate balance of ecosystem interdependences. For instance, above we discussed how anthropogenic stressors such as naphthalene (Krang 2007) or fenarimol (Jacobson and Sundelin 2006) can directly effect survival or mating success of amphipods. Decreasing the population of such basal organisms can cause cascading effects on the animals relying on such animals for oxygenation of the soil and for food. While the direct effect may be obvious, cascading effects on higher trophic levels can be overlooked (Clotfelter et al. 2004; Clements and Rohr 2009; Tuomainen and Candolin 2011). These effects can be overshadowed by the complexity of ecosystems and by synergistic interactions between multiple contaminants and between contaminants and natural stressors (e.g., Kuiken et al. 1994; Boone and Semlitsch 2003; LaFiandra et al. 2008; Brooks et al. 2009; Pestana et al. 2009). Adding animal behavior as a factor to understanding ecosystem interactions significantly complicates the challenge of managing conservation efforts, but it is one for which we must account if we are to fully appreciate the scope of the detrimental effects of anthropogenic stressors.

Conflating Individual Behavioral Variation and Contaminant Effects

We have reviewed some of the many ways that contaminants might affect sexual development, courtship, sexual behaviors and mating. Not surprisingly, this is but a fraction of the ways in which contaminants disrupt natural behavior. Indeed, contaminants affect behavior in many other domains, including predator/prey (e.g., Boone and Semlitsch 2003; Brooks et al. 2009; Pestana et al. 2009; Riddell et al. 2005; Weis et al. 1999), non-courtship communication (Gorissen et al. 2005; Venerosi et al. 2006), learning and memory (Schantz et al. 2001; Fischer et al. 2008), and social behaviors (Schantz et al. 1992; Aldridge et al. 2005; Engell et al. 2006; Palanza et al. 2002; Wisniewski et al. 2005). As such, the influence of these factors is broad and should be considered by anyone interested in the study of

animal behavior or behavioral ecology. It should be clear that contaminants of almost any type appear to affect behavior, and all have the potential to compromise data and mislead interpretations if their effects were not considered. We have provided several examples above demonstrating that low (ecologically 'safe') levels of an array of contaminants can influence natural behavior in the field leading to and including mating, which may otherwise be interpreted as variation.

Some of the examples discussed above highlight that signals, or the reception of signals, are highly susceptible to several forms of human activity. Animal communication is among the largest sub-disciplines within the study of animal behavior. Characterization of signal types and reception of signals most often occurs in the field. The degree to which study sites are touched by the various forms of human activity, which may include commonly used pesticides, fertilizers, or fuel discharge, could prove to be more important than previously appreciated.

As behavioral ecologists, we often attribute different dialects within species or regional differences in neural phenotype with the evolution of song (e.g., Nelson et al. 2001). However the results we discuss above indicate that behavioral ecologists should be wary of the influence of contaminants on courtship behavior when drawing conclusions such as these. Contaminants such as PCBs (like Aroclor 1248), organochlorine insecticides (like DDT), and heavy metals (like mercury) are ubiquitous and persistent in nature (Klassen 2001). The effects that these contaminants can assert on neural development could have a significant and lasting impact on the ability of wild animals to produce stereotypical species-specific courtship signals (recall Gorissen et al. 2005; Hallinger et al. 2010; Iwaniuk et al. 2006). The influence of contaminants on courtship is not trivial. However, because the extent to which contaminants are present may vary by site, contaminants themselves could account for a significant portion of the 'natural' variation that has been attributed to population differences reported in the literature. Indeed, great care should be taken when choosing study sites. Comparative studies or studies of individual or geographic variation are particularly susceptible to biases rooted in anthropogenic contaminants, and scientists with these interests may be well served to screen for such confounding effects as conclusions are drawn about sexually selected differences between or within groups.

Fitness Reducing Effects of Contaminant via Sexually Selected Behaviors

Disruption of the naturally selected form of communication can have long-term effects on mating and fitness, and could lead to species extinction through missed signals or potential unexpected hybridizing events through poor signal discrimination (Bradbury and Vehrencamp 1998). When mate preferences break down, as in the cases discussed in fish ornamentation and display, the strength of sexual selection is thrown off balance, which can have unexpected and often maladaptive consequences. The example of diamond back moths above provides one such example of maladaptive selection. Not only might the effects of contaminants on courtship signals mislead interpretations of the basic form and function of such signals, but they may also assert a strong influence on the continued evolution of these signals. Awareness of and vigilance for these potential influences is crucial if behavioral ecologists are to truly understand the study systems with which they work. On the other hand, understanding that such effects may lead to maladaptive behavior provides an alternative mechanistic explanation of confusing data when theory fails to provide a clear account.

Laboratory Artifacts

Scientists performing behavioral research in the laboratory should be equally attentive to the potential for environmental stressors to affect animals used in laboratory studies (Clotfelter

et al. 2004). For example, consider that the majority of laboratory rodents are housed in polycarbonate cages. Polycarbonate plastic contains BPA. We have discussed the estrogenic-like effects that BPA can assert on behavior and sexual development throughout this review. Housing animals in such conditions significantly increases the potential for exposure to this compound, which – as we have discussed above – has the potential to modify sexual behavior substantially. Furthermore, it is common practice to supplement laboratory animals (particularly exotic species) with non-commercial diets or provide environmental enrichment to the animals. However, many of these materials could have been exposed to pesticides, metals, PCBs and dioxins, among other things. A careful balance between providing environmental enrichment (a crucial factor for lab-reared animals) and the potential exposure to the swath of contaminants that could interfere with natural behavior must be considered. Some contaminants, like DES or vinclozolin, have genetic and epigenetic effects (Colborn et al. 1993; Anway et al. 2008). Thus, laboratory animals have the potential to develop behavioral dysfunctions even without direct exposure. Ultimately, it is important that ethologists are fully aware of the contaminants to which their experimental animals are exposed, and how they might affect behavior. An acknowledgment of such effects places behavioral research in a stronger position to draw conclusions when assessing empirical results. The potential for contaminants to influence behavior in the field or lab, further emphasizes the need for comparative work in which lab studies are field verified, and field studies are tested under controlled laboratory conditions (Beach 1950; Wolff 2003; Zala and Penn 2004).

Viewing Contaminants as a Cryptic Selection Pressure

Obviously mating is crucial for reproductive success. One of the basic tenets of the theory of natural selection is that animals that leave more offspring will shape the reproductive pool in subsequent generations. In the middle of the last century, the discovery of industrial melanism taught us that human activities can profoundly impact the process of natural selection (Kettlewell 1955; Tinbergen 1958). In doing so we learned a great deal about the mechanisms of evolution. Contaminants have the potential to serve in the same way; to both influence the trajectory of evolution while teaching us about the mechanisms that contribute to this process. Whether in the lab or in the wild, animals exposed to environmental stressors are experiencing unnatural pressures that can shape the evolutionary trajectory of a population or limit the ability to relate conclusions from laboratory experiments to evolutionary processes (see above for specific examples and discussion of their implications). When certain breeding pools are exposed over time to such pressures, those populations are likely to (naturally or artificially) evolve differently than others that are unexposed. While most environmental contaminants should act indiscriminately on natural selection, the influence of this class of artificial selective pressures can have profound effects on the composition of the gene pool in similar ways as genetic drift, mutation, and catastrophism. This is particularly true if they directly affect mate attraction or mating behavior. They may also act synergistically with these other factors to enhance or reduce the effects of each other. Thus, anthropogenic contaminants represent a significant class of selective pressures, though they are not often considered as such. In this sense, anthropogenic compounds embody a cryptic form of selective pressure that must be considered carefully and that could have important repercussions and potentially confounding effects on conclusions regarding our understanding of natural behavior.

Acknowledgments

We would like to thank Carlos Alonso-Alvarez and two anonymous reviewers for their helpful suggestions and thoughtful comments, which improved the quality of this review. We also thank the National Science Foundation Graduate Research Fellowship Program for supporting TDB.

References

- Aldridge J, Levin E, Seidler F, Slotkin T. Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. *Environ Health Perspect.* 2005; 113(5):527–531.10.1289/ehp.7867 [PubMed: 15866758]
- Alix A, Cortesero AM, Nenon JP, Anger JP. Selectivity assessment of chlorfenvinphos reevaluated by including physiological and behavioral effects on an important beneficial insect. *Environ Toxicol Chem.* 2001; 20(11):2530–2536. [PubMed: 11699779]
- Aller, RC. Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures. In: Blackburn, TH.; Sorensen, J., editors. *Nitrogen cycling in coastal marine environments*. John Wiley and Sons Ltd; Chichester, NY: 1988. p. 301-338.
- Alonso-Alvarez C, Galvan I. Free Radical Exposure Creates Paler Carotenoid-Based Ornaments: A Possible Interaction in the Expression of Black and Red Traits. *Plos One.* 2011; 6(4)10.1371/journal.pone.0019403
- Alonso-Alvarez C, Perez-Rodriguez L, Ester Ferrero M, Garcia de-Blas E, Casas F, Mougeot F. Adjustment of female reproductive investment according to male carotenoid-based ornamentation in a gallinaceous bird. *Behavioral Ecology and Sociobiology.* 2012; 66(5):731–742.10.1007/s00265-012-1321-8
- Andersson, M. *Sexual selection*. Princeton University Press; Princeton, New Jersey: 1994.
- Anway MD, Rekow SS, Skinner MK. Comparative anti-androgenic actions of vinclozolin and flutamide on transgenerational adult onset disease and spermatogenesis. *Reproductive Toxicology.* 2008; 26(2):100–106.10.1016/j.reprotox.2008.07.008 [PubMed: 18762243]
- Anway MD, Skinner MK. Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease. *Reproductive Biomedicine Online.* 2008; 16(1):23–25. [PubMed: 18252044]
- Arellano-Aguilar O, Garcia CM. Exposure to pesticides impairs the expression of fish ornaments reducing the availability of attractive males. *Proceedings of the Royal Society B-Biological Sciences.* 2008; 275(1640):1343–1350.10.1098/rspb.2008.0163
- Arnold AP, Breedlove SM. Organizational and activational effects of sex steroids on brain and behavior: A reanalysis. *Horm Behav.* 1985; 19:469–498. [PubMed: 3910535]
- Arrhenius F, Hansson S. Food-Consumption of Larval, Young and Adult Herring and Sprat in the Baltic Sea. *Mar Ecol-Prog Ser.* 1993; 96(2):125–137.
- ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological profile for mirex and chlordecone.* 1995.
- ATSDR. *Toxicological profile for chlorfenvinphos.* 1997a.
- ATSDR. *Toxicological profile for chlorpyrifos.* 1997b.
- ATSDR. *Toxicological profile for chlorinated dibenzo-p-dioxins: draft for public comment, update.* 1998.
- ATSDR. *Toxicological profile for polychlorinated biphenyls: draft for public comment, update.* 2000a.
- ATSDR. *Toxicological profile for toluene.* 2000b.
- ATSDR. *Toxicological profile for methyl parathion.* 2001.
- ATSDR. *Toxicological profile for methoxychlor.* 2002.
- ATSDR. *Toxicological profile for atrazine: draft for public comment, update.* 2003a.
- ATSDR. *Toxicological profile for malathion: draft for public comment, update.* 2003b.
- ATSDR. *Toxicological profile for pyrethrins and pyrethroids: draft for public comment, update.* 2003c.
- Baatrup E, Junge M. Antiandrogenic pesticides disrupt sexual characteristics in the adult male guppy (*Poecilia reticulata*). *Environ Health Perspect.* 2001; 109(10):1063–1070. [PubMed: 11675272]
- Baerends GP, Brouwer R, Waterbolk HT. Ethological studies on *Lebistes reticulatus* (Peters) 1. An analysis of the male courtship pattern. *Behavior.* 1955; 8:249–334.

- Ball GF, Riters LV, Balthazart J. Neuroendocrinology of song behavior and avian brain plasticity: Multiple sites of action of sex steroid hormones. *Front Neuroendocrin.* 2002; 23(2):137–178.10.1006/Frne.2002.0230
- Barfield RJ, Chen JJ. Activation of estrous behavior in ovariectomized rats by intracerebral implants of estradiol benzoate. *Endocrinology.* 1977; 101(6):1716–1725. [PubMed: 338289]
- Baum MJ, Vreeburg JT. Copulation in castrated male rats following combined treatment with estradiol and dihydrotestosterone. *Science.* 1973; 182(4109):283–285.10.1126/science.182.4109.283 [PubMed: 4742734]
- Bayley M, Junge M, Baatrup E. Exposure of juvenile guppies to three antiandrogens causes demasculinization and a reduced sperm count in adult males. *Aquat Toxicol.* 2002; 56(4):227–239. [PubMed: 11856573]
- Beach FA. The snark was a boojum. *American Psychologist.* 1950; 5(4):115–124.10.1037/h0056510
- Bell A. Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback, *Gasterosteus aculeatus*. *Anim Behav.* 2001; 62:775–780.10.1006/anbe.2001.1824
- Berglund A, Rosenqvist G, Svensson I. Mate choice, fecundity and sexual dimorphism in two pipefish species (*Syngnathidae*). *Behavioral Ecology and Sociobiology.* 1986; 19(4):301–307.
- Bertolasio J, Fyfe S, Snyder BW, Davis AM. Neonatal injections of methoxychlor decrease adult rat female reproductive behavior. *Neurotoxicology.* 2011; 32(6):809–813.10.1016/j.neuro.2011.06.007 [PubMed: 21726579]
- Birkhoj M, Nellemann C, Jarfelt K, Jacobsen H, Andersen H, Dalgaard M, Vinggaard A. The combined antiandrogenic effects of five commonly used pesticides. *Toxicol Appl Pharmacol.* 2004; 201(1):10–20.10.1016/j.taap.2004.04.016 [PubMed: 15519604]
- Blazer VS, Iwanowicz LR, Iwanowicz DD, Smith DR, Young JA, Hedrick JD, Foster SW, Reeser SJ. Intersex (testicular oocytes) in smallmouth bass from the Potomac River and selected nearby drainages. *Journal of Aquatic Animal Health.* 2007; 19(4):242–253.10.1577/h07-031.1 [PubMed: 18333481]
- Boone M, Semlitsch R. Interactions of bullfrog tadpole predators and an insecticide: predation release and facilitation. *Oecologia.* 2003; 137(4):610–616.10.1007/s00442-003-1394-1 [PubMed: 14505025]
- Borg B, Mayer I. Androgens and behaviour in the three-spined stickleback. *Behaviour.* 1995; 132:1025–1035.
- Bradbury, JW.; Vehrencamp, SL. *Principles of Animal Communication.* 1. Sinauer Associates; Sunderland Massachusetts: 1998.
- Brenowitz EA, Margoliash D, Nordeen KW. An introduction to birdsong and the avian song system. *J Neurobiol.* 1997; 33(5):495–500. [PubMed: 9369455]
- Brien SE, Heaton JPW, Racz WJ, Adams MA. Effects of an environmental anti-androgen on erectile function in an animal penile erection model. *Journal of Urology.* 2000; 163(4):1315–1321.10.1016/s0022-5347(05)67770-1 [PubMed: 10737536]
- Brooks A, Gaskell P, Maltby L. Sublethal effects and predator-prey interactions: implications for ecological risk assessment. *Environ Toxicol Chem.* 2009; 28(11):2449–2457. [PubMed: 19572771]
- Brooks B, Chambliss C, Stanley J, Ramirez A, Banks K, Johnson R, Lewis R. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ Toxicol Chem.* 2005; 24(2):464–469.10.1897/04-081R.1 [PubMed: 15720009]
- Cant MA. A model for the evolution of reproductive skew without reproductive suppression. *Anim Behav.* 1998; 55:163–169.10.1006/anbe.1997.0589 [PubMed: 9480682]
- Carson, R. *Silent Spring.* Houghton Mifflin Co; New York, NY: 1962.
- Casarett, L.; Doull, J. *Toxicology: the basic science of poisons.* MacMillan; New York: 1975.
- Christiansen S, Scholze M, Dalgaard M, Vinggaard A, Axelstad M, Kortenkamp A, Hass U. Synergistic Disruption of External Male Sex Organ Development by a Mixture of Four Antiandrogens. *Environ Health Perspect.* 2009; 117(12):1839–1846.10.1289/ehp.0900689 [PubMed: 20049201]
- Chu S, Metcalfe C. Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid

- chromatography-tandem mass spectrometry. *Journal of Chromatography*. 2007; 1163(1–2):112–118.10.1016/j.chroma.2007.06.014
- Clark JT, Jimenez B, Evans SL, Barrow R, Winfree M, Mrotek JJ. Cadmium-induced sexual dysfunction does not involve increased hepatic metabolism of testosterone nor increased circulating levels of corticosterone. *Physiol Behav*. 1994; 56(5):975–981. [PubMed: 7824600]
- Clements WH, Rohr JR. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environ Toxicol Chem*. 2009; 28(9):1789–1800. [PubMed: 19358627]
- Clotfelter E, Bell A, Levering K. The role of animal behaviour in the study of endocrine-disrupting chemicals. *Anim Behav*. 2004; 68:665–676.10.1016/j.anbehav.2004.05.004
- Colborn T, Saal FSV, Soto AM. Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans. *Environ Health Perspect*. 1993; 101(5):378–384. [PubMed: 8080506]
- Cordier S. Evidence for a role of paternal exposures in developmental toxicity. *Basic & Clinical Pharmacology & Toxicology*. 2008; 102(2):176–181.10.1111/j.1742-7843.2007.00162.x [PubMed: 18226072]
- Cox C. Glyphosate herbicide factsheet. *Journal of Pesticide Reform*. 2004; 24(4):10–15.
- Crews D. Epigenetics, brain, behavior, and the environment. *Horm-Int J Endocrinol*. 2010; 9(1):41–50.
- Crews D, Moore M. Evolution of mechanisms controlling mating behavior. *Science*. 1986; 231:121–125. [PubMed: 3941893]
- Csoka AB, Szyf M. Epigenetic side-effects of common pharmaceuticals: A potential new field in medicine and pharmacology. *Medical Hypotheses*. 2009; 73(5):770–780.10.1016/j.mehy.2008.10.039 [PubMed: 19501473]
- Curtis JT, Hood AN, Chen Y, Cobb GP, Wallace DR. Chronic metals ingestion by prairie voles produces sex-specific deficits in social behavior: An animal model of autism. *Behav Brain Res*. 2010; 213(1):42–49.10.1016/j.bbr.2010.04.028 [PubMed: 20433873]
- Daughton C, Ternes T. Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ Health Perspect*. 1999; 107:907–938.10.2307/3434573 [PubMed: 10592150]
- Dauwe T, Eens M. Melanin- and carotenoid-dependent signals of great tits (*Parus major*) relate differently to metal pollution. *Naturwissenschaften*. 2008; 95(10):969–973.10.1007/s00114-008-0400-1 [PubMed: 18506415]
- De Silva P, Samayawardhena LA. Effects of chlorpyrifos on reproductive performances of guppy (*Poecilia reticulata*). *Chemosphere*. 2005; 58(9):1293–1299.10.1016/j.chemosphere.2004.10.030 [PubMed: 15667849]
- Deng J, Liu CS, Yu LQ, Zhou BS. Chronic exposure to environmental levels of tribromophenol impairs zebrafish reproduction. *Toxicol Appl Pharmacol*. 2010; 243(1):87–95.10.1016/j.taap.2009.11.016 [PubMed: 19931292]
- Devlin RH, Nagahama Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture*. 2002; 208:191–364.
- DeVoogd, TJ.; Lauay, C. Emerging biopsychology of the avian song system. In: Blass, E., editor. *Handbook of Behavioral Neurobiology*. Vol. 13. Kluwer Academic/Plenum Publishers; New York: 2001. p. 357-392.
- Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocr Rev*. 2009; 30(4):293–342.10.1210/Er.2009-0002 [PubMed: 19502515]
- Dodds EC, Lawson W. Synthetic oestrogenic agents without the phenanthrene nucleus. *Nature*. 1936; 137:996.
- Edwards DA, Pfeifle JK. Hormonal-control of receptivity, proceptivity and sexual motivation. *Physiol Behav*. 1983; 30(3):437–443. [PubMed: 6683412]
- Eeva T, Sillanpaa S, Salminen J, Nikkinen L, Tuominen A, Toivonen E, Pihlaja K, Lehtikainen E. Environmental Pollution Affects the Plumage Color of Great Tit Nestlings through Carotenoid Availability. *Ecohealth*. 2008; 5(3):328–337.10.1007/s10393-008-0184-y [PubMed: 18704585]
- Eibl-Eibesfeldt, I. *Ethology: The biology of behavior*. Holt, Rinehart & Winston Inc; New York, New York: 1970.

- Eliyahu D, Applebaum S, Rafaeli A. Moth sex-pheromone biosynthesis is inhibited by the herbicide diclofop. *Pest Biochem Physiol.* 2003; 77(2):75–81.10.1016/S0048-3575(03)00101-9
- Emlen ST, Oring LW. Ecology, Sexual Selection, and Evolution of Mating Systems. *Science.* 1977; 197(4300):215–223. [PubMed: 327542]
- Engell MD, Godwin J, Young LJ, Vandenberg JG. Perinatal exposure to endocrine disrupting compounds alters behavior and brain in the female pine vole. *Neurotoxicol Teratol.* 2006; 28(1): 103–110.10.1016/j.ntt.2005.10.002 [PubMed: 16307867]
- Environmental Protection Agency (EPA). List D, Case 4052. 1991. Reregistration eligibility document inorganic nitrate/nitrite (sodium and potassium nitrates).
- EPA. Prevention, pesticides and toxic substances: Fenitrothion facts. 2000a.
- EPA. Prevention, pesticides and toxic substances: Reregistration Eligibility Decision Facts, Diclofop-methyl. 2000b.
- EPA. Prevention, pesticides and toxic substances: Reregistration Eligibility Decision Facts, Vinclozolin. 2000c.
- EPA. Fenarimol Summary Document Registration Review: Initial Docket. 2007.
- EPA. Glyphosate Summary Document Registration Review: Initial Docket. 2009.
- EPA. Carbaryl Summary Document Registration Review: Initial Docket. 2010.
- Eroschenko VP, Amstislavsky SY, Schwabel H, Ingermann RL. Altered behaviors in male mice, male quail, and salamander larvae following early exposures to the estrogenic pesticide methoxychlor. *Neurotoxicol Teratol.* 2002; 24(1):29–36. [PubMed: 11836069]
- Erskine MS. SOLICITATION BEHAVIOR IN THE ESTROUS FEMALE RAT - A REVIEW. *Horm Behav.* 1989; 23(4):473–502.10.1016/0018-506X(89)90037-8 [PubMed: 2691387]
- Fischer C, Fredriksson A, Eriksson P. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. *Toxicology.* 2008; 244(2–3):157–165.10.1016/j.tox.2007.11.006 [PubMed: 18155821]
- Flaherty CM, Dodson SI. Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction. *Chemosphere.* 2005; 61(2):200–207.10.1016/j.chemosphere.2005.02.016 [PubMed: 16168743]
- Fleeger J, Carman K, Nisbet R. Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment.* 2003; 317(1–3):207–233.10.1016/S0048-9697(03)00141-4 [PubMed: 14630423]
- Ford A, Martins I, Fernandes T. Population level effects of intersexuality in the marine environment. *Science of the Total Environment.* 2007; 374(1):102–111.10.1016/j.scitotenv.2006.12.046 [PubMed: 17270251]
- Fort DJ, Thomas JH, Rogers RL, Noll A, Spaulding CD, Guiney PD, Weeks JA. Evaluation of the developmental and reproductive toxicity of methoxychlor using an anuran (*Xenopus tropicalis*) chronic exposure model. *Toxicol Sci.* 2004; 81(2):443–453.10.1093/toxsci/kfh230 [PubMed: 15272137]
- Frederick P, Jayasena N. Altered pairing behaviour and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. *Proceedings of the Royal Society B-Biological Sciences.* 2011; 278(1713):1851–1857.10.1098/rspb.2010.2189
- Fry D, Toone C. DDT-induced feminization of gull embryos. *Science.* 1981; 213(4510):922–924.10.1126/science.7256288 [PubMed: 7256288]
- Frye C, Bo E, Calamandrei G, Calza L, Dessi-Fulgheri F, Fernandez M, Fusani L, Kah O, Kajta M, Le Page Y, Patisaul H, Venerosi A, Wojtowicz A, Panzica G. Endocrine Disruptors: A Review of Some Sources, Effects, and Mechanisms of Actions on Behaviour and Neuroendocrine Systems. *J Neuroendocrinol.* 2012; 24(1):144–159.10.1111/j.1365-2826.2011.02229.x [PubMed: 21951193]
- Gagne F, Blaise C, Pellerin J, Pelletier E, Strand J. Health status of *Mya arenaria* bivalves collected from contaminated sites in Canada (Saguenay Fjord) and Denmark (Odense Fjord) during their reproductive period. *Ecotoxicology and Environmental Safety.* 2006; 64(3):348–361.10.1016/j.ecoenv.2005.04.007 [PubMed: 15939472]
- Galvan I, Alonso-Alvarez C. The expression of melanin-based plumage is separately modulated by exogenous oxidative stress and a melanocortin. *Proceedings of the Royal Society B-Biological Sciences.* 2009; 276(1670):3089–3097.10.1098/rspb.2009.0774

- Garamszegi LZ, Eens M. Brain space for a learned task: strong intraspecific evidence for neural correlates of singing behavior in songbirds. *Brain Res Rev.* 2004; 44(2–3):187–193.10.1016/j.brainresrev.2003.12.001 [PubMed: 15003393]
- Gibson R, Smith M, Spary C, Tyler C, Hill E. Mixtures of estrogenic contaminants in bile of fish exposed to wastewater treatment works effluents. *Environ Sci Technol.* 2005; 39(8):2461–2471.10.1021/es048892g [PubMed: 15884336]
- Gillooly J, Ophir A. The energetic basis of acoustic communication. *Proceedings of the Royal Society B-Biological Sciences.* 2010; 277(1686):1325–1331.10.1098/rspb.2009.2134
- Gorissen L, Snoeijs T, Van Duyse E, Eens M. Heavy metal pollution affects dawn singing behaviour in a small passerine bird. *Oecologia.* 2005; 145(3):504–509. [PubMed: 16133189]
- Gray GD, Smith ER, Davidson JM. Hormonal-regulation of penile erection in castrated male rats. *Physiol Behav.* 1980; 24(3):463–468.10.1016/0031-9384(80)90237-1 [PubMed: 7375566]
- Gray L, Ostby J, Ferrell J, Rehnberg G, Linder R, Cooper R, Goldman J, Slott V, Laskey J. A dose-response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. *Fundamental and Applied Toxicology.* 1989; 12(1):92–108.10.1016/0272-0590(89)90065-1 [PubMed: 2925022]
- Gray LE, Ostby JS. In-utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicol Appl Pharmacol.* 1995; 133(2):285–294. [PubMed: 7645025]
- Grue CE, Gibert PL, Seeley ME. Neurophysiological and behavioral changes in non-target wildlife exposed to organophosphate and carbamate pesticides: Thermoregulation, food consumption, and reproduction. *Am Zool.* 1997; 37(4):369–388.
- Guillette LJ, Crain DA, Rooney AA, Pickford DB. Organization versus activation-The role of endocrine-disrupting contaminants (EDCs) during embryonic-development in wildlife. *Environ Health Perspect.* 1995; 103:157–164. [PubMed: 8593864]
- Guillette LJ, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental Abnormalities of the Gonad and Abnormal Sex-Hormone Concentrations in Juvenile Alligators from Contaminated and Control Lakes in Florida. *Environ Health Perspect.* 1994; 102(8):680–688. [PubMed: 7895709]
- Haegele M, Hudson R. Reduction of courship behavior induced by DDE in male ringed turtle doves. *Wilson Bulletin.* 1977; 89(4):593–601.
- Hahn ME, Karchner SI, Shapiro MA, Perera SA. Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AHR1 and AHR2) and the PAS family. *Proc Natl Acad Sci U S A.* 1997; 94(25):13743–13748. [PubMed: 9391097]
- Hallinger KK, Zabransky DJ, Kazmer KA, Cristol DA. Birdsong differs between mercury-polluted and reference sites. *Auk.* 2010; 127(1):156–161.
- Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): Laboratory and field evidence. *Environ Health Perspect.* 2003; 111(4):568–575.10.1289/Ehp.5932 [PubMed: 12676617]
- Hayes TB. There is no denying this: Defusing the confusion about atrazine. *Bioscience.* 2004; 54(12):1138–1149.
- Hayes TB, Anderson LL, Beasley VR, de Solla SR, Iguchi T, Ingraham H, Kestemont P, Kniewald J, Kniewald Z, Langlois VS, Luque EH, McCoy KA, Munoz-de-Toro M, Oka T, Oliveira CA, Orton F, Ruby S, Suzawa M, Tavera-Mendoza LE, Trudeau VL, Victor-Costa AB, Willingham E. Demasculinization and feminization of male gonads by atrazine: Consistent effects across vertebrate classes. *J Steroid Biochem.* 2011; 127(1–2):64–73.10.1016/J.jsbmb.2011.03.015
- Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc Natl Acad Sci U S A.* 2002; 99(8):5476–5480.10.1073/Pnas.082121499 [PubMed: 11960004]
- Hayes TB, Khoury V, Narayan A, Nazir M, Park A, Brown T, Adame L, Chan E, Buchholz D, Stueve T, Gallipeau S. Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). *Proc Natl Acad Sci U S A.* 2010; 107(10):4612–4617.10.1073/pnas.0909519107 [PubMed: 20194757]

- Heaton JPW, Varrin SJ. Effects of castration and exogenous testosterone supplementation in an animal-model of penile erection. *Journal of Urology*. 1994; 151(3):797–800. [PubMed: 8309009]
- Henry T, Black M. Acute and chronic toxicity of fluoxetine (selective serotonin reuptake inhibitor) in western mosquitofish. *Arch Environ Contam Toxicol*. 2008; 54(2):325–330.10.1007/s00244-007-9018-0 [PubMed: 17763886]
- Hinck J, Blazer V, Schmitt C, Papoulias D, Tillitt D. Widespread occurrence of intersex in black basses (*Micropterus* spp.) from US rivers, 1995–2004. *Aquat Toxicol*. 2009; 95(1):60–70.10.1016/j.aquatox.2009.08.001 [PubMed: 19717194]
- Hogan NS, Duarte P, Wade MG, Lean DRS, Trudeau VL. Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): Identifying critically vulnerable periods of development. *General and Comparative Endocrinology*. 2008; 156(3):515–523.10.1016/j.ygcen.2008.03.011 [PubMed: 18430423]
- Hoogesteijn AL, Kollias GV, Quimby FW, De Caprio AP, Winkler DW, DeVoogd TJ. Development of a brain nucleus involved in song production in zebra finches (*Taeniopygia guttata*) is disrupted by Aroclor 1248. *Environ Toxicol Chem*. 2008; 27(10):2071–2075. [PubMed: 18380522]
- Hoshina Y, Takeo T, Nakano K, Sato T, Sakuma Y. Axon-Sparing Lesion of the Preoptic Area Enhances Receptivity and Diminishes Proceptivity among Components of Female Rat Sexual-Behavior. *Behav Brain Res*. 1994; 61(2):197–204. [PubMed: 8037867]
- Houtman C, Van Oostveen A, Brouwer A, Lamoree M, Legler J. Identification of estrogenic compounds in fish bile using bioassay-directed fractionation. *Environ Sci Technol*. 2004; 38(23):6415–6423.10.1021/es049750p [PubMed: 15597899]
- Howdeshell K, Hotchkiss A, Thayer K, Vandenberg J, vom Saal F. Environmental toxins - Exposure to bisphenol A advances puberty. *Nature*. 1999; 401(6755):763–764. [PubMed: 10548101]
- Huang Y, Zhu LY, Liu GX. The effects of bis(tributyltin) oxide on the development, reproduction and sex ratio of calanoid copepod *Pseudodiaptomus marinus*. *Estuarine Coastal and Shelf Science*. 2006; 69(1–2):147–152.10.1016/j.ecss.2006.04.010
- Hued A, Oberhofer S, Bistoni M. Exposure to a Commercial Glyphosate Formulation (Roundup (R)) Alters Normal Gill and Liver Histology and Affects Male Sexual Activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). *Arch Environ Contam Toxicol*. 2012; 62(1):107–117.10.1007/s00244-011-9686-7 [PubMed: 21643816]
- Ikedo M, Tamura M, Yamashita J, Suzuki C, Tomita T. Repeated in utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure affects male gonads in offspring, leading to sex ratio changes in F-2 progeny. *Toxicol Appl Pharmacol*. 2005; 206(3):351–355.10.1016/j.taap.2004.11.019 [PubMed: 16039946]
- Ishihara K, Warita K, Tanida T, Sugawara T, Kitagawa H, Hoshi N. Does paternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affect the sex ratio of offspring? *Journal of Veterinary Medical Science*. 2007; 69(4):347–352.10.1292/jvms.69.347 [PubMed: 17485921]
- Iwaniuk AN, Koperski DT, Cheng KM, Elliott JE, Smith LK, Wilson LK, Wylie DRW. The effects of environmental exposure to DDT on the brain of a songbird: Changes in structures associated with mating and song. *Behav Brain Res*. 2006; 173(1):1–10.10.1016/j.bbr.2006.05.026 [PubMed: 16828177]
- Jacobsen P, Christiansen S, Boberg J, Nellemann C, Hass U. Combined exposure to endocrine disrupting pesticides impairs parturition, causes pup mortality and affects sexual differentiation in rats. *International Journal of Andrology*. 2010; 33(2):434–441.10.1111/j.1365-2605.2009.01046.x [PubMed: 20487043]
- Jacobson T, Sundelin B. Reproductive effects of the endocrine disruptor fenarimol on a baltic amphipod *Monoporeia affinis*. *Environ Toxicol Chem*. 2006; 25(4):1126–1131. [PubMed: 16629152]
- Jarfelt K, Dalgaard M, Hass U, Borch J, Jacobsen H, Ladefoged O. Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. *Reproductive Toxicology*. 2005; 19(4):505–515.10.1016/j.reprotox.2004.11.005 [PubMed: 15749265]

- Jayasena N, Frederick P, Larkin I. Endocrine disruption in white ibises (*Eudocimus albus*) caused by exposure to environmentally relevant levels of methylmercury. *Aquat Toxicol.* 2011; 105(3–4): 321–327.10.1016/j.aquatox.2011.07.003 [PubMed: 21801696]
- Johnstone RA, Cant MA. Reproductive skew and indiscriminate infanticide. *Anim Behav.* 1999a; 57:243–249.10.1006/anbe.1998.0952 [PubMed: 10053092]
- Johnstone RA, Cant MA. Reproductive skew and the threat of eviction: a new perspective. *P Roy Soc Lond B Bio.* 1999b; 266(1416):275–279.
- Jones AG, Avise JC. Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. *Mol Ecol.* 1997; 6(3): 203–213. [PubMed: 9076975]
- Jones AG, Walker D, Avise JC. Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. *P Roy Soc Lond B Bio.* 2001; 268(1485):2531–2535.10.1098/Rspb.2001.1841
- Jones DP. Redefining oxidative stress. *Antioxid Redox Signal.* 2006; 8(9–10):1865–1879.10.1089/ars.2006.8.1865 [PubMed: 16987039]
- Katsiadaki I, Scott AP, Mayer I. The potential of the three-spined stickleback (*Gasterosteus aculeatus* L.) as a combined biomarker for oestrogens and androgens in European waters. *Mar Environ Res.* 2002; 54(3–5):725–728. [PubMed: 12408643]
- Kawata M. Roles of steroid hormones and their receptors in structural organization in the nervous system. *Neuroscience Research.* 1985; 24:1–46. [PubMed: 8848287]
- Keller L, Reeve HK. Partitioning of reproduction in animal societies. *Trends Ecol Evol.* 1994; 9:98–102. [PubMed: 21236786]
- Kettlewell HBD. Selection experiments on industrial melanism in the *Lepidoptera*. *Heredity.* 1955; 9:321–342.
- Kidd K, Blanchfield P, Mills K, Palace V, Evans R, Lazorchak J, Flick R. Collapse of a fish population after exposure to a synthetic estrogen. *Proc Natl Acad Sci U S A.* 2007; 104(21): 8897–8901.10.1073/pnas.0609568104 [PubMed: 17517636]
- Kirby M, Allen Y, Dyer R, Feist S, Katsiadaki I, Matthiessen P, Scott A, Smith A, Stentiford G, Thain J, Thomas K, Tolhurst L, Waldock M. Surveys of plasma vitellogenin and intersex in male flounder (*Platichthys flesus*) as measures of endocrine disruption by estrogenic contamination in United Kingdom estuaries: Temporal trends, 1996 to 2001. *Environ Toxicol Chem.* 2004; 23(3): 748–758.10.1897/03-166 [PubMed: 15285369]
- Klassen, C. Casarett & Doull's Toxicology: The Basic Science of Poisons. 6. McGraw-Hill Companies, Inc; United States of America: 2001.
- Kokko H, Johnstone RA. Social queuing in animal societies: a dynamic model of reproductive skew. *P Roy Soc Lond B Bio.* 1999; 266(1419):571–578.
- Krang A, Dahstrom M. Effects of a candidate antifouling compound (medetomidine) on pheromone induced mate search in the amphipod *Corophium volutator*. *Marine Pollution Bulletin.* 2006; 52(12):1776–1783.10.1016/j.marpolbul.2006.09.015 [PubMed: 17070855]
- Krang AS. Naphthalene disrupts pheromone induced mate search in the amphipod *Corophium volutator* (Pallas). *Aquat Toxicol.* 2007; 85(1):9–18.10.1016/j.aquatox.2007.07.012 [PubMed: 17850896]
- Kristensen T, Baatrup E, Bayley M. 17 alpha-ethinylestradiol reduces the competitive reproductive fitness of the male guppy (*Poecilia reticulata*). *Biol Reprod.* 2005; 72(1):150–156.10.1095/biolreprod.104.033001 [PubMed: 15355877]
- Kuiken T, Bennett PM, Allchin CR, Kirkwood JK, Baker JR, Lockyer CH, Walton MJ, Sheldrick MC. PCBs, cause of death and body condition in harbor porpoises (*Phocoena-phocoena*) from British waters. *Aquat Toxicol.* 1994; 28(1–2):13–28.10.1016/0166-445x(94)90017-5
- Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg P, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology.* 1998; 139(10):4252–4263. [PubMed: 9751507]
- Laessig SA, Auger AP, McCarthy MM, Silbergeld EK. Effects of prenatal chlordecone on sexually differentiated behavior in adult rats. *Neurotoxicol Teratol.* 2007; 29(2):255–263.10.1016/j.ntt.2006.10.003 [PubMed: 17169529]

- LaFiandra EM, Babbitt KJ, Sower SA. Effects of atrazine on anuran development are altered by the presence of a nonlethal predator. *J Toxicol Env Health Part A*. 2008; 71(8):505–511.10.1080/15287390801907442 [PubMed: 18338285]
- Lange R, Hutchinson TH, Croudace CP, Siegmund F, Schweinfurth H, Hampe P, Panter GH, Sumpter JP. Effects of the synthetic estrogen 17 alpha-ethinylestradiol on the life-cycle of the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem*. 2001; 20(6):1216–1227. [PubMed: 11392131]
- Lingappa, S.; Basavanagoud, K.; Kulkarni, KA.; Patil, RS.; Kambrekar, DN. Disease Management of Fruits and Vegetables. Vol. 1. Kluwer Academic Publishers; Netherlands: 2004. Threat to vegetable production by Diamondback moth and its management strategies; p. 357-396.
- Lopes C, Charles S, Vollat B, Garric J. Toxicity of ivermectin on cladocerans: comparison of toxic effects on daphnia and ceriodaphnia species. *Environ Toxicol Chem*. 2009; 28(10):2160–2166. [PubMed: 19456204]
- Lye C, Bentley M, Galloway T. Effects of 4-nonylphenol on the endocrine system of the shore crab, *Carcinus maenas*. *Environ Toxicol*. 2008; 23(3):309–318.10.1002/tox.20344 [PubMed: 18214899]
- Madsen EL, Mann CL, Bilotta SE. Oxygen limitations and aging as explanations for the field persistence of naphthalene in coal tar-contaminated surface sediments. *Environ Toxicol Chem*. 1996; 15(11):1876–1882.
- McCoy KA, Bortnick LJ, Campbell CM, Hamlin HJ, Guillette LJ, St Mary CM. Agriculture Alters Gonadal Form and Function in the Toad *Bufo marinus*. *Environ Health Perspect*. 2008; 116(11):1526–1532.10.1289/ehp.11536 [PubMed: 19057706]
- McDonald P, Beyer C, Newton F, Brien B, Baker R, Tan HS, Sampson C, Kitching P, Greenhil R, Pritchard D. Failure of 5Alpha-dihydrotestosterone to initiate sexual behaviour in castrated male rat. *Nature*. 1970; 227(5261):964-&.10.1038/227964a0 [PubMed: 5449005]
- Meisel, R.; Sachs, B. The physiology of male sexual behavior. In: Knobil, E.; Neil, JD., editors. The physiology of reproduction. 2nd ed. Raven press; New York: 1994.
- Michael RP, Zumpe D, Bonsall RW. Estradiol administration and the sexual activity of castrated rhesus monkeys (*Macaca mulatta*). *Horm Behav*. 1990; 24(1):71–88.10.1016/0018-506x(90)90028-v [PubMed: 2328971]
- Moniz AC, Cruz-Casallas PE, Salzgeber SA, Varoli FMF, Spinosa HS, Bernardi MM. Behavioral and endocrine changes induced by perinatal fenvalerate exposure in female rats. *Neurotoxicol Teratol*. 2005; 27(4):609–614.10.1016/j.ntt.2005.05.005 [PubMed: 16005607]
- Monje L, Varayoud J, Munoz-de-Toro M, Luque EH, Ramos JG. Neonatal exposure to bisphenol A alters estrogen-dependent mechanisms governing sexual behavior in the adult female rat. *Reproductive Toxicology*. 2009; 28(4):435–442.10.1016/j.reprotox.2009.06.012 [PubMed: 19577632]
- Moore A, Waring C. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). *Aquat Toxicol*. 2001; 52(1):1–12.10.1016/S0166-445X(00)00133-8 [PubMed: 11163426]
- Musatov S, Chen W, Pfaff DW, Kaplitt MG, Ogawa S. RNAi-mediated silencing of estrogen receptor in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. *Proc Natl Acad Sci U S A*. 2006; 103(27):10456–10460.10.1073/pnas.0603045103 [PubMed: 16803960]
- Nash JP, Kime DE, Van der Ven LTM, Wester PW, Brion F, Maack G, Stahlschmidt-Allner P, Tyler CR. Long-term exposure to environmental concentrations of the pharmaceutical ethinylestradiol causes reproductive failure in fish. *Environ Health Perspect*. 2004; 112(17):1725–1733.10.1289/ehp.7209 [PubMed: 15579420]
- Nelson DA, Khanna H, Marler P. Learning by instruction or selection: Implications for patterns of geographic variation in bird song. *Behaviour*. 2001; 138:1137–1160.
- Norris KJ. Female choice and the evolution of the conspicuous plumage coloration of monogamous male great tits. *Behavioral Ecology and Sociobiology*. 1990; 26(2):129–138.
- Oduma JA, Wango EO, Makawiti DW, Einerjensen N, Oduorokelo D. Effects of graded doses of the pesticide heptachlor on body-weight, mating success, estrous-cycle, gestation length and litter

- size in laboratory rats. *Comp Biochem Physiol C-Pharmacol Toxicol Endocrinol*. 1995; 110(2): 221–227. [PubMed: 7599970]
- Ogawa S, Olazabal UE, Parhar IS, Pfaff DW. Effects of intrahypothalamic administration of antisense DNA for progesterone-receptor messenger RNA on reproductive behavior and progesterone-receptor immunoreactivity in female rats. *J Neurosci*. 1994; 14(3):1766–1774. [PubMed: 8126569]
- Oka T, Tooi O, Mitsui N, Miyahara M, Ohnishi Y, Takase M, Kashiwagi A, Shinkai T, Santo N, Iguchi T. Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*. *Aquat Toxicol*. 2008; 87(4):215–226.10.1016/j.aquatox.2008.02.009 [PubMed: 18395276]
- Ottinger MA, Wu JM, Hazelton JL, Abdelnabi MA, Thompson N, Quinn ML, Donoghue D, Schenck F, Ruscio M, Beavers J, Jaber M. Assessing the consequences of the pesticide methoxychlor: neuroendocrine and behavioral measures as indicators of biological impact of an estrogenic environmental chemical. *Brain Res Bull*. 2005; 65(3):199–209.10.1016/j.brainresbull.2004.11.019 [PubMed: 15811582]
- Palanza P, Morellini F, Parmigiani S, vom Saal FS. Ethological methods to study the effects of maternal exposure to estrogenic endocrine disrupters - A study with methoxychlor. *Neurotoxicol Teratol*. 2002; 24(1):55–69.10.1016/s0892-0362(01)00191-x [PubMed: 11836072]
- Pandey S. Effects of Hypophysectomy on Testis and Secondary Sex Characters of Adult Guppy *Poecilia Reticulata* Peters. *Can J Zoolog*. 1969; 47(5):775-&.
- Partridge C, Boettcher A, Jones AG. Short-term exposure to a synthetic estrogen disrupts mating dynamics in a pipefish. *Horm Behav*. 2010; 58(5):800–807.10.1016/j.yhbeh.2010.08.002 [PubMed: 20708009]
- Patisaul H, Adewale H. Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. *Frontiers in Behavioral Neuroscience*. 2009;3.10.3389/neuro.08.010.2009 [PubMed: 19503748]
- Pestana JLT, Loureiro S, Baird DJ, Soares A. Fear and loathing in the benthos: Responses of aquatic insect larvae to the pesticide imidacloprid in the presence of chemical signals of predation risk. *Aquat Toxicol*. 2009; 93(2–3):138–149.10.1016/j.aquatox.2009.04.008 [PubMed: 19477535]
- Pleim ET, Brown TJ, Maclusky NJ, Etgen AM, Barfield RJ. DILUTE ESTRADIOL IMPLANTS AND PROGESTIN RECEPTOR INDUCTION IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS - CORRELATION WITH RECEPTIVE BEHAVIOR IN FEMALE RATS. *Endocrinology*. 1989; 124(4):1807–1812. [PubMed: 2924724]
- Pocock VJ, Sales GD, Wilson CA, Milligan SR. Effects of perinatal octylphenol on ultrasound vocalization, behavior and reproductive physiology in rats. *Physiol Behav*. 2002; 76(4–5):645–653. [PubMed: 12127004]
- Quinn MJ, Summitt CL, Ottinger MA. Consequences of in ovo exposure to p,p -DDE on reproductive development and function in Japanese quail. *Horm Behav*. 2008; 53(1):249–253.10.1016/j.yhbeh.2007.10.004 [PubMed: 18021774]
- Reik W, Walter J. Genomic imprinting: Parental influence on the genome. *Nature Reviews Genetics*. 2001; 2:21–32.
- Riddell DJ, Culp JM, Baird DJ. Behavioral responses to sublethal cadmium exposure within an experimental aquatic food web. *Environ Toxicol Chem*. 2005; 24(2):431–441.10.1897/04-026r.1 [PubMed: 15720005]
- Rubin BS, Barfield RJ. Progesterone in the ventromedial hypothalamus facilitates estrous behavior in ovariectomized, estrogen-primed rats. *Endocrinology*. 1983; 113:797–804. [PubMed: 6683623]
- Rudegeair, T. The reproductive behavior and ecology of the white ibis (*Eudocimus albus*). University of Florida; Gainesville, FL: 1975.
- Safe SH. Polychlorinated-Biphenyls (Pcbs) - Environmental-Impact, Biochemical and Toxic Responses, and Implications for Risk Assessment. *Crit Rev Toxicol*. 1994; 24(2):87–149. [PubMed: 8037844]
- Samanta SK, Singh OV, Jain RK. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *Trends Biotechnol*. 2002; 20(6):243–248.10.1016/s0167-7799(02)01943-1 [PubMed: 12007492]

- Sanchez W, Sremski W, Piccini B, Palluel O, Maillot-Marechal E, Betoulle S, Jaffal A, Ait-Aissa S, Brion F, Thybaud E, Hinfrey N, Porcher JM. Adverse effects in wild fish living downstream from pharmaceutical manufacture discharges. *Environment International*. 2011; 37(8):1342–1348.10.1016/j.envint.2011.06.002 [PubMed: 21722962]
- Schantz SL, Ferguson SA, Bowman RE. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on behavior of monkeys in peer groups. *Neurotoxicol Teratol*. 1992; 14(6):433–446. [PubMed: 1488038]
- Schantz SL, Gasior DM, Polverejan E, McCaffry RJ, Sweeney AM, Humphrey HEB, Gardiner JC. Impairments of memory and learning in older adults exposed to polychlorinated biphenyl via consumption of great lakes fish. *Environ Health Perspect*. 2001; 109(6):605–611. [PubMed: 11445515]
- Schug T, Janesick A, Blumberg B, Heindel J. Endocrine disrupting chemicals and disease susceptibility. *J Steroid Biochem*. 2011; 127(3–5):204–215.10.1016/j.jsbmb.2011.08.007
- Sebire M, Katsiadaki I, Taylor N, Maack G, Tyler C. Short-term exposure to a treated sewage effluent alters reproductive behaviour in the three-spined stickleback (*Gasterosteus aculeatus*). *Aquat Toxicol*. 2011; 105(1–2):78–88.10.1016/j.aquatox.2011.05.014 [PubMed: 21684244]
- Sebire M, Scott AP, Tyler CR, Cresswell J, Hodgson DJ, Morris S, Sanders MB, Stebbing PD, Katsiadaki I. The organophosphorous pesticide, fenitrothion, acts as an anti-androgen and alters reproductive behavior of the male three-spined stickleback, *Gasterosteus aculeatus*. *Ecotoxicology*. 2009; 18(1):122–133.10.1007/s10646-008-0265-2 [PubMed: 18807270]
- Secondi J, Hinot E, Djalout Z, Sourice S, Jadas-Hecart A. Realistic nitrate concentration alters the expression of sexual traits and olfactory male attractiveness in newts. *Funct Ecol*. 2009; 23(4):800–808.10.1111/j.1365-2435.2009.01558.x
- Seuront L. Hydrocarbon Contamination Decreases Mating Success in a Marine Planktonic Copepod. *Plos One*. 2011; 6(10)10.1371/journal.pone.0026283
- Shuster, SM.; Wade, MJ. Mating systems and strategies. Princeton University Press; Princeton, New Jersey: 2003.
- Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Horm Behav*. 2003; 44(2):132–139.10.1016/S0018-506x(03)00124-7 [PubMed: 13129485]
- Tierney K, Baldwin D, Hara T, Ross P, Scholz N, Kennedy C. Olfactory toxicity in fishes. *Aquat Toxicol*. 2010; 96(1):2–26.10.1016/j.aquatox.2009.09.019 [PubMed: 19931199]
- Tietjen WJ. Pesticides affect the mating behavior of *Rabidosia rabida* (Araneae, Lycosidae). *J Arachnol*. 2006; 34(2):285–288.
- Tinbergen N. The curious behavior of the stickleback. *Scientific American*. 1952; 187:22–26.10.1038/scientificamerican1252-22
- Tinbergen, N. Curious Naturalists. Basic Books; New York, New York: 1958.
- Truss M, Beato M. Steroid hormone receptors: Interaction with deoxyribonucleic acid and transcription factors. *Endocr Rev*. 1993; 14:459–479. [PubMed: 8223341]
- Tsai MJ, Omalley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annual Review of Biochemistry*. 1994; 63:451–486.
- Tuomainen U, Candolin U. Behavioural responses to human-induced environmental change. *Biological Reviews*. 2011; 86(3):640–657.10.1111/j.1469-185X.2010.00164.x [PubMed: 20977599]
- Uphouse L. Single injection with chlordecone reduces behavioral receptivity and fertility of adult-rats. *Neurobehavioral Toxicology and Teratology*. 1986; 8(2):121–126. [PubMed: 2423894]
- Vandenberg L, Maffini M, Sonnenschein C, Rubin B, Soto A. Bisphenol-A and the Great Divide: A Review of Controversies in the Field of Endocrine Disruption. *Endocr Rev*. 2009; 30(1):75–95.10.1210/er.2008-0021 [PubMed: 19074586]
- Vehrencamp, SL. The roles of individual, kin and group selection in the evolution of sociality. In: Marler, P.; Vandenbergh, J., editors. *Social Behaviour and Communication*. Plenum Press; New York, New York: 1979. p. 351-394.
- Venerosi A, Calamandrei G, Ricceri L. A social recognition test for female mice reveals behavioral effects of developmental chlorpyrifos exposure. *Neurotoxicol Teratol*. 2006; 28(4):466–471.10.1016/j.ntt.2006.05.003 [PubMed: 16814983]

- Weis JS, Smith GM, Zhou T. Altered predator prey behavior in polluted environments: implications for fish conservation. *Environ Biol Fishes*. 1999; 55(1–2):43–51.10.1023/a:1007496528012
- Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal developments in mammals. *Physiological Reviews*. 2007; 87:1–28. [PubMed: 17237341]
- Wisniewski AB, Cernetich A, Gearhart JP, Klein SL. Perinatal exposure to genistein alters reproductive development and aggressive behavior in male mice. *Physiol Behav*. 2005; 84(2): 327–334.10.1016/j.physbeh.2004.12.008 [PubMed: 15708785]
- Wolff JO. Laboratory studies with rodents: Facts or artifacts? *Bioscience*. 2003; 53(4):421–427.10.1641/0006-3568(2003)053[0421:lswrfo]2.0.co;2
- Woodruff T, Carlson A, Schwartz J, Giudice L. Proceedings of the Summit on Environmental Challenges to Reproductive Health and Fertility: executive summary. *Fertil Steril*. 2008; 89(2): 281–300.10.1016/j.fertnstert.2007.10.002 [PubMed: 18275883]
- Woodward AR, Percival HF, Jennings ML, Moore CT. Low clutch viability of American alligators on Lake Apopka. *Florida Scientist*. 1993; 56(1):52–62.
- Wu MV, Manoli DS, Fraser EJ, Coats JK, Tollkuhn J, Honda SI, Harada N, Shah NM. Estrogen masculinizes neural pathways and sex-specific behaviors. *Cell*. 2009; 139(1):61–72. [PubMed: 19804754]
- Wyatt, TD. Pheromones and animal behavior: communication by smell and taste. Cambridge University Press; Cambridge, United Kingdom: 2003.
- Xu Z, Cao GC, Dong SL. Changes of Sex Pheromone Communication Systems Associated with Tebufenozide and Abamectin Resistance in Diamondback Moth, *Plutella xylostella* (L.). *J Chem Ecol*. 2010; 36(5):526–534.10.1007/s10886-010-9785-3 [PubMed: 20407810]
- Zahavi, A.; Zahavi, A. The Handicap Principle: A Missing Piece of Darwin's Puzzle. Oxford University Press; 1997.
- Zala S, Penn D. Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. *Anim Behav*. 2004; 68:649–664.10.1016/j.anbehav.2004.01.005
- Zama AM, Uzumcu M. Epigenetic effects of endocrine-disrupting chemicals on female reproduction: An ovarian perspective. *Front Neuroendocrin*. 2010; 31(4):420–439.10.1016/j.yfrne.2010.06.003

Table 1

Common chemical contaminants with effects on mating behavior. Bold text indicates large classes of contaminants. Specific examples and common sources for contaminant classes are provided.

Contaminant Class & Examples	Sources of Contaminant
Metals	
Elemental, inorganic, and organic forms	
Cadmium ¹	Batteries, metal coatings, metal alloys, metal smelting, and fish and seafood consumption
Lead ²	Batteries, ammunition, paint, and industrial emissions
Mercury ³	Batteries, vaccines, thermometers, dentistry, metal smelting, and fish and seafood consumption
Pesticides	
Abamectin ⁴	Natural fermentation product of bacteria used as an insecticide in agricultural settings
Organophosphate insecticides	
Parathion and Methyl-Parathion ⁵	Banned in some countries, restricted to use in agriculture in others
Chlorfenvinphos ⁶	Banned in some countries, used in agriculture in others
Chlorpyrifos ⁷	Agricultural settings and golf courses
Fenitrothion ⁸	Agricultural, and residential settings
Malathion ⁹	Agricultural, and residential settings
Organochlorine insecticides	
Dichlorodiphenyltrichloroethane (DDT) ¹⁰	Banned in nearly every country worldwide. Where not banned, used in agricultural settings and in disease control
Methoxychlor ¹¹	Banned in many countries, used in agriculture in others
Heptachlor ¹²	Banned in many countries, used in agriculture and to control fire ants in power transformers in others
Chlordecone ¹³	Banned in many countries, used in agriculture in others
Carbamate insecticides	
Carbaryl ¹⁴	Used in forestry, agriculture and on turf
Pyrethroid insecticides	
Fenvalerate ¹⁵	Agricultural and veterinary settings
Cypermethrin ¹⁵	Agricultural and residential settings
Fungicides	
Captan ¹⁶	Agricultural and residential settings
Vinclozolin ¹⁷	Agricultural settings and turf
Fenarimol ¹⁸	Agricultural settings and turf
Herbicides	
Atrazine ¹⁹	Agricultural settings
Diclofop-methyl ²⁰	Agricultural settings and golf courses
Glyphosate ²¹	Forestry and agricultural and residential settings
Agent Orange ²	Vietnam War

Contaminant Class & Examples	Sources of Contaminant
Biocides Medetomidine ^{22, 23}	Veterinary settings, used as an anti-fouling compound in paints
Pharmaceuticals & Personal Care Products	
Contraceptives Ethinyl estradiol (EE2) ²⁴	Used to prevent pregnancy
Antidepressants/Psychiatric drugs Diazepam ²⁴	Used to treat anxiety
Fluoxetine ²⁴	Used to treat depression
Anti-inflammatory drugs/Analgesics Acetaminophen ²⁴	Used for pain alleviation
Ibuprofen ²⁴	Used for pain alleviation
Lipid-regulating drugs Bezafibrate ²⁴	Used to treat hyperlipidaemia
Fenofibrate ²⁴	Used to treat hyperlipidaemia
Anti-hypertension drugs Verapamil ²⁴	Used to treat hypertension
Bisoprolol ²⁴	Used to treat hypertension
Musks Musk xylene ²⁴	Perfumery
Galaxolide ²⁴	Perfumery
Industrial compounds	
Polychlorinated biphenyls (PCBs) Aroclor 1248 ²⁵	Used in many commercial applications (i.e., hydraulic equipment, plasticizers, paints, plastics, as an insulating agent).
Bisphenol A (BPA) ²⁶	Industrial chemical used in polycarbonate plastic and epoxy resins.
Octylphenol ²⁷	Used in production of paints, plastic, pesticides and detergents.
Nitrates Ammonium nitrate ²⁸	Fertilizers
By-Products	
Polycyclic aromatic hydrocarbons (PAHs) Naphthalene ²⁹	By-products of burning organic compounds.
Toluene ³⁰	Found in gasoline, used in the production of paints, adhesives, cleaning agents, and synthetic fragrances
Polychlorinated dibenzo-dioxins (PCDD) 2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin (TCDD) ²⁵	By-products of burning chlorine-containing products
Polychlorinated dibenzofurans (PCDF) 2,3,7,8-tetrachlorodibenzofuran ²⁵	By-products of burning chlorine-containing products

References:

¹Friberg et al. 1986

- ²Klassen 2001
- ³Clarkson et al. 2003
- ⁴Lasota & Dybas 1991
- ⁵ATSDR (Agency for Toxic Substances and Disease Registry) 2001
- ⁶ATSDR 1997a
- ⁷ATSDR 1997b
- ⁸EPA (Environmental Protection Agency) 2000a
- ⁹ATSDR 2003b
- ¹⁰National Toxicology Program, Department of Health and Human Services 2011
- ¹¹ATSDR 2002
- ¹²World Health Organization 2006
- ¹³ATSDR 1995
- ¹⁴EPA 2010
- ¹⁵ATSDR 2003c
- ¹⁶National Pesticide Information Center 2000
- ¹⁷EPA 2000c
- ¹⁸EPA 2007
- ¹⁹ATSDR 2003a
- ²⁰EPA 2000b
- ²¹EPA 2009
- ²²Cullen 1996
- ²³Dahlstrom et al. 2000
- ²⁴Daughton & Ternes 1999
- ²⁵Safe 1990
- ²⁶Vandenberg et al. 2009
- ²⁷Ying et al. 2002
- ²⁸EPA 1991
- ²⁹Samanta et al. 2002
- ³⁰ATSDR 2000b.