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# Prenatal exposure to low doses of atrazine affects mating behaviors in male guppies

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ABSTRACT

Performing appropriate mating behaviors is crucial to male reproductive success, especially in species where mating is predominantly via female mate choice. Mating behaviors are hormonally regulated and may be sexually selected traits: courtship displays are selected via mate choice, while forced copulations and aggressive behaviors are selected for via intrasexual competition. Endocrine disrupting compounds interfere with proper hormonal functioning in exposed animals. Exposures during developmentally crucial life stages can have irreversible effects lasting through adulthood. I tested the effects of prenatal exposure to environmentally relevant doses of a commonly used herbicide, atrazine (1 and 13.5 μg/L) on mating behaviors in male guppies. Guppies were used as a model organism to test the effects of atrazine exposure on wildlife reproductive health. Adult female guppies were mated and exposed to the treatments throughout the gestation period, and offspring born to them were raised without further treatment. At adulthood, the males were tested for the effects of prenatal exposure on their mating behaviors such as courtship displays, gonopodium swings, forced copulatory attempts, and competitive and aggressive behaviors towards rivals who were not exposed to atrazine. I also tested female preference for treated males compared to control males. Atrazine-exposed males were less likely to perform the mating behaviors, and performed them less frequently, than control males. Atrazine exposure also made males less aggressive towards rivals. Females preferred untreated males over atrazine-treated males. In all cases, a non-monotonic pattern was seen, highlighting the significance of low-dose exposures.


## Introduction

Mating behaviors can be characterized as any behaviors that influence an individual's reproductive success. In many animal species, females choose mates by assessing courtship behaviors (Edvardsson and Arnqvist, 2000; Lopez, 1998; Rebar et al., 2009; Sargent et al., 1998). Males may also gain some fertilizations by employing other strategies like forced copulations (Clutton-Brock and Parker, 1995). In species with high male–male competition, aggression between competing males may be a factor influencing access to females and monopoly of fertilizations (Bertram et al., 2011; Brown et al., 2007; Hurtado-Gonzales and Uy, 2010). Males performing sub-optimally in any of these mating behaviors will suffer reduced mating and reproductive success. In most cases, mating behaviors in many animal species are hormonally regulated, and are especially under the control of gonadal hormones (Ball and Balthazart, 2004; Balthazart et al., 1990; Bell, 2001). Altered levels of gonadal hormones lead to altered expression of mating behaviors (reviewed in Shenoy and Crowley, 2011).

Endocrine disrupting compounds (EDCs) are a class of compounds that interfere with proper hormonal functioning in exposed animals. Common types of EDCs include organochlorides, organophosphates, polychlorinated biphenyls (PCBs), phthalates, synthetic hormones and hormone-blockers, and phytoestrogens; common sources of EDCs include pesticides, industrial effluents, pulp mill effluents, plastics and sewage. Animals may be exposed directly by living in contaminated soil or water, or indirectly by eating contaminated prey (Markman et al., 2007, 2008; Park et al., 2009; Walters et al., 2010). EDC concentrations can differ temporally within a region, thus exposing animals only during certain life stages. Often, this can coincide with developmentally crucial periods, and the effects of disruption during these stages can be manifested at later life stages. Because most animals are sensitive to physiological disruptors at early life stages, exposure during this period can have important implications to development and therefore fitness. Embryonic exposure particularly can lead to organizational effects (Guillette et al., 1995). Latent, and sometimes transgenerational, effects of early developmental stage exposures to some EDCs have been examined in humans, model organisms, and wildlife (reviewed in Anway and Skinner, 2006; Colborn et al., 1993; Fenton, 2006; Guillette et al., 1995), but few of these address reproductive behaviors.

I tested the effects of prenatal exposure to atrazine on mating behaviors in male guppies (*Poecilia reticulata*). Here, I particularly exposed guppies to the disruptor only during embryonic development to examine the latent effects manifested at adulthood. Atrazine was chosen as

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the focal contaminant to study because it is the second most commonly used pesticide in the US (Grube et al., 2011), and so exposure to this herbicide is common for many wildlife species in the US. The half-life of atrazine in surface waters can be over 700 days (Comber, 1999; Solomon et al., 1996), an interval that can span a part or all of the life of many aquatic and semi-aquatic animals. Depending on the type of crop and application rate, atrazine concentrations in water bodies around agricultural fields are expected to be in the range of 19–194 ppb over a 90 day period (US EPA, 2006). It has been hypothesized that atrazine induces aromatization of testosterone to estradiol (Fan et al., 2007; Hayes, 2005), but this mechanism has been debated (Hecker et al., 2005). Several studies have demonstrated the feminizing effects of atrazine in amphibians (Hayes et al., 2002a, 2002b, 2003, 2010), fish (Shenoy, 2012), and other animal taxa (Rohr and McCoy, 2010). Shenoy (2012) showed that long-term exposure of adult male guppies to atrazine altered mating behaviors, which is possibly an activational effect. The effects of atrazine-exposure during early life stages have been studied on various end-points at later life stages, such as immunocompetence (Rohr et al., 2013; Rowe et al., 2006), development of reproductive tract (Stanko et al., 2010), survival, desiccation risk, and moisture-regulating behaviors (Rohr and Palmer, 2005, 2013); but the link between embryonic exposure to atrazine and the development (and later manifestation) of sexual behaviors is not clear.

I hypothesized that exposure to environmentally relevant doses of atrazine during embryonic development would have the following effects at adulthood: (1) reduced likelihood and frequency of males performing mating behaviors such as courtship displays, gonopodium swings, and forced copulatory attempts (these were considered behaviors related to mating effort); (2) reduced frequency of behaviors related to mating effort and male–male aggression in the presence of competing males; and (3) reduced attractiveness of exposed males in the context of female mate choice. The second hypothesis was tested because it is ecologically relevant to examine behaviors in the context of intra-sexual competition for mates. Also, in a realistic mating arena, one could expect that different individuals would be impacted by EDCs to different degrees because of the differential spatial and temporal distribution of contaminants, and because individuals from different locations may converge at a breeding site. To account for the fact that males exposed to varying degrees would be competing with each other, and females may choose between such males, I tested the second and third hypotheses by pairing treated males with those that were not exposed to the contaminants. This also standardized the condition of each experimental male's opponent. These hypotheses test the effects of low dose exposures at early life stages on wildlife reproductive health.

## Methods

### Study system

I used guppies (*P. reticulata*) as a model organism to test my hypotheses. Guppies are small tropical fish native to Trinidad and parts of South America. They have distinct sexual dimorphism, their mating signals and behaviors have been well characterized (Houde, 1997), and the role of sex hormones in the expression of these traits has been explored (Hallgren et al., 2006; Jayasooriya et al., 2002). Further, guppies have been used for testing similar questions in other ecotoxicological studies (Baatrup and Junge, 2001; Kristensen et al., 2005; Toft and Baatrup, 2001).

Guppies are especially useful for testing hypotheses related to sexual selection. Females are viviparous and hence suited for testing the effects of maternal exposures. Males have different colored spots on their body and fins (Houde, 1997); they perform characteristic courtship displays (called "sigmoid" displays) and attempt forced copulations. Mating is predominantly through female mate choice: females respond to courtship displays and to males with larger and brighter orange spots (Houde, 1997; Kodric-Brown and Nicoletto, 2001), but avoid forced copulatory attempts (Evans et al., 2003; Houde, 1997). Fertilization is internal, and males have a modified anal fin called the gonopodium, which is used as a copulatory organ. Males frequently swing their gonopodium forward (which I term a "gonopodium swing"), and this appears to increase in frequency during mating or aggressive interactions. Displaying the copulatory organ as an ornament is common in poeciliids (Basolo, 1995; Langerhans et al., 2005; Schlosberg et al., 1949). Gonopodium swings are different from gonopodium thrusts (Houde, 1997) in that the swings are a forward movement of the gonopodium without an attempt to make contact with the female's vent; these actions can occur even when the male is far from the female, courting a female, or displaying to a rival male. A gonopodium thrust on the other hand is a copulatory attempt where the male thrusts his gonopodium towards the female's vent in an attempt to transfer sperm (Houde, 1997), which may or may not be preceded by courtship.

Adult female guppies used for this study were descendants of wild-caught guppies from Trinidad. Three populations – Aripo Upper River, Aripo Lower River, and Guanapo Upper River – were equally represented in all treatments to account for geographic and genetic variation.

### Treatments

40 adult female guppies were randomly assigned to one of the four treatment levels at 10 fish per level. Before the treatment was administered, each female was housed with a separate male on each of four consecutive days to improve brood characteristics (Edvardsson and Arnqvist, 2000). The treatment levels included a negative control (no manipulation), dimethylsulfoxide (DMSO, 83.3 μL/L) as the solvent control, atrazine low-dose (1 μg/L), and atrazine high-dose (15 μg/L). A solvent control was used because atrazine was dissolved in DMSO; all treatments received the same concentration of DMSO. Atrazine concentrations used were based on US EPA estimated environmental concentrations (US EPA, 2006). Concentration of atrazine in the water column in three randomly selected jars per treatment was ascertained by liquid phase extraction with methylene chloride following an adaptation of the US EPA Method 619 (US EPA, 1993) – which produced 95% recovery of the target compound – and analyzed by gas chromatography/mass spectrometry. Water was changed twice a week with static renewal of chemicals. The average concentration at the end of 4 days was determined to be 13.56 μg/L for the high-dose with negligible loss over 4 days. I was unable to analyze atrazine concentrations in the low-dose treatments, but in other experiments (Shenoy, 2012), I have found there to be negligible loss over 4 days for this concentration as well. No atrazine was detected in the control samples. Atrazine (98% purity) was purchased from Chem Service, Inc., through Fisher Scientific. Treatments continued until a brood was produced (which ranged from 6 to 88 days) to simulate a long-term exposure. Offspring born to treated females were raised to adulthood with no further treatment.

### Animal care

During the period of the study, all female adult fish that were being treated were housed separately in individual glass bowls with 3 L of aged (for at least 24 h), pre-aerated, carbon filtered, conditioned water (conditioned with AmQuel® and NovAqua® by Kordon, LLC). Fish were fed with tropical fish flake food once each day and brine shrimp nauplii once each day in *ad libitum* quantities. Room temperature was maintained at an average of 25 °C; the light:dark cycle was set to 12:12 h. Mortality was recorded every day. Bowls were checked twice daily for offspring.

Offspring born to treated females were immediately removed from the mother's bowl. Brood sizes ranged from 1 to 16, with the number of males per brood ranging from 1 to 7. At early developmental stages (before development of sex-specific traits), each brood was housed together in a plastic tank with 6 L of aged, pre-aerated, carbon filtered,