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Risky business: Changes in boldness behavior in male Siamese fighting fish, *Betta splendens*, following exposure to an antiandrogen[☆]

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ABSTRACT

Components of boldness, such as activity level and locomotion, influence an individual's ability to avoid predators and acquire resources, generating fitness consequences. The presence of endocrine disrupting chemicals (EDCs) in the aquatic environment may affect fitness by changing morphology or altering behaviors like courtship and exploration. Most research on EDC-generated behavioral effects has focused on estrogen mimics and reproductive endpoints. Far fewer studies have examined the effects of other types of EDCs or measured non-reproductive behaviors. EDCs with antiandrogenic properties are present in waterways yet we know little about their effects on exposed individuals although they may produce effects similar to those caused by estrogen mimics because they act on the same hormonal pathway. To examine the effects of antiandrogens on boldness, this study exposed male Siamese fighting fish, *Betta splendens*, to a high or low dose of one of two antiandrogens, vinclozolin or flutamide, and observed behavior in three boldness assays, both before and after exposure. Overall, antiandrogen exposure increased boldness behavior, especially following exposure to the higher dose. Whether or not antiandrogen exposure influenced boldness, as well as the nature and intensity of the effect, was assay-dependent. This demonstrates the importance of studying EDC effects in a range of contexts and, at least within this species, suggests that antiandrogenic compounds may generate distinct physiological effects in different situations. How and why the behavioral effects differ from those caused by exposure to an estrogen mimic, as well as the potential consequences of increased activity levels, are discussed.

Exposure to an antiandrogen, regardless of dose, produced elevated activity levels and altered shoaling and exploration in male Siamese fighting fish. These modifications may have fitness consequences.

1. Introduction

Human-induced environmental changes, such as eutrophication and climate change, may bring about behavioral changes in organisms that experience these phenomena, which could impact survival or reproduction (Blois et al., 2013; Sih, 2013; Wong and Candolin, 2015). One behavior that might have significant fitness implications if altered is boldness, the propensity to engage in risky behavior (Martel and Dill, 1995). Changes in boldness may affect the ability to find resources (Wilson et al., 1994; Dingle and Holyoak, 2001), dispersal rate (Cote et al., 2010), and predator avoidance (Werner and Anholt, 1993; Martel and Dill, 1995) among other behaviors and may impact species survival (Sih et al., 2012). For example, urbanization causes delicate skinks, *Lampropholis delicate*, to have elevated foraging and locomotion rates (Moule et al., 2016). Additionally, because boldness is linked to activity level and exploration, any changes in boldness could influence the likelihood that the affected individual continues to encounter the threat. One type of human-induced environmental change that has received particular attention is the presence of manmade pharmaceuticals, personal care products, and other pollutants in the environment. If exposure to one of these contaminants makes an individual more active, it would have an increased exposure encounter rate, which could result in an even greater change in boldness (Brodin et al., 2013; Montiglio and Royauté, 2014). Therefore, studying behavioral changes in aquatic organisms following exposure is especially important as even subtle changes may have significant impacts on

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survival and reproduction.

Endocrine disrupting chemicals (EDCs) enter the aquatic ecosystem from industrial, agricultural and domestic sources where they have the ability to interfere with the morphology, physiology, and behavior of organisms. The bulk of the studies on EDCs have focused on compounds with estrogenic properties; however, EDCs with antiandrogenic properties may also generate adverse effects in wildlife and humans (Söffker and Tyler, 2012). There are a number of reasons for why studies that expose aquatic organisms to antiandrogens rather than estrogen mimics are less common. Antiandrogenic compounds are less prevalent in waterways than compounds with estrogenic properties (WHO/UNEP, 2013; De Falco et al., 2015), which could lead to a misconception that these types of compounds are not currently a threat to wildlife or the environment. This is inaccurate as studies on bodies of water in the United Kingdom find that antiandrogens in the aquatic environment do indeed pose a significant risk to exposed individuals (Johnson et al., 2007; Pottinger et al., 2013). Additionally, it is often assumed that antiandrogenic and estrogenic EDCs have similar effects on exposed individuals, or that antiandrogen exposure has lesser consequences. Both antiandrogens and estrogen mimics act within the reproductive systems, targeting different points along the same hormonal pathway. Estrogen mimics influence estrogen receptors (Danzo, 1998; Mueller, 2004) while antiandrogens exert their effects by acting directly on androgen receptors, thereby reducing the synthesis and circulation of androgen hormones (Wilson et al., 2007). A recent study suggested that this may not be the method by which antiandrogens exert their effects and they may instead work by amplifying the feminizing effects of estrogen mimics (Lange et al., 2015). Finally, much of the work on antiandrogens has centered on secondary sexual characteristics. In such cases, minimal changes are found or the results are so variable across the species tested that no generalizations can be made (Wells and Van Der Kraak, 2000; Wilson et al., 2007; Green et al., 2015). The variation in findings could be due to differences in doses or exposure regimens across studies or life history differences among the species tested. These complications may make it appear that antiandrogen exposure is relatively harmless, yet this may not be the case when other endpoints, such as behavior, are examined.

In fact, studies on the effects of antiandrogen exposure do find that compounds with antiandrogenic properties may influence reproductive endpoints. The two antiandrogenic EDCs that have received the most attention are flutamide and vinclozolin. Flutamide is an antiandrogen used in chemotherapy to treat prostate cancer (Hotchkiss et al., 2002) while vinclozolin is an environmental pesticide with antiandrogenic properties (e.g. Comoretto et al., 2007; McKinlay et al., 2008). Exposure to antiandrogens may negatively affect testicular tissue (Tokarz, 1987; Jensen et al., 2004), sperm production (Bayley et al., 2003; Kinnberg and Toft, 2003), and alter male-typical behaviors like aggression and courtship (Baatrup and Junge, 2001; Bayley et al., 2002; Sebire et al., 2011). For example, chronic exposure 100 $\mu g\,L^{-1}$ or higher of flutamide reduced nest building and courtship and 500 $\mu g\,L^{-1}$ flutamide decreased spiggin production in male threespine stickleback (Sebire et al., 2008). Some studies suggest that other social behaviors may also be affected by antiandrogens (e.g. Flynn et al., 2001; Hotchkiss et al., 2003; Satre et al., 2009) but we are unaware of any research that has been done into whether activity level is impacted aside from one study in female rats (Flynn et al., 2001).

Siamese fighting fish, *Betta splendens*, is a common study system for both behavioral and ecotoxicology studies (e.g. Bronstein, 1984; Clotfelter et al., 2010; Stevenson et al., 2011; Dzieweczynski and Hebert, 2013). Siamese fighting fish have behaviors that are easy to observe and quantify, especially in terms of courtship and aggression (Simpson, 1968). Additionally, the endocrine system is evolutionary conserved across vertebrates (Bonett, 2016), and findings in this species may be applicable to other aquatic vertebrates. Exposing male Siamese fighting fish to an estrogen mimic produces many negative effects including reduced gonad size and sperm motility (Montgomery et al., 2012), suppressed male-typical behaviors (Clotfelter and Rodriguez, 2006; Dzieweczynski et al., 2014), and decreased behavioral variability and boldness (Dzieweczynski and Hebert, 2013; Hebert et al., 2014). Finally, the SSRI fluoxetine decreases aggression and boldness in this species (Lynn et al., 2007; Dzieweczynski and Hebert, 2012; Forsatkar et al., 2014; Dzieweczynski et al., 2016). These prior studies indicate that contaminants have adverse effects on Siamese fighting fish behavior and exposure to antiandrogens could produce similar effects.

Given the lack of studies that examine the potential behavioral consequences of antiandrogen exposure, the current study examines how various fitness-related behaviors in male Siamese fighting fish change following exposure to one of two compounds with antiandrogenic properties, flutamide and vinclozolin. Male Siamese fighting fish, *Betta splendens*, were randomly assigned to one of five experimental exposure groups: control, low and high vinclozolin (VINC), and low and high flutamide (FLUT). All subjects were then run through a series of three behavioral assays both before and after 21 days of exposure. We hypothesized that flutamide and vinclozolin would decrease behaviors related to boldness, such as activity level and time spent shoaling, in all of the assays. The reasons for this prediction were twofold. First, a previous study found that the estrogen mimic 17α-ethinylestradiol caused males to be less bold (Hebert et al., 2014). Second, estrogen mimics and antiandrogens target the same hormonal pathway and we, therefore, expected them to generate similar results. In relation to whether one of the two antiandrogens would have the same degree of influence, we did not have a specific prediction. Some studies find that flutamide is more potent than vinclozolin (Jolly et al., 2009) and others find the opposite (Pottinger et al., 2013), so we did not know if these antiandrogens will exert similar or dissimilar effects in our study.

2. Methods

2.1. Subjects

Domesticated Siamese fighting fish (*Betta splendens*) of the veil tail strain were purchased from a breeder at www.liveaquaria.com. A sample size of 150 male subjects was obtained (September 2015: 70, January 2016: 30; September 2016: 50) from this online breeder (sizes: 56.47–59.22 mm total length; 1.04–1.68 g) as well as 20 females that served as shoal fish and were purchased as needed throughout the experiment. Males were housed individually in 475 mL opaque containers to minimize stress resulting from the constant presence of male conspecifics. The females that were used as shoal fish were housed in 475 mL transparent containers until the start of testing because they prefer the presence of other females over isolation (Snekser et al., 2006). Just prior to testing, the females were placed in larger tanks of three females so that they would form shoals. For at least two weeks before testing, fish were acclimated to laboratory conditions (26 °C and a 12:12 light:dark cycle) and received complete water changes weekly during this period. Subjects were fed Hikari Bio-Pure freeze-dried bloodworms *ad libitum* every other day. No food was given the day before testing to increase motivation to explore the environments.