Hormones and Behavior 70 (2015) 85–91

Contents lists available at ScienceDirect

Hormones and Behavior

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

Sex in troubled waters: Widespread agricultural contaminant disrupts reproductive behaviour in fish

Michael G. Bertram [a,*], Minna Saaristo [a,b], John B. Baumgartner [c], Christopher P. Johnstone [a], Mayumi Allinson [d], Graeme Allinson [d,e], Bob B.M. Wong [a]

[a] School of Biological Sciences, Monash University, Victoria, Australia
[b] Department of Biosciences, Åbo Akademi University, Turku, Finland
[c] ARC Centre of Excellence for Environmental Decisions, School of Botany, The University of Melbourne, Victoria, Australia
[d] Centre for Aquatic Pollution Identification and Management (CAPIM), The University of Melbourne, Bio21 Institute, Victoria, Australia
[e] Department of Environment and Primary Industries (DEPI), Victoria, Australia

ARTICLE INFO

Article history:
Received 4 October 2014
Revised 11 March 2015
Accepted 13 March 2015
Available online 19 March 2015

Keywords:
Endocrine disrupting chemical
EDC
Hormonal growth promotant
17β-Trenbolone
Trenbolone acetate
Guppy
Poecilia reticulata
Behavioural ecotoxicology
Sexual selection
Reproductive behaviour

ABSTRACT

Chemical pollution is a pervasive and insidious agent of environmental change. One class of chemical pollutant threatening ecosystems globally is the endocrine disrupting chemicals (EDCs). The capacity of EDCs to disrupt development and reproduction is well established, but their effects on behaviour have received far less attention. Here, we investigate the impact of a widespread androgenic EDC on reproductive behaviour in the guppy, *Poecilia reticulata*. We found that short-term exposure of male guppies to an environmentally relevant concentration of 17β-trenbolone—a common environmental pollutant associated with livestock production—influenced the amount of male courtship and forced copulatory behaviour (sneaking) performed toward females, as well as the receptivity of females toward exposed males. Exposure to 17β-trenbolone was also associated with greater male mass. However, no effect of female exposure to 17β-trenbolone was detected on female reproductive behaviour, indicating sex-specific vulnerability at this dosage. Our study is the first to show altered male reproductive behaviour following exposure to an environmentally realistic concentration of 17β-trenbolone, demonstrating the possibility of widespread disruption of mating systems of aquatic organisms by common agricultural contaminants.

Introduction

Chemical pollutants have accumulated in ecosystems globally, endangering wildlife, ecosystem function and human health (Schwarzenbach et al., 2006). One class of chemical pollutant, known as endocrine disrupting chemicals (EDCs), comprises environmental contaminants with the capacity to disrupt the natural hormonal functioning of organisms (Colborn et al., 1993). Endocrine disruptors are of particular concern given their extreme potency, with exposure to concentrations as low as nanograms per litre having deleterious effects, as well as the propensity of some EDCs to bioaccumulate, persist temporally and act transgenerationally (Diamanti-Kandarakis et al., 2009). Conventionally, studies in ecotoxicology have focussed on direct mortality and chronic sub-lethal effects of EDCs on development and reproduction (Melvin and Wilson, 2013). However, EDCs can also induce alarming changes in behaviour. Indeed, the particular sensitivity of behaviour to EDCs has driven recent interest in behavioural ecotoxicology as a tool for investigating endocrine disruption at environmentally relevant pollutant concentrations (reviewed in Melvin and Wilson, 2013). Existing studies in behavioural ecotoxicology typically focus on EDCs that disrupt gonadal steroid signalling by interacting with vertebrate estrogen or androgen receptors, as chemical interference with this pathway has the potential to disrupt sexual selection (e.g., Saaristo et al., 2009). However, the vast majority of these efforts have concentrated on EDCs with estrogenic activity. This is surprising because the handful of studies that have considered androgenic EDCs suggest that they are also capable of markedly altering animal behaviour (e.g., Hoffmann and Kloas, 2012).

An androgenic EDC of particular concern is 17β-trenbolone, the most bioactive metabolite of trenbolone acetate, a hormonal growth promotant used extensively in livestock production around the world (Kolodziej et al., 2013). Trenbolone acetate is a powerful steroid, with androgenic and anabolic potency 15–50 times greater than testosterone (Kolodziej et al., 2013; Neumann, 1976). Its metabolite 17β-trenbolone acts as a powerful androgen receptor agonist in the environment, is highly temporally persistent (with a half-life of approximately 260 days;

* Corresponding author at: School of Biological Sciences, Monash University, Senior Zoology Bldg. 18, Wellington Road, Clayton Campus, Victoria 3800, Australia.
E-mail address: michael.g.bertram@monash.edu (M.G. Bertram).

http://dx.doi.org/10.1016/j.yhbeh.2015.03.002
0018-506X/

Schiffer et al., 2001) and has been repeatedly detected in aquatic environments associated with feedlot operations. Detected concentrations of 17β-trenbolone range from ≤20 ng/L in diffuse run-off (Durhan et al., 2006), to as high as 162 ng/L in fields directly receiving effluent (Gall et al., 2011). Recent studies report that exposure to 17β-trenbolone adversely impacts physiological and morphological endpoints in fish species (e.g., Morthorst et al., 2010). However, despite the potency and widespread global use of 17β-trenbolone, very little is known about its effects on behaviour. This is concerning as the ability of animals to produce and maintain behaviour appropriate to their environment is fundamental for survival and reproduction, so that disruption of these behaviours can have dire ecological and evolutionary consequences (reviewed in Candolin and Wong, 2012).

The mating system of the guppy, *Poecilia reticulata*, is ideal for investigating the effects of 17β-trenbolone on reproductive behaviour. The guppy is a small, live-bearing freshwater fish native to north-eastern South America that has a widespread global distribution, precipitated by numerous deliberate and accidental introductions (Lindholm et al., 2005). Importantly, throughout their range, guppies have the potential to be exposed to 17β-trenbolone, as they are known to inhabit water bodies receiving agricultural waste (e.g., Araújo et al., 2009; López-Rojas and Bonilla-Rivero, 2000; Widianarko et al., 2000). Male guppies employ two alternate mating strategies, either soliciting copulations from females through courtship ('sigmoid displays') or coercing copulations through unsolicited 'sneaking' behaviour (Luyten and Liley, 1991). The latter involves males surreptitiously approaching females from behind to insert their gonopodium (a modified anal fin serving as an intromittent organ) into the female's genital pore (Luyten and Liley, 1991). Female guppies are choosy and are known, for example, to prefer males possessing greater orange pigmentation (Houde, 1987). By preferentially associating with certain males over others, females are able to directly influence mating outcomes (Shohet and Watt, 2004).

Here we test the hypothesis that short-term (21-day) exposure to an environmentally relevant concentration of 17β-trenbolone (22 ng/L) alters male and female reproductive behaviour in guppies. A short-term exposure duration was employed as agricultural pollutants often enter aquatic habitats in pulses and these temporally discrete contamination events can have persistent consequences (García et al., 2011; Morthorst et al., 2010).

Materials and methods

Ethical statement

The research detailed in this paper was approved by the Biological Sciences Animal Ethics Committee of Monash University (permit number: BSCI/2013/09) and complies with all relevant State and Federal laws of Australia.

Animal housing

This study used laboratory-reared descendants of wild caught guppies from Alligator Creek (19° 26 18 S, 146° 57 01 E), Queensland, Australia. Sexually mature guppies reared in large mixed-sex holding tanks (90 cm × 45 cm × 45 cm) were assumed to be non-virginal given the near-constant mating pressure exerted by males in mixed-sex populations (Houde, 1997; Magurran and Seghers, 1994). Non-virgin fish were used to simulate mixed-sex wild populations, and because mate choice in virgin females can be indiscriminate (Pitcher et al., 2003). Fish were separated by sex into 81 L housing tanks (60 cm × 45 cm × 30 cm) and acclimated to laboratory conditions (25–27 °C; 12:12 h light regime) for 2 months. Fish were fed once daily (Otohime Hirame larval diet; 580–910 μm).

Exposure set-up and monitoring

A flow-through exposure design was used, as described by Saaristo et al. (2013). Fish were assigned to identical 54 L separate-sex aquaria (60 cm × 30 cm × 30 cm), which were monitored for temperature ($\bar{x}$ = 26.38 °C, SD = 0.52 °C) and flow-through rates ($\bar{x}$ = 18.88 mL/min, SD = 0.59 mL/min). In total, 308 fish were randomly assigned to one of seven 17β-trenbolone-exposure tanks, or one of seven unexposed tanks containing fresh water (22 fish per tank). Exposed and unexposed aquaria each comprised four male tanks and three female tanks, with a surplus of fish exposed to ensure adequate sample sizes for each treatment.

The concentration of 17β-trenbolone used ($\bar{x}$ = 22 ng/L, SD = 14.55 ng/L, n = 28) was monitored following Saaristo et al. (2013), with some modifications, using a commercial enzyme-linked immunosorbent assay (ELISA). Weekly water samples were drawn according to the protocol detailed by Saaristo et al. (2013).

Behaviour trials

To investigate the impact of 17β-trenbolone on the reproductive behaviour of guppies, four treatments were employed: (1) unexposed male paired with unexposed female (control; hereafter UU; n = 18), (2) unexposed male with exposed female (UE; n = 19), (3) exposed male with unexposed female (EU; n = 18), and (4) exposed male with exposed female (EE; n = 20). Fish were taken at random and equally from each exposure tank and allocated to behavioural trials (n = 75), which took place in 54 L observation tanks (60 cm × 30 cm × 30 cm) containing fresh water (i.e. water free from 17β-trenbolone). Trials involved a 5-minute period of acclimation, before both fish were released from holding containers and allowed to freely interact, while their behaviour was video-recorded for 15 min. Fish were euthanized immediately after trials using an overdose (40 mg/L) of anaesthetic clove oil, following which morphological and colouration analyses were conducted.

Reproductive behaviours (see Supplementary materials Table S1 for detailed descriptions) were quantified from video recordings using the event-recording software JWatcher V1.0 (Blumstein and Daniel, 2007). Briefly, for males, we counted the number of courtship bouts performed (i.e., male orienting in front of the female and performing courtship displays), as well as the number of sneaking attempts (i.e., male surreptitiously approaching the female from behind for forced copulation). For females, we counted the number of times that a female actively associated with the male, a frequently used measure of mating intent in poeciliid fishes (e.g., Kahn et al., 2010), including guppies (e.g., Shohet and Watt, 2004).

Morphological analysis

After behavioural trials, we measured the length of males and females (±0.01 mm). Males were also weighed (±0.0001 g), and an index of male condition was derived from a regression of the mass (g) of all males against their standard length (mm). This male Condition Index was calculated as the residuals from the least squares regression line (i.e., weight = −0.164 + 0.016 × length).

Colouration analysis

Because female guppies typically prefer males with greater orange pigmentation (e.g., Houde, 1987)—including in the population from which fish were sourced for the present study (Brooks and Endler, 2001; Gamble et al., 2003)—the percentage of males' body surface containing orange pigments was assessed using photographic colouration analysis, performed immediately after behavioural trials. After euthanasia, fish were photographed on the right side in a