



Exposure to an agricultural contaminant, 17 β -trenbolone, impairs female mate choice in a freshwater fish



Patrick Tomkins^{a,*}, Minna Saaristo^{a,b}, Mayumi Allinson^c, Bob B.M. Wong^a

^a School of Biological Sciences, Monash University, Victoria, Australia

^b Department of Biosciences, Åbo Akademi University, Turku, Finland

^c Centre for Aquatic Pollution Identification and Management (CAPIM), School of Chemistry, The University of Melbourne, Victoria, Australia

ARTICLE INFO

Article history:

Received 29 June 2015

Received in revised form

15 September 2015

Accepted 29 September 2015

Available online 9 October 2015

ABSTRACT

Despite the pivotal role sexual selection plays in population dynamics and broader evolutionary processes, the impact of chemical pollution on female mate choice is poorly understood. One group of chemical contaminants with the potential to disrupt the mechanisms of female mate choice is endocrine disrupting chemicals (EDCs); a broad class of environmental pollutants that can interfere with the endocrinology of organisms at extremely low concentrations. Recent research has revealed that estrogenic EDCs can affect female mate choice in fish, but the impact of androgenic EDC exposure is yet to be studied. To address this, we investigated the effects of an environmentally relevant concentration of trenbolone – an androgenic steroid used as a growth promoter in the cattle industry – on female mate choice in wild-caught guppies (*Poecilia reticulata*). We exposed male and female guppies to 17 β -trenbolone for 21 days (measured concentration 4 ng/L) via a flow-through system, and found that trenbolone-exposed female guppies spent less time associating with males, and were less choosy, compared to unexposed females. In contrast, trenbolone had no impact on male reproductive behavior or morphology. This is the first study to show that androgenic EDC exposure can disrupt female mate choice, highlighting the need for studies to investigate the behavioral impacts of environmental contaminants on both sexes.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Sexual selection, by influencing the quality and quantity of offspring produced, has important implications for population dynamics, ecosystem function, and broader evolutionary processes (reviewed in Candolin and Wong, 2012). One of its key mechanisms is female mate choice (Andersson and Simmons, 2006), which is known to confer a suite of direct (i.e., material) and indirect (i.e., genetic) benefits to choosy individuals (Andersson, 1994; Kokko et al., 2003). The traits that females use to evaluate male quality are finely attuned to the local environmental conditions in which they have evolved (Wong and Candolin, 2015). As a result, the mechanisms of female mate choice are particularly vulnerable to anthropogenic, environmental change. Although, the majority of studies have focused on how altering the physical and auditory environment can interfere with these mechanisms (e.g., Slabbekoorn and Peet, 2003; Wong et al., 2007), changing the

chemical environment can also disrupt female mate choice (e.g., Fisher et al., 2006). This is not surprising given the profound influence chemical pollution can have on morphology, physiology and behavior (Clotfelter et al., 2004; Ward et al., 2008; Diamanti-Kandarakis et al., 2009), and the pivotal role chemical communication plays in the reproduction of a wide range of taxa (Johansson and Jones, 2007).

One group of chemical contaminants with the capacity to disrupt female mate choice is endocrine disrupting chemicals (EDCs). EDCs are ubiquitous in the environment and possess several characteristics that make them particularly concerning. They can persist in ecosystems, affect organisms at extremely low concentrations, have a tendency to bioaccumulate in the environment, and can act transgenerationally (Anway and Skinner, 2006; Crews et al., 2007; Walker and Gore, 2011). There is a plethora of research documenting the environmental impacts of EDCs, with studies traditionally concentrating on morphological and physiological effects. However, with the recognition of behavior as a particularly sensitive and powerful biomarker of EDC contamination (Melvin and Wilson, 2013), an increasing number of studies are also turning their attention to understand the behavioral impacts of EDCs. Recent research has revealed that EDCs can affect a range of behaviorally important

* Corresponding author at: School of Biological Sciences, Monash University Clayton Campus, Victoria, Australia.

E-mail address: patrick.tomkins@monash.edu (P. Tomkins).

processes, including cognition, boldness, sociality and reproduction (for a review see Clotfelter et al., 2004; Frye et al., 2012; Soffker and Tyler, 2012).

Surprisingly, despite growing interest in the impacts of EDCs on behavior, relatively little is known about how EDCs affect sexual selection and, more specifically, female mate choice. Studies have recently revealed, for instance, that EDC exposure can affect female mate choice in fish (Coe et al., 2008; Saaristo et al., 2009a). However, these studies have largely focused on only one group of EDCs—those with estrogenic properties. In comparison, the influence of androgenic EDC exposure on sexual selection has yet to be investigated.

One androgenic EDC with the potential to impact sexual selection is trenbolone, a synthetic steroid commonly used in many parts of the world to accelerate growth rates in beef cattle (Lange et al., 2002; Khan et al., 2008; Morthorst et al., 2010). Trenbolone enters the environment as 17 β -trenbolone, and has been detected in aquatic environments associated with cattle feedlots at concentrations ranging from <20 ng/L (Durhan et al., 2006) to as high as 162 ng/L (Gall et al., 2011). 17 β -trenbolone is an extremely stable compound, with a half-life of up to 260 days measured in animal waste (Durhan et al., 2006), and is particularly potent, binding to androgen receptors with three times the affinity of testosterone (Khan et al., 2008). Exposure to 17 β -trenbolone can have severe implications for the sexual morphology of fish (eg: reduced fecundity in fathead minnows, *Pimephales promelas*: Ankley et al., 2003; Jensen et al., 2006; sex reversal in zebrafish, *Danio rerio*: Larsen and Baatrup, 2010), but its behavioral consequences are poorly understood. Although, recent research has found that trenbolone can influence the reproductive behavior of male and female Poeciliid fishes (Saaristo et al., 2013; Bertram et al., 2015), it is still unknown whether these behavioral changes may impact the mechanisms of sexual selection and female mate choice.

Our study species was the guppy (*Poecilia reticulata*)—a small, viviparous fish commonly found in freshwater environments contaminated with EDCs (López-Rojas and Bonilla-Rivero, 2000; Widianarko et al., 2000). Guppies are native to north-eastern South America but have become invasive throughout the world as a result of both deliberate and accidental introductions (Lindholm et al., 2005). The mating system of the guppy makes them an ideal candidate for investigating the effects of EDCs on sexual selection. Female guppies are choosy and actively associate with preferred males, which can have a direct influence on mating outcomes (Godin and Briggs, 1996; Shenoy, 2012). Females are known to favor a number of male traits including increased orange pigmentation, size, and display rate (Endler, 1980; Brooks and Caithness, 1995; Kodric-Brown and Nicoletto, 2001), all of which are honest indicators of male quality.

Here we test the hypothesis that short-term exposure to an environmentally relevant concentration of trenbolone will impact female mate choice in guppies. The male traits that females use to choose mates are under androgenic control (Wilson, 1999; Emerson, 2000), meaning they are likely to be influenced by trenbolone. This, combined with the fact that trenbolone is known to affect reproduction in Poeciliids, suggests that exposure should also affect female mate choice.

2. Methods

2.1. Collection and housing

Guppies were collected with dip nets from Alligator Creek (19°26'17.94" S, 146°57'1.09" E) in Queensland, Australia. Alligator Creek is a rainforest-fed stream located in the pristine Bowling Green Bay National Park. Water samples taken from this site over

consecutive years revealed no contamination from estrogenic or androgenic EDCs (ALS global, unpublished data), thus ensuring that fish used in this study were from an uncontaminated source. Fish were transported to Monash University via airfreight and were acclimated to laboratory conditions (26 °C, 12:12 h light regime) for 2 months prior to exposure. Fish were fed *ad libitum* once daily with commercial fish pellets (Otohime Hirame larval diet; 580–910 μ m).

2.2. Exposure

After acclimation, fish were exposed to trenbolone for 21 days, as previous experiments have shown that exposure periods ranging from 14 to 28 days are sufficient to induce behavioral changes (Bayley et al., 1999; Bell, 2001; Martinović et al., 2007; Majewski et al., 2002; Maunder et al., 2007; Oshima et al., 2003; Saaristo et al., 2009a,b). Furthermore, EDCs often enter the environment in pulses and may only remain in waterways for a short period of time (Diamanti-Kandarakis et al., 2009), meaning short exposure times are ecologically meaningful.

Guppies were exposed to trenbolone via a flow-through system based on the design of Saaristo et al. (2013). This system included eight 54 L (60 cm \times 30 cm \times 30 cm) tanks: 4 control tanks (containing a solvent control) and 4 trenbolone-exposed tanks (containing 17 β -trenbolone). A total of 160 fish (100 males, 60 females) were separated by sex and distributed between these eight tanks (i.e., 2 control and 2 trenbolone-exposed tanks for each sex). Guppies in the trenbolone-exposed tanks were exposed to trenbolone at a nominal concentration of 15 ng/L (measured concentration = 4 ng/L; see below for details on how trenbolone concentrations were monitored), while guppies in the control tanks were exposed to a solvent control (0.000013% ethanol—see below for details). All exposure tanks were maintained at a constant temperature between 25 and 27 °C, and fish were fed daily *ad libitum*.

Water entered these exposure tanks via a mixing tank, which received a constant flow of fresh, filtered tap water and a constant flow of either trenbolone (in the case of the trenbolone-exposed tanks) or the solvent solution (in the case of the control tanks) from a stock tank via a peristaltic pump (Watson Marlow 323 U/MC). Water was channeled into the exposure tanks using silicone tubing, and flow rates were kept constant (2.25 L/h) using flow meters (BES Flowmeters, MPB Series 1200) and adjustable valves.

2.3. Stock solution preparation

The trenbolone stock solution was created by first dissolving 17 β -trenbolone (4,9,11-estratrien-17-ol-3-one; Novachem, Germany) in 100% ethanol (solvent) to create a stock standard of 300 mg/L. This stock standard was then diluted to 600 μ g/L using deionized water, resulting in a solvent concentration of 0.2%. The stock solution was further diluted in the mixing tank in the flow-through system to achieve the desired nominal 17 β -trenbolone concentration of 15 ng/L (measured concentration: \bar{x} = 4 ng/L, SD = 1.4 ng/L, n = 14). A solvent solution of 0.2% was used in the control tanks, which was diluted in the exposure system to a concentration of 0.000013%.

2.4. Water analysis

In order to monitor trenbolone concentrations in the exposure tanks, and to ensure there was no contamination of control tanks, a 100 mL water sample was taken from each of the exposure tanks weekly and analysed using enzyme-linked immunosorbent assay (Trenbolone ELISA, EuroProxima, Arnhem, The Netherlands). For a detailed description of the ELISA testing protocol, see Saaristo et al. (2013).

2.5. Behavior trials

The impact of trenbolone exposure on female mate choice was investigated using a dichotomous choice experiment, which is a standard method used for investigating mate choice preferences in guppies (Pilastro et al., 2004; Jeswiet et al., 2012). A 51 L tank (60 cm × 30 cm × 24 cm) was split into three compartments using clear, perforated plastic dividers to allow water flow and full visual and chemical contact between compartments. A single female was placed in the middle compartment in a clear plastic cylinder, while a single male was placed in each of the end compartments. After a 10-min acclimation period, the cylinder was removed and the fish were allowed to interact for 15 min. All trials were filmed using a digital video recorder (Canon Powershot S110).

Two trial combinations were used to investigate the impacts of trenbolone on female mate choice: (1) an unexposed female was given a choice between an exposed and an unexposed male (hereafter referred to as ‘unexposed female trial’), and (2) an exposed female was given a choice between an exposed and an unexposed male (hereafter referred to as ‘exposed female trial’). There was no difference in the weight or length of exposed and unexposed males (weight: $t = 1.35$, $df = 58.95$, $p = 0.18$; length: $t = 1.05$, $df = 59.95$, $p = 0.33$, see Table 1 for details) or exposed and unexposed females (weight: $t = 0.18$, $df = 28.93$, $p = 0.86$; length: $t = 0.46$, $df = 28.64$, $p = 0.65$, see Table 1 for details) in either of the trial combinations. A total of 31 trials were conducted ($n = 15$ unexposed female trials, $n = 16$ exposed female trials).

Female preference was determined by quantifying the amount of time spent within a 5 cm ‘preference zone’ of either male compartment. Association time is commonly used as a measure of female preference in guppies (Kodric-Brown, 1985, 1989; Karino and Shinjo, 2004; Pilastro et al., 2004) and has been shown to be an accurate indicator of female mate choice in Poeciliid fishes (Walling et al., 2010). The courtship behavior (i.e., time spent conducting sigmoid displays and orienting their body toward that of the female) of both exposed and unexposed male fish was also quantified. We calculated the total time that males spent courting as females were in view of the males throughout the entire trial, meaning males courted even if females were not directly associating with them. Male and female behaviors were quantified using JWatcher v 1.0.

2.6. Morphological analysis

Male and female weight and length was measured directly after behavior trials. Males were also photographed on their right side in a standardized fashion (Nikon D90, shutter speed = 1/250, Nikon AF Micro-Nikkor 60 mm f/2.8D), and the resultant pictures analysed using Photoshop (CS6 version 13.0 Extended) to determine the total percentage of the body containing orange pigments. For a detailed description of the coloration analysis method, see Bertram et al. (2015).

2.7. Statistical analysis

All data were analyzed using the statistical program ‘R’ (version 2.13.1; R Development Team, 2011). Data was checked for normality and homogeneity of variance, and transformed where necessary. Independent samples t -tests were used to compare the amount of time that unexposed and exposed females spent associating with males. The amount of time that females spent associating with each male, and the courtship behavior of exposed and unexposed males, were compared in each treatment using paired t -tests. The weight and length of males and females was compared using independent samples t -tests, as was the percentage of orange pigmentation of males.

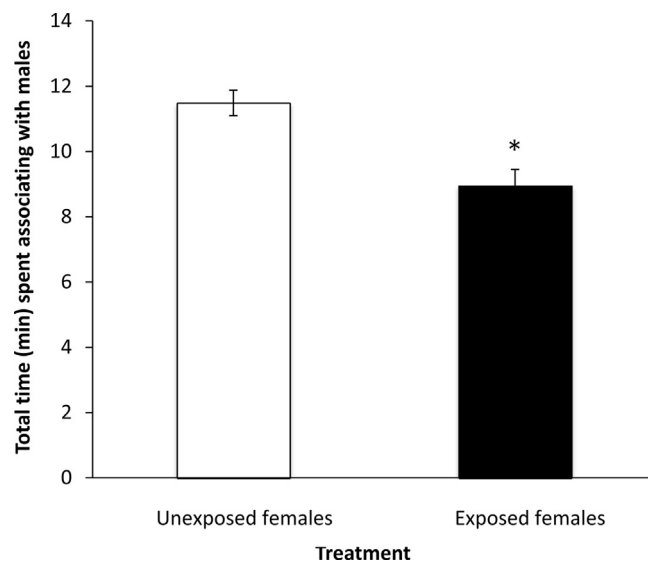


Fig. 1. Mean total time (\pm S.E.) that unexposed females ($n = 15$) and exposed ($n = 16$) females ($n = 16$) spent associating with males.

2.8. 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.

3. Results

3.1. Female behavior

Exposed females spent significantly less time associating with males than unexposed females ($t = 4.03$, $df = 28.05$, $p < 0.001$, Fig. 1). When they were associating with males, unexposed females spent significantly more time associating with unexposed males than exposed males ($t = 3.14$, $df = 14$, $p < 0.01$, Fig. 2), while exposed females showed no preference for either exposed or unexposed males ($t = 0.44$, $df = 15$, $p = 0.67$, Fig. 2).

3.2. Male behavior and morphology

There was no difference in the total time that exposed and unexposed males spent courting in both the unexposed female trial ($t = -0.66$, $df = 14$, $p = 0.52$, Table 2) and the exposed female trial ($t = -0.56$, $df = 15$, $p = 0.58$, Table 2). There was also no difference in the percentage of orange pigmentation between exposed and unexposed males in either treatment (unexposed female trial: $t = -0.37$, $df = 23.29$, $p = 0.71$, exposed female trial: $t = 0.1819$, $df = 29.81$, $p = 0.86$, Table 2).

4. Discussion

This is the first study to show that an androgenic agricultural contaminant can affect female mate choice. We found that unexposed females spent more time associating with males than trenbolone-exposed females, and also showed a preference for unexposed males over trenbolone-exposed males. Exposed females, on the other hand, showed no preference for either male. Surprisingly, trenbolone exposure had no impact on any of the male traits or behaviors examined in this study, with no difference observed in male body size, percentage of orange pigmentation, or courtship behavior of exposed and unexposed fish.

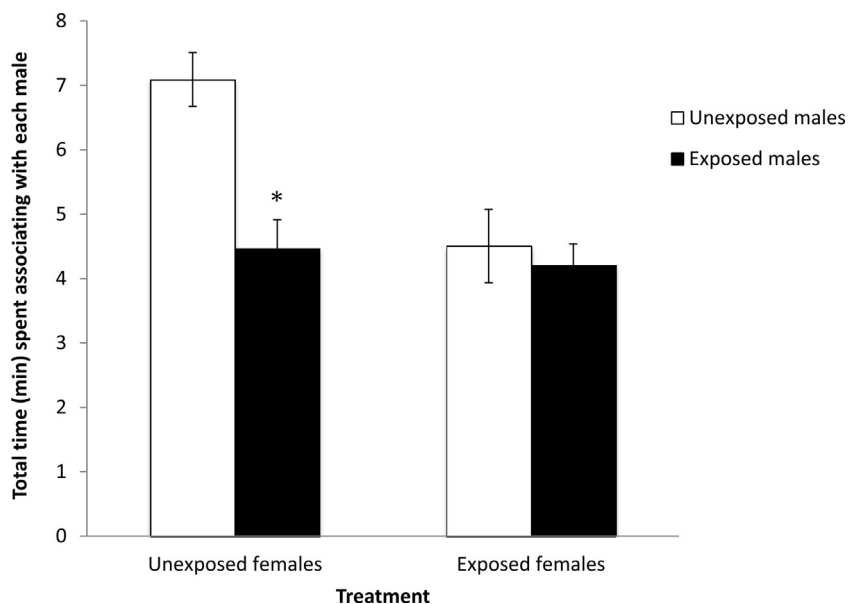


Fig. 2. Mean total time (\pm S.E.) that unexposed females ($n=15$) and exposed females ($n=16$) spent associating with exposed and unexposed males.

Trenbolone-exposed females spent less time associating with males than unexposed females, indicating a decreased desire to mate, and were also less choosy than unexposed females. Previous research has shown that trenbolone exposure can suppress estrogenic activity in female fish, causing varying levels of masculinization. Specifically, Ankley et al. (2003) observed reduced concentrations of vitellogenin and β -estradiol in trenbolone-exposed female fathead minnows, which was correlated with the development of male morphological characteristics. Trenbolone-induced masculinization has also been observed in female mosquitofish (*Gambusia holbrooki*; Sone et al., 2005) and zebra fish (Morthorst et al., 2010). Hence, it is conceivable that females in our study were masculinized to some degree, which could have reduced their desire to mate and made them less choosy.

In contrast to the exposed females, unexposed female guppies were choosy, and associated more with unexposed than exposed males. However, we did not find any differences between exposed and unexposed males in traits that have previously been found to influence female mate choice in guppies (i.e., color: Endler, 1980;

display rate: Kodric-Brown and Nicoletto, 2001). Although, it is not clear in this experiment what cue(s) unexposed females may have been using to discriminate against exposed males and to preferentially associate with unexposed males, previous research has shown that chemical cues play an important role in Poeciliid reproduction, including mate choice in guppies (Guevara-Fiore et al., 2009, 2010). It is possible that trenbolone exposure altered the chemical cues of male guppies in this study, which may explain female preference for unexposed males. This warrants further investigation into the effect of trenbolone exposure on chemical cues and its subsequent impact on reproductive behavior.

Trenbolone is a potent androgen agonist, meaning it has the potential to intensify the expression of male sexual traits by stimulating androgen production (Schiffer et al., 2001; Hotchkiss et al., 2008). This process of xenoandrogen-induced 'hypermasculinization' has been observed in multiple species. For example, androgenic EDC-exposed African clawed frogs, *Xenopus laevis*, exhibited increased levels of androgen-dependent male mate calling (Hoffmann and Kloas, 2010), while androgenic EDC

Table 1
Total length and weight of females and males (mean \pm S.E.) used in behavior trials (TB—trenbolone).

Trial combination	n	Mean length (mm) \pm S.E.	Mean weight (mm) \pm S.E.
Unexposed female trial			
Unexposed males	15	21.22 \pm 0.402	0.09 \pm 0.002
TB-exposed males	15	21.58 \pm 0.359	0.10 \pm 0.004
Unexposed females	15	24.14 \pm 0.663	0.16 \pm 0.016
Exposed female trial			
Unexposed males	16	21.68 \pm 0.365	0.09 \pm 0.004
TB-exposed males	16	21.35 \pm 0.450	0.10 \pm 0.003
TB-exposed females	16	24.61 \pm 0.769	0.16 \pm 0.016

Table 2
Total percentage of orange pigmentation and total time spent courting by males used in behavior trials (TB—trenbolone).

Trial combination	n	Mean% orange pigmentation \pm S.E.	Mean time spent courting \pm S.E. (min)
Unexposed female trial			
Unexposed males	15	9.20 \pm 0.678	7.12 \pm 0.929
TB-exposed males	15	9.66 \pm 1.341	7.82 \pm 0.578
Exposed female trial			
Unexposed males	16	9.68 \pm 1.101	6.58 \pm 0.838
TB-exposed males	16	9.37 \pm 1.078	7.25 \pm 0.981

exposure was found to increase the intensity of male sexual behaviors in several cyprinid fish species (Belanger et al., 2010). Why, then, were the male sexual traits in this experiment not influenced by trenbolone exposure? It has been well established that EDCs can influence the percentage of orange coloration in male guppies (Bayley et al., 2002, 2003; Shenoy 2012; Tian et al., 2012). However, the vast majority of these studies exposed guppies before they became sexually mature, while in this study only mature adults were exposed. Male courtship behavior was also unaffected by trenbolone exposure, but this result is more difficult to interpret. Recent studies investigating the impact of trenbolone on the courtship behavior of male Poeciliids have yielded contrasting results. Saaristo et al. (2013) found that short-term exposure to an environmentally relevant concentration of trenbolone (6 ng/L, 21 day exposure) had no impact on the reproductive behavior of male mosquitofish, while Bertram et al. (2015) found that exposure to 22 ng/L of trenbolone for 21 days had a significant impact on the amount of time that male guppies spent courting and conducting coercive mating behaviors. This suggests that more research is required in order to gain a more comprehensive understanding of the impact of trenbolone on reproductive behavior.

In conclusion, this is the first study to show that an androgenic EDC can impact sexual selection by disrupting the mechanisms of female mate choice. We found that females exposed to an environmentally relevant concentration of trenbolone associated with males less than unexposed females and were also less choosy. Female mate choice can have important consequences at the population level by influencing both the quality and quantity of offspring produced (Wong and Candolin, 2015), highlighting the need for studies to investigate the behavioral impacts of environmental contaminants on both sexes.

Acknowledgements

We thank Bruce Tomkins for his assistance in the field and during the exposure period. Funding for this research was provided by the Academy of Finland (265269, to MS), and the Australian Research Council (DP130100385, to BMW).

References

- Andersson, M., Simmons, L.W., 2006. Sexual selection and mate choice. *Trends Ecol. Evol.* 21, 296–302.
- Andersson, M., 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Ankley, G.T., Jensen, K.M., Makynen, E.A., Kahl, M.D., Korte, J.J., Hornung, M.W., Wilson, V.S., 2003. Effects of the androgenic growth promoter 17- β -trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environ. Toxicol. Chem.* 22, 1350–1360.
- Anway, M.D., Skinner, M.K., 2006. Epigenetic transgenerational actions of endocrine disruptors. *Endocrinol.* 147, 43–49.
- Bayley, M., Nielsen, J.R., Baatrup, E., 1999. Guppy sexual behavior as an effect biomarker of estrogen mimics. *Ecotoxicol. Environ. Saf.* 43, 68–73.
- Bayley, M., Junge, M., Baatrup, E., 2002. Exposure of juvenile guppies to three antiandrogens causes demasculinization and a reduced sperm count in adult males. *Aquat. Toxicol.* 56, 227–239.
- Bayley, M., Larsen, P.F., Baekegaard, H., Baatrup, E., 2003. The effects of vinclozolin, an anti-androgenic fungicide, on male guppy secondary sex characters and reproductive success. *Biol. Reprod.* 69, 1951–1956.
- Belanger, R.M., Pachkowski, M.D., Stacey, N.E., 2010. Methyltestosterone-induced changes in electro-olfactogram responses and courtship behaviors of cyprinids. *Chem. Senses* 35, 65–74.
- Bell, A.M., 2001. Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback, *Gasterosteus aculeatus*. *Anim. Behav.* 62, 775–780.
- Bertram, M.G., Saaristo, M., Baumgartner, J.B., Johnstone, C.P., Allinson, M., Allinson, G., Wong, B.B.M., 2015. Sex in troubled waters: Widespread agricultural contaminant disrupts reproductive behavior in fish. *Horm. Behav.* 70, 85–91.
- Brooks, R., Caithness, N., 1995. Female choice in a feral guppy population: are there multiple cues? *Anim. Behav.* 50, 301–307.
- Candolin, U., Wong, B.M., 2012. Sexual selection in changing environments: consequences for individuals and populations. In: Candolin, U., Wong, B.M. (Eds.), *Behavioural Responses to a Changing World: Mechanisms and Consequences*. Oxford University Press, United Kingdom, pp. 201–213.
- Clotfelter, E.D., Bell, A.M., Levering, K.R., 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. *Anim. Behav.* 68, 665–676.
- Coe, T.S., Hamilton, P.B., Hodgson, D., Paull, G.C., Stevens, J.R., Sumner, K., Tyler, C.R., 2008. An environmental estrogen alters reproductive hierarchies: disrupting sexual selection in group-spawning fish. *Environ. Sci. Technol.* 42, 5020–5025.
- Crews, D., Gore, A.C., Hsu, T.S., Dangleben, N.L., Spinetta, M., Schallert, T., Skinner, M.K., 2007. Transgenerational epigenetic imprints on mate preference. *Proc. Natl. Acad. Sci. U. S. A.* 104, 5942–5946.
- Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. *Endocr. Rev.* 30, 293–342.
- Durhan, E.J., Lambright, C.S., Makynen, E.A., Lazorchak, J., Hartig, P.C., Wilson, V.S., Ankley, G.T., 2006. Identification of metabolites of trenbolone acetate in androgenic runoff from a beef feedlot. *Environ. Health Perspect.* 114, 65.
- Emerson, S.B., 2000. Vertebrate secondary sexual characteristics—physiological mechanisms and evolutionary patterns. *Am. Nat.* 156, 84–91.
- Endler, J.A., 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 76–91.
- Fisher, H., Wong, B.M., Rosenthal, G., 2006. Alteration of the chemical environment disrupts communication in a freshwater fish. *Proc. R. Soc. B: Biol. Sci.* 1591, 1187–1193.
- Frye, C., Bo, E., Calamandre, G., Calza, L., Dessì-Fulgheri, F., Fernandez, M., Le Page, Y., 2012. Endocrine disruptors: a review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. *J. Neuroendocrinol.* 24, 144–159.
- Gall, H.E., Sassman, S.A., Lee, L.S., Jafvert, C.T., 2011. Hormone discharges from a Midwest tile-drained agroecosystem receiving animal wastes. *Environ. Sci. Technol.* 45, 8755–8764.
- Godin, J.G., Briggs, S.E., 1996. Female mate choice under predation risk in the guppy. *Anim. Behav.* 51, 117–130.
- Guevara-Fiore, P., Skinner, A., Watt, P.J., 2009. Do male guppies distinguish virgin females from recently mated ones? *Anim. Behav.* 77, 425–431.
- Guevara-Fiore, P., Stapley, J., Krause, J., Ramnarine, I.W., Watt, P.J., 2010. Male mate-searching strategies and female cues: how do male guppies find receptive females? *Anim. Behav.* 79, 1191–1197.
- Hotchkiss, A.K., Rider, C.V., Blystone, C.R., Wilson, V.S., Hartig, P.C., Ankley, G.T., Gray, L.E., 2008. Fifteen years after “Wingspread”—environmental endocrine disruptors and human and wildlife health: where we are today and where we need to go. *Toxicol. Sci.* 105, 235–259.
- Jensen, K.M., Makynen, E.A., Kahl, M.D., Ankley, G.T., 2006. Effects of the feedlot contaminant 17 α -trenbolone on reproductive endocrinology of the fathead minnow. *Environ. Sci. Technol.* 40, 3112–3117.
- Jeswiet, S.B., Lee-Jenkins, S.S., Godin, J.G.J., 2012. Concurrent effects of sperm competition and female quality on male mate choice in the Trinidadian guppy (*Poecilia reticulata*). *Behav. Ecol.* 23, 195–200.
- Johannson, B., Jones, T., 2007. The role of chemical communication in mate choice. *Biol. Rev.* 82, 265–289.
- Karino, K., Shinjo, S., 2004. Female mate preference based on male orange spot patterns in the feral guppy *Poecilia reticulata* in Japan. *Ichthyol. Res.* 51, 316–320.
- Khan, S.J., Roser, D.J., Davies, C.M., Peters, G.M., Stuetz, R.M., Tucker, R., Ashbolt, N.J., 2008. Chemical contaminants in feedlot wastes: concentrations effects and attenuation. *Environ. Int.* 34, 839–859.
- Kodric-Brown, A., Nicoletto, P.F., 2001. Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behav. Ecol. Sociobiol.* 50, 346–351.
- Kodric-Brown, A., 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* 17, 199–205.
- Kodric-Brown, A., 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav. Ecol. Sociobiol.* 25, 393–401.
- Kokko, H., Brooks, R., Jennions, M.D., Morley, J., 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. B: Biol. Sci.* 270, 653–664.
- López-Rojas, H., Bonilla-Rivero, A.L., 2000. Anthropogenically induced fish diversity reduction in Lake Valencia Basin, Venezuela. *Biodivers. Conserv.* 9, 757–765.
- Lange, I.G., Daxenberger, A., Schiffer, B., Witters, H., Ibarreta, D., Meyer, H.H.D., 2002. Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. *Anal. Chim. Acta* 473, 27–37.
- Larsen, M.G., Baatrup, E., 2010. Functional behavior and reproduction in androgenic sex reversed zebrafish (*Danio rerio*). *Environ. Toxicol. Chem.* 29, 1828–1833.
- Lindholm, A.K., Breden, F., Alexander, H.J., Chan, W.K., Thakurta, S.G., Brooks, R., 2005. Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Mol. Ecol.* 14, 3671–3682.
- Majewski, A.R., Blanchfield, P.J., Palace, V.P., Wautier, K., 2002. Waterborne 17 alpha-ethynylestradiol affects aggressive behaviour of male fathead minnows (*Pimephales promelas*) under artificial spawning conditions. *Water Qual. Res. J. Can.* 37, 697–710.
- Martinović, D., Hogarth, W.T., Jones, R.E., Sorensen, P.W., 2007. Environmental estrogens suppress hormones, behavior, and reproductive fitness in male fathead minnows. *Environ. Toxicol. Chem.* 26, 271–278.
- Maunder, R.J., Matthiessen, P., Sumpter, J.P., Pottinger, T.G., 2007. Impaired reproduction in three-spined sticklebacks exposed to ethinyl estradiol as juveniles. *Biol. Reprod.* 77, 999–1006.
- Melvin, S.D., Wilson, S.P., 2013. The utility of behavioral studies for aquatic toxicology testing: a meta-analysis. *Chemosphere* 93, 2217–2223.

- Morthorst, J.E., Holbech, H., Bjerregaard, P., 2010. Trenbolone causes irreversible masculinization of zebrafish at environmentally relevant concentrations. *Aquat. Toxicol.* 98, 336–343.
- Oshima, Y., Kang, I.J., Kobayashi, M., Nakayama, K., Imada, N., Honjo, T., 2003. Suppression of sexual behavior in male Japanese medaka (*Oryzias latipes*) exposed to 17 β -estradiol. *Chemosphere* 50, 429–436.
- Pilastro, A., Simonato, M., Bisazza, A., Evans, J.P., 2004. Cryptic female preference for colorful males in guppies. *Evolution* 58, 665–669.
- Saaristo, M., Craft, J.A., Lehtonen, K.K., Bjork, H., Lindstrom, K., 2009a. Disruption of sexual selection in sand gobies (*Pomatoschistus minutus*) by 17 α -ethinyl estradiol, an endocrine disruptor. *Horm. Behav.* 55, 530–537.
- Saaristo, M., Craft, J.A., Lehtonen, K.K., Lindstrom, K., 2009b. Sand goby (*Pomatoschistus minutus*) males exposed to an endocrine disrupting chemical fail in nest and mate competition. *Horm. Behav.* 56, 315–321.
- Saaristo, M., Tomkins, P., Allinson, M., Allinson, G., Wong, B.B.M., 2013. An androgenic agricultural contaminant impairs female reproductive behaviour in a freshwater fish. *PLoS One* 8, e62782.
- Schiffer, B., Daxenberger, A., Meyer, K., Meyer, H., 2001. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. *Environ. Health Perspect.* 109, 1145.
- Shenoy, K., 2012. Environmentally realistic exposure to the herbicide atrazine alters some sexually selected traits in male guppies. *PLoS One* 7, e30611.
- Slabbekoorn, H., Peet, M., 2003. Ecology: birds sing at a higher pitch in urban noise. *Nature* 424, 267.
- Sone, K., Hinago, M., Itamoto, M., Katsu, Y., Watanabe, H., Urushitani, H., Iguchi, T., 2005. Effects of an androgenic growth promoter 17 β -trenbolone on masculinization of mosquitofish (*Gambusia affinis*). *Gen. Comp. Endocrinol.* 143, 151–160.
- Soffker, M., Tyler, C. R. Endocrine disrupting chemicals and sexual behaviors in fish – a critical review on effects and possible consequences. 42, 2012, 653–668.
- Tian, H., Li, Y., Wang, W., Wu, P., Ru, S., 2012. Exposure to monocrotophos pesticide during sexual development causes the feminization/demasculinization of the reproductive traits and a reduction in the reproductive success of male guppies (*Poecilia reticulata*). *Toxicol. Appl. Pharm.* 263, 163–170.
- Walker, D.M., Gore, A.C., 2011. Transgenerational neuroendocrine disruption of reproduction. *Nat. Rev. Endocrinol.* 7, 197–207.
- Walling, C.A., Royle, N.J., Lindström, J., Metcalfe, N.B., 2010. Do female association preferences predict the likelihood of reproduction? *Behav. Ecol. Sociobiol.* 64, 541–548.
- Ward, A.J., Duff, A.J., Horsfall, J.S., Currie, S., 2008. Scents and scents-ability: pollution disrupts social recognition and shoaling in fish. *Proc. R. Soc. Lond. B* 275, 101–105.
- Widianarko, B., Van Gestel, C.A.M., Verweij, R.A., Van Straalen, N.M., 2000. Associations between trace metals in sediment, water, and guppy, *Poecilia reticulata* (Peters), from urban streams of Semarang, Indonesia. *Ecotoxicol. Environ. Saf.* 46, 101–107.
- Wilson, J.D., 1999. The role of androgens in male gender role behaviour. *Endocr. Rev.* 20, 726–737.
- Wong, B.B.M., Candolin, U., 2015. Behavioral responses to changing environments. *Behav. Ecol.* 26, 665–673.
- Wong, B.B.M., Candolin, U., Lindström, K., 2007. Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. *Am. Nat.* 170, 184–189.