Environmental Pollution 234 (2018) 223–230

Contents lists available at ScienceDirect

Environmental Pollution

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

Nest-defense behaviors in fathead minnows after lifecycle exposure to the antidepressant venlafaxine☆

Joanne L. Parrott [a,*], Chris D. Metcalfe [b]

[a] Water Science and Technology Directorate, Environment and Climate Change Canada, Burlington, Ontario, L7S 1A1, Canada
[b] Water Quality Centre, Trent University, Peterborough, Ontario, K9J 7B8, Canada

ARTICLE INFO

Article history:
Received 18 July 2017
Received in revised form
8 November 2017
Accepted 11 November 2017

Keywords:
Venlafaxine
Fathead minnow
Behavior
Antidepressant

ABSTRACT

Venlafaxine is an antidepressant and anti-anxiety drug that has been detected in municipal wastewater at low μg/L concentrations. In this study, the nest-defense behavior of adult male fathead minnows (*Pimephales promelas*) was observed in fish exposed for a full lifecycle to venlafaxine nominal concentrations of 0.88, 8.8, and 88 μg/L (i.e. 1, 9.3, 75 μg/L mean measured concentrations). Nest-defense behaviors quantified were the time taken to contact a dummy intruder fish (on a flexible stick, held near each nest) and the number of contacts made during a 1 min period. In male fathead minnows exposed to venlafaxine over a full lifecycle at environmentally relevant nominal concentrations (i.e. 0.88 and 8.8 μg/L) no significant effects were observed in behavior. However, in males exposed over a full lifecycle to the highest concentration of venlafaxine (i.e. 88 μg/L), nest-defense behaviors were increased in males with empty nests, as shown by the significantly elevated percentage of empty-nest males that made contact with the dummy intruder fish (89%) relative to the lower percentage of contacts (65%) among the Control males ($p = 0.046$). Lifecycle exposure to high venlafaxine (88 μg/L) caused males to over-protect their empty nests. Environmental venlafaxine concentrations are approximately 70 x lower than this, so it is unlikely that behavioral changes from venlafaxine exposure would occur in the environment. Normal nest defense behaviours in control males varied, depending on whether they were protecting empty nests or nests with eggs. Compared to Control males with empty nests, more Control males with eggs in their nests made contact with the dummy intruder fish ($p = 0.014$), contact was faster (i.e. <10 s, $p = 0.011$), and they hit the dummy intruder fish more times in 1 min ($p = 0.031$) This study is the first to assess reproductive behaviors in fish exposed to an antidepressant over a full lifecycle.

1. Introduction

Venlafaxine is an antidepressant and antianxiety drug that has been detected in the effluents of wastewater treatment plants (WWTP) at concentrations in the low μg/L range (Arnnok et al., 2017; Roberts et al., 2016). For example, venlafaxine was detected in two WWTP effluents that discharge to the Niagara River at concentrations of 0.26 μg/L and 0.94 μg/L, respectively (Arnnok et al., 2017), and was present at concentrations up to 0.362 μg/L (mean 0.162 μg/L) in WWTP effluents discharging into the Grand River, ON (Couperus et al., 2016). The concentrations of some of the biologically active transformation products of venlafaxine, such as the N-desmethyl metabolite can be even higher in WWTP effluents, with total venlafaxine concentrations up to 2–4 μg/L (Arnnok et al., 2017; Metcalfe et al., 2010). Lower concentrations of venlafaxine have been detected in surface waters downstream of WWTPs, at 0.01–0.46 μg/L in a large river (Lajeunesse et al., 2008), and 0.9–1.1 μg/L in smaller rivers (Metcalfe et al., 2010; Schultz and Furlong, 2008; Schultz et al., 2010). From a survey of small streams in the southeastern United States, Bradley et al. (2016) reported concentrations of the transformation product, N-desmethyl venlafaxine of up to 1 μg/L, with mean concentrations of 0.03 μg/L.

The effects of venlafaxine have been assessed in acute and chronic tests with fish using a variety of biological endpoints. Exposure of fathead minnows (*Pimephales promelas*) to venlafaxine for 21 d reduced survival, but the response was not concentration-dependent, with about 36% mortality in a treatment at 0.3 μg/L and

☆ This paper has been recommended for acceptance by Klaus Kummerer.
* Corresponding author. Environment and Climate Change Canada, 867 Lakeshore Road, Burlington, ON, L7S 1A1, Canada.
E-mail address: joanne.parrott@canada.ca (J.L. Parrott).

https://doi.org/10.1016/j.envpol.2017.11.049

18% mortality at 1.1 μg/L (Schultz et al., 2011). Our previous study on fathead minnows exposed over a full cycle to venlafaxine showed no effects in fish exposed at environmentally relevant concentrations, but fish from the highest venlafaxine treatment (i.e. 88 μg/L nominal) produced 46% more eggs per female than control fish (Parrott and Metcalfe, 2017). In contrast, adult zebrafish (*Danio rerio*) exposed to 5–6 μg/L venlafaxine for 7 weeks had reduced egg production (Galus et al., 2013).

Venlafaxine has been shown to affect fish behavior in short-term exposures. The escape responses of larval fathead minnows were slowed after exposure to 5 μg/L venlafaxine for 12 d (Painter et al., 2009). Hybrid striped bass (*Morone saxatilis* x *Morone chrysops*) exposed to 0.36–4.65 μg/L venlafaxine for 3–6 d took longer to capture their prey (Bisesi et al., 2014). Adult mosquitofish (*Gambusia holbrooki*) exposed to 100 μg/L venlafaxine for 7 d had disrupted circadian rhythm and decreased swimming during daylight (Melvin, 2017). Venlafaxine exposure at 1 μg/L for 7 d reduced feeding in rainbow trout (*Oncorhynchus mykiss*) and increased plasma cortisol concentrations in subordinate fish (Melnyk-Lamont et al., 2014).

Venlafaxine has also been shown to affect invertebrate behaviours. Exposure of common cuttlefish (*Sepia officinalis*) to 0.1 μg/L venlafaxine for 20 d decreased their camouflage ability (Bidel et al., 2016). In marine snails (*Chlorostoma funebralis*) exposed to 157 μg/L venlafaxine for 4 h, 90% of the animals detached from the substrate, while exposure to 31 μg/L caused no significant detachment (Fong and Molnar, 2013). Fong et al. (2015) reported that brief exposure to venlafaxine (31–157 μg/L) increased crawling speed in two species of marine snail, the oyster drill (*Urosalpinx cinerea*) and the American starsnail (*Lithopoma americanum*). Marine mud snails (*Ilyanassa obsolete*) exposed to 31 μg/L venlafaxine for 2 h took longer to right themselves after being inverted (Fong et al., 2017).

Male fathead minnows have a complex reproductive behavior in which they aggressively defend a nest that contains eggs that they have fertilized (Cole and Smith, 1987, 1992; Unger, 1983; Unger and Sargent, 1988). Researchers have recently begun to assess nest-defense and nest-tending in fathead minnows to determine if there are behavioral impacts from exposure to environmental contaminants (Hoover et al., 2013; Lorenzi et al. 2012, 2016; Schoenfuss et al., 2016; Schultz et al., 2012; Weinberger and Klaper, 2014).

"Dummy" fish have been used to assess defensive and nest-tending behaviors in several species of fish (Barlow et al., 1986; Barlow and Siri, 1994; Dzieweczynski, 2011; Dzieweczynski et al., 2006; Dzieweczynski and Hebert, 2012; Lehtonen, 2014; Rowland, 1975; Slovin and Rowland, 1978; Sowersby et al., 2017). In our previous study with fathead minnows exposed to venlafaxine over a full lifecycle (Parrott and Metcalfe, 2017), we observed that the exposed male minnows would frequently "hit" the gloved hands of research personnel checking for and collecting the eggs that were laid under tile nests. In order to evaluate the potential effects of venlafaxine on male nest-defense behavior, we introduced a dummy intruder fish near each male's nest and quantified the number of contacts and the time to contact when the dummy intruder was positioned on a flexible stick near each male's nest tile. We validated these behavioral assessments by determining if there were differences in protective behaviors between the male fish from the Control treatment with empty nests compared to the Control males with eggs in their nests. The objective of the present study was to evaluate whether exposure of fathead minnows to venlafaxine over a full lifecycle would alter the behaviors of male fish that were defending nests with eggs, as well as the behaviors of male fish guarding empty nests. The lowest venlafaxine exposure concentration was chosen to represent an environmentally-relevant concentration (0.88 μg/L) which is close to concentrations of venlafaxine in MWWEs, and approximates the highest venlafaxine concentrations detected in surface waters. The two higher venlafaxine fish exposure concentrations were 10x and 100x this environmentally-relevant concentration.

2. Materials and methods

2.1. Venlafaxine exposures

As described previously (Parrott and Metcalfe, 2017), fathead minnows were exposed over a full lifecycle to venlafaxine using a flow-through system with a modified Mount & Brungs-type diluter at nominal concentrations of 0 (Control), 0.88, 8.8, and 88 μg/L. The mean ± standard deviation measured concentrations in the exposure tanks were 1.00 (n = 2), 9.26 ± 1.9 (n = 4), and 75.2 ± 19.5 (n = 4) μg/L, respectively, as reported previously (Parrott and Metcalfe, 2017). The stock solution was made up from venlafaxine-HCl (CAS#99300-78-4, 99.9% purity, lot number A-1237-145) purchased from SynFine Research (Richmond Hill, ON, Canada). The flow-through system consisted of a series of 12 L glass aquaria, each with 3 vol changes per day. Venlafaxine was not detected in the Control tanks at concentrations above the Limit of Detection (LOD). The LOD was 0.02 μg/L and the Limit of Quantification (LOQ) was 0.07 μg/L.

Exposures of fathead minnows were from the fertilized egg stage through to 162–163 days post-hatch (dph). There were 8 replicate control tanks and 4 replicate tanks for each venlafaxine exposure concentration. Each tank contained 5 females and 3 males during the breeding phase of the experiment (i.e. after 106 days post hatch). Data on number of fish in each tank and survival over time are shown in Supplemental Data S3. There were three breeding tiles per tank, for one tile nest per breeding male. Tanks were aerated and covered, and the temperature was maintained by a water bath. The photoperiod was 16 h light:8 h dark, with dawn and dusk dimming. Water quality was measured weekly and was stable during the exposures. Temperatures ranged from 24.2 to 24.4 °C, mean dissolved oxygen ranged from 8.3 to 8.4 mg/L, mean pH ranged from 7.79 to 7.84, and mean conductivity ranged from 367 to 370 μS/cm, as reported previously (Parrott and Metcalfe, 2017). Data on the dilution water quality is shown in Supplemental Data (Table S1).

2.2. Nest-defense behaviors

During the breeding phase of the experiment (90–162 dph), there were 3 mature males and 5 mature females per tank, and each tank had 3 PVC breeding tiles under which the eggs were laid. As reported in the description of our previous study (Parrott and Metcalfe, 2017), tiles were checked daily for presence of eggs so that daily egg production could be calculated. During the checking for eggs, the male fathead minnow males would often "hit" the gloved hand of research personnel, and many of the male fish would persist in hitting repeatedly. To quantify these nest-defense behaviors, we designed a procedure using a dummy intruder fish (Fig. 1) introduced into the tank with the males and we set up trials to assess each adult male's nest-protecting behaviors over a period of 18 days. These behavioral trials were performed after the enumeration of egg production had ceased.

The nest defense behaviors of male fathead minnows were assessed with five independent observers, each measuring the time to contact with a dummy intruder fish and by counting the number of contacts made during a 1 min time interval. This behavioral assessment was conducted with adult male fathead minnows at the 140 dph to 158 dph stage of development that were exposed over a full lifecycle in treatments with venlafaxine at 0.88, 8.8, and 88 μg/L