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Chronic fluoxetine exposure alters movement and burrowing in adult freshwater mussels

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ABSTRACT

The antidepressant fluoxetine is commonly found in aquatic fauna living near or downstream from point-sources of municipal waste effluent. Continuous release of fluoxetine results in increased effective exposure duration in surface waters, resulting in a chronic exposure for animals downstream, particularly in effluent dominated ecosystems. Fluoxetine is known to cause disruptions in reproductive behavior of freshwater mussels (order Unionoida), including stimulating release of gametes, parturition of glochidia (larvae), and changes in lure display and foot protrusion. However, the ecological relevance of these effects at environmental concentrations is unknown. We conducted a 67-d exposure of adult *Lampsilis fasciola* to fluoxetine concentrations of 0, 0.5, 2.5, and 22.3 μg/L and assessed impacts on behavior (lateral movement, burrowing, and filtering) and metabolism (glycogen storage and respiration). Mussels treated with 2.5 and 22.3 μg/L fluoxetine displayed mantle lures significantly ($p < 0.05$) more than controls. Animals treated with 22.3 μg/L fluoxetine were statistically more likely to have shorter time-to-movement, greater total movement, and initiate burrowing sooner than control animals. These observations suggest that increased activity of mussels exposed to fluoxetine may result in increased susceptibility to predators and may lead to a reduction in energy stores.

1. Introduction

Evidence continues to grow of the prevalence of pharmaceuticals and personal care products (PPCPs) in the environment (Boxall et al., 2012; Brooks et al., 2012). Particularly in effluent dominated aquatic ecosystems (Brooks et al., 2006), continual releases of PPCPs increase effective exposure duration (Ankley et al., 2007), which leads to chronic exposure, bioaccumulation, and thus risks to aquatic organisms (Brooks et al., 2005; Ramirez et al., 2009; Bringolf et al., 2010). Because pharmaceuticals are designed to be biologically active, there is growing concern of the effects on non-target organisms and ecosystems (Boxall et al., 2012; Brausch et al., 2012), and for potential effects on humans from trace pharmaceutical concentrations in drinking water (Boxall et al., 2012; Daughton and Ruhoy, 2013), particularly related to antibiotic resistence (Pruden et al., 2013). Such concerns have prompted research on assessment of better wastewater treatment practices (Styrishave et al., 2011; Lajeunesse et al., 2012), and stimulated questions related to more sustainable drug designs and the potential overuse of pharmaceuticals in modern society (Daughton, 2002; Brooks et al., 2012; Valenti et al., 2012; Daughton and Ruhoy, 2013).

Among the most studied pharmaceuticals in the environment (PIEs: Boxall et al., 2012) is a class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs). These drugs are relatively stable in the environment, resistant to hydrolysis and photolysis in laboratory studies (Kwon and Armbrust, 2006), and are found to accumulate in sediments and biota (Brooks et al., 2005; Chu and Metcalfe, 2007; Bringolf et al., 2010; Schultz et al., 2010). SSRIs are known to affect reproduction, foraging, stress responses, and locomotion of fish and invertebrates (Brooks et al., 2003). SSRI activity in aquatic fauna is due to the conservation of the active serotonin reuptake transporter throughout vertebrates (Valenti et al., 2009, 2012; Mennigen et al., 2011), and documented importance of serotonin receptors in the neuroendocrine

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system of invertebrates (Newcomb et al., 2006; Spitzer et al., 2008; Meechonkit et al., 2010). Though concentrations of SSRIs in the environment (e.g. <1 μg/L) are typically well below acute toxicity levels to native fauna (Kolpin et al., 2002; Ramirez et al., 2009; Brooks et al., 2012), primary metabolites also show bioactivity similar to the parent compound (Fong and Molnar, 2008), and cocktails of multiple SSRIs may result in additive or synergistic effects that are greater than the combined effects of independently tested compounds (Henry and Black, 2007; Styrishave et al., 2011). Thus, the number of chemicals and metabolites in the environment capable of inducing a biological response may be larger than predicted or measured thresholds from laboratory studies (Oakes et al., 2010).

In the present study, we investigated the effects of chronic exposure (67 d) to fluoxetine on adult freshwater mussel behavior and physiology. Fluoxetine is the prototype SSRI, highly prescribed worldwide (Brooks et al., 2003) and commonly found among PIEs (Kolpin et al., 2002). Freshwater mussels of the order Unionoida are among the most imperiled taxa worldwide (Haag and Williams, 2013), and water quality concerns and pollution are considered an important challenge to their recovery (Downing et al., 2010). Freshwater mussels are often the most sensitive taxa to acute toxicity from aquatic pollutants (Raimondo et al., 2008), including fluoxetine (Hazelton et al., 2013). Serotonin is an important neurotransmitter to unionids (Meechonkit et al., 2010). And serotonergic effects, such as changes in serotonin, dopamine, cyclooxygenase, and monamine oxidase activity, have been reported in mussels exposed to wastewater effluent at threshold concentrations equivalent to those at distances of 4–5 km downstream of a municipal discharge (Gagne et al., 2004). Known effects on freshwater mollusks include release of gametes and parturition of larvae (Fong, 1998; Cunha and Machado, 2001; Gagne et al., 2004; Bringolf et al., 2010), changes in embryonic or larval development (Gust et al., 2009; Hazelton et al., 2013), increased mantle lure displays (Cunha and Machado, 2001; Bringolf et al., 2010; Hazelton et al., 2013), and an apparent loss of control of the muscular foot (Cunha and Machado, 2001; Fong and Molnar, 2013; Hazelton et al., 2013). However, the importance of these behaviors in an environmental context is not well understood. Therefore, the current study examined the influence of fluoxetine (target concentrations 0, 0.5, 5.0, 50 μg/L) on movement, burrowing behavior, respiration, algal clearance rates, and glycogen storage in the wavy-rayed lampmussel (*Lampsilis fasciola*), in an effort to better understand potential adverse outcomes (Ankley et al., 2010) related to behavioral and physiological effects in unionid bivalves.

2. Methods

2.1. Animal care & experimental aquaria

One year-old female *L. fasciola* (mean ± std. dev.: length = 42.8 ± 1.9 mm, width = 19.0 ± 2.8 mm, height = 28.3 ± 1.1 mm) were cultured from wild brood stock at the Alabama Aquatic Biodiversity Center in natural pond water and shipped to the University of Georgia Aquatic Ecology Laboratory by overnight courier on April 18, 2012. On arrival, animals were tagged (with cyanoacrylate adhesive) with individually coded Hallprint shellfish tags (Hallprint Inc., Hindmarsh Valley, South Australia). Animals were acclimated over 48 h to filtered (<25 μm) pond water through two 50% dilutions of shipping water, and eventually maintained in a 530-L Living Stream (Frigid Units, Toledo, OH) equipped with partial flow-through of filtered pond water. Natural food in the pond water was supplemented twice weekly with a 500-mL solution of commercial shellfish food (stock solution included 6 mL/L *Nannochloropsis* and 14 mL/L Shell Fish Diet; Reed Mariculture, Campbell, CA).

Behavioral experiments were conducted in 19-L glass aquaria (39 cm L × 19.6 cm W × 23 cm D). Four liters of Quikrete Premium Play Sand (Quikrete, Atlanta, GA) were spread to an approximately uniform depth (5 cm) in each aquaria. Sand was triple washed with dechlorinated tap water through a 300 μm sieve to reduce turbidity from small particles during water changes. Four replicate aquaria ($N = 4$) were assigned to each treatment concentration (nominal fluoxetine treatment levels: 0, 0.5, 5, 50 μg/L) containing two mussels each for a total of 16 experimental aquaria. Two additional aquaria with no mussels were assigned to each treatment group as control tanks for algal clearance and fluoxetine uptake endpoints.

To facilitate identification between the two individual mussels in an aquarium, each was tagged with a 7-cm segment of floating fly fishing line colored either yellow or orange. The tag was located on the posterior-dorsal quadrant of the right valve, extending along the anterior–posterior axis. Longer segments of floating fly fishing line have been used successfully in movement studies of freshwater mussels and this method does not appear to harm the animal or affect burrowing activity (Newton et al., 2012). Tests were conducted in standardized-reconstituted moderately hard water (USEPA, 2002), and animals were gradually introduced to test water through a series of dilutions. On August 10, animals were introduced to aerated test aquaria containing reconstituted soft water (32 mg/L $CaCO_3$ hardness, 30 mg/L $CaCO_3$ alkalinity; USEPA, 2002) for 48 h, followed by 100% water renewals of increasing hardness (48 h: 64 mg/L $CaCO_3$ hardness, 32 mg/L $CaCO_3$ alkalinity; 72 h: 74 mg/L $CaCO_3$ hardness, 54 mg/L $CaCO_3$ alkalinity). Final moderately hard dilution water (USEPA, 2002) was reached on August 14 and used for the remainder of the acclimation period and experiment (mean ± std. dev. hardness = 86.6 ± 8.4 mg/L $CaCO_3$, mean ± std. dev. alkalinity = 63.21 ± 9.69 mg/L $CaCO_3$).

2.2. Experimental procedure

Our experimental design incorporated a 67 day exposure (day 1 = 9/4/2012, day 67 = 11/10/2012) to fluoxetine with 72 h static renewal and retreatment cycles ($N = 19$ treatment cycles). Mussels were allowed to acclimate to the 72 h renewal schedule during seven 100% water changes at 72 h intervals in the absence of fluoxetine treatment from August 14 to September 4. Mussels were handled in the same manner during acclimation water changes as they were throughout the remainder of the experiment. Behavioral data (detailed below) were collected during a subset of seven treatment cycles (starting on exposure days: 1, 3, 6, 9, 41, 55, 64), at 8 timepoints (0, 1, 3, 6, 12, 24, 8, 72 h) within each treatment cycle. Algal clearance data were collected during a subset of six treatment cycles (starting on exposure days: 1, 9, 12, 24, 36, 56). Flow-through respirometry and tissue glycogen were measured following the 67-day exposure.

During each water change and 100% fluoxetine retreatment, (1) the mussels were removed from the aquaria; (2) water and organic deposition on the sediment was siphoned and discarded; (3) water was replaced and sediment was graded to a uniform depth; (4) mussels were replaced on top of the sediment on their left valve with their anterior–posterior axis oriented along the narrow width of the tank. Animals marked with yellow tags were placed at ~13 cm along the length of the tank, and individuals with orange tags were placed at ~26 cm. Both animals were placed approximately along the midline of the tank width, which served as a starting observation point, after which all behavior and movement could be measured. To further acclimate test animals to the static water renewal procedures, four 72 h treatment cycles and water changes were conducted prior to introducing fluoxetine exposures on September 4. During a water change on day 18 an incidental mortality occurred to one animal in the 0.5 μg/L treatment, this animal was subsequently removed from all experimental analyses.