THE EFFECTS OF ANTIDEPRESSANTS APPEAR TO BE RAPID AND AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS

ALEX T. FORD*† and PETER P. FONG‡

†Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Ferry Road, Portsmouth, United Kingdom
‡Department of Biology, Gettysburg College, Gettysburg, Pennsylvania, USA

Abstract: The effects of antidepressants on wildlife are currently raising some concern because of an increased number of publications indicating biological effects at environmentally relevant concentrations (<100 ng/L). These results have been met with some scepticism because of the higher concentrations required to detect effects in some species and the perceived slowness to therapeutic effects recorded in humans and other vertebrates. Because their mode of action is thought to be by modulation of the neurotransmitters serotonin, dopamine, and norepinephrine, aquatic invertebrates that possess transporters and receptors sensitive to activation by these pharmaceuticals are potentially affected by them. The authors highlight studies on the effects of antidepressants, particularly on crustacean and molluskan groups, showing that they are susceptible to a wide variety of neuroendocrine disruptions at environmentally relevant concentrations. Interestingly, some effects observed in these species can be observed within minutes to hours of exposure. For example, exposure of amphipod crustaceans to several selective serotonin reuptake inhibitors can invoke changes in swimming behavior within hours. In mollusks, exposure to selective serotonin reuptake inhibitors can induce spawning in male and female mussels and foot detachment in snails within minutes of exposure. In the light of new studies indicating effects on the human brain from selective serotonin reuptake inhibitors using magnetic resonance imaging scans, the authors discuss possible reasons for the discrepancy in former results in relation to the read-across hypothesis, variation in biomarkers used, modes of uptake, phylogenetic distance, and the affinity to different targets and differential sensitivity to receptors. *Environ Toxicol Chem* 2016;35:794–798. © 2015 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals, Inc. on behalf of SETAC.

Keywords: Selective serotonin reuptake inhibitor   Pharmaceutical   Pollution   Neuroendocrine

INTRODUCTION

Several recent studies have raised concerns that antidepressants in aquatic ecosystems may be an environmental concern [1–6]. Prescriptions for antidepressants have been increasing rapidly in some countries [7], with studies indicating that antidepressants are taken by 1 in 10 of the population [8]. These drugs are used to treat a wide range of conditions, such as depression, anxiety, and bipolar disorders [9]. A wide range of antidepressants are currently in medical use, which include some of the older prescribed tricyclic compounds (e.g., amitriptyline), the selective serotonin reuptake inhibitors (e.g., fluoxetine), the serotonin and norepinephrine reuptake inhibitors (e.g., venlaflaxine), and the serotonin antagonist and reuptake inhibitors (e.g., trazodone).

Concentrations of antidepressants in water bodies vary considerably but have been detected in freshwater [3,10–14], groundwater [15], and seawater [16]. In arid and semiarid parts of the world, ephemeral streams can be dominated by municipal and/or industrial effluent discharges, particularly in urbanized watersheds [17]. Therefore, some aquatic organisms are likely to be receiving relatively high and constant exposure to serotonergic and neurologically active drugs. Furthermore, recent studies have shown the capacity of aquatic organisms to bioaccumulate these compounds [18–21]. Despite the widespread presence of antidepressants in the aquatic environment, their bioactive properties (both neruological and hormonal), their capacity to bioaccumulate in tissues, and relatively similar prescription rates of the concentraceptive pill, it was recently highlighted that the body of research on synthetic estrogen exposure hugely outweighs the amount currently known for neurological drugs [22].

EFFECTS IN WILDLIFE AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS

The concentrations of antidepressants in the aquatic environment range from nanograms to micrograms per liter, with most studies reporting concentrations below 100 ng/L. The scientific literature has increased in the number of publications highlighting effects of antidepressants observed at very low environmentally relevant concentrations [6]. These include induction of spawning in bivalves [23,24]; altered cyclic adenosine $3',5'$-monophosphate/protein kinase A pathway and serotonin (5-hydroxytryptamine [5-HT]) expression in mussels [25]; altered mobility in snails [26]; altered memory, cognitive function, and ability to camouflage in cuttlefish [27,28]; induced phototaxis and altered activity in amphipods [4,29–31]; gene expression of putative serotonergic pathways in amphipods [31]; and altered reproduction [21], activity [32], and embryonic/development endpoints [33] in fish. Therefore, one might conclude that the effects of these compounds are diverse and potentially impact a wide range of invertebrate and vertebrate phyla.

Fong and Ford [6] recently highlighted that many of these studies report nonmonotonic concentration–response curves

[6,31–33]. The low-dose effects reported by some studies have been questioned as to whether they are in fact artifacts and whether they are repeatable [34]. Several studies have also been criticized because of limitations in study design, including use of novel biomarkers, large interspecies variability, nominal concentrations, and low numbers of concentrations used [34,35]. Therefore, calls [22] have been made for laboratories to repeat their studies and those of others to appropriately assess the risk posed by these compounds. Vandenburg et al. [36] recently conducted a large review of cell culture, animal, and epidemiology studies and concluded that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and endocrine-disrupting chemicals. They went on to suggest that fundamental changes in chemical testing and safety determination are needed to protect human health. Accepting some of the limitations of recent studies, it seems reasonable to assume that hormetic effects might also be found in serotonergic drugs.

ARE RAPID EFFECTS THAT UNUSUAL?

One of the most intriguing results of some of the reported studies is that effects can sometimes be observed in very short periods of time [31]. Zebra mussels can be significantly induced to spawn within minutes of both fluoxetine and fluvoxamine exposure at concentrations as low as 300 ng/L and 430 ng/L, respectively. For example, Fong [23] found that 70% of male zebra mussels could be induced to spawn in 1 h or less in 1 nM (430 ng/L) fluvoxamine. Altered oocyte and spermatozoan densities were observed in zebra mussels exposed to fluoxetine at 20 ng/L and 200 ng/L following several days of exposure [24]. Several studies have looked at the effects of fluoxetine on activity measurements in amphipods and similarly found effects within very short time frames [6,29–31]. For example, within less than 2 h of exposure, the freshwater amphipod *Gammarus pulex* display altered activity measured following exposure to fluoxetine at low concentrations [29,30]. The experimental protocol used a 30-min acclimation period followed by a 1.5-h recording using electrical conductance induced by the organism's movement. The greatest effects on activity were observed at 10 ng/L to 100 ng/L fluoxetine. In another study using the marine/estuarine amphipod *Echinogammarus marinus*, the authors recorded increased positive phototaxis and decreased geotaxis following fluoxetine exposure for 1 wk, with the greatest effects observed at 10 ng/L to 100 ng/L [4]. These behavioral effects were also observed following 2-wk and 3-wk exposures. The behavioral effects recorded in the amphipods corresponded to those when exposed to serotonin (5-HT) or infected with serotonin-modulating parasites. Using an alternative method of behavioral analysis, the activity of *E. marinus* was recorded using Daniovision (Noldus) with Ethovion XT software (Ver 8.1) following exposure to the selective serotonin reuptake inhibitors sertraline and fluoxetine [31]. Significant effects on amphipod activity (velocity in millimeters per second) were recorded after 1 d for fluoxetine and both 1 h and 1 d for sertraline. Similarly, the greatest effects were observed at 100 ng/L, with exposed organisms displaying elevated velocities under both dark and light conditions. Following 8-d exposure, there was a significant down-regulation of genes with putative serotonergic function for fluoxetine (but not sertraline) at 1 ng/L and 10 ng/L. It is important to note that neither fluoxetine nor sertraline elicited effects on velocity after 8 d. Therefore, albeit with nominal concentrations and the relatively few studies done to date, there is some repeatability in the low-dose effects observed.

Although we believe many of the observed effects can be attributed to different modes of action and not exclusively to 5-HT reuptake inhibition, it is important to mention the role of pH on the toxicokinetics and uptake of antidepressants. Several recent studies have highlighted that changes in pH can strongly influence the ionization of antidepressants, resulting in different uptake rates and consequently toxicity [37–42]. Noteworthy is the increased toxicity observed at higher pH.

Although the pH of the medium is undoubtedly important because the hydrophobicity of the compound would affect its ability to cross membranes and enter cells, the route of uptake and the mechanism of action would determine the target tissues and cell membranes to cross. The route of uptake of antidepressants in aquatic vertebrates such as fishes is likely through the gills or oral cavity. Once in the blood, and if capable, they would cross the blood–brain barrier, enter the brain, and exert action by blocking reuptake of 5-HT there. Aquatic anurans, on the other hand, would be capable of gill or cutaneous uptake before the antidepressant enters the blood. Brooks [43] reported that, using probabilistic hazard assessment and fish plasma modeling approaches, selective serotonin reuptake inhibitors and tricyclic antidepressants are predicted to result in therapeutic hazard to fish (internal fish plasma level equalling mammalian therapeutic dose) when exposed to water (inhalational) at or below 1 µg/L. However, Brooks [43] also stated that because of data limitations we do not know the internal doses of therapeutic or side effects of drugs in fish or invertebrates.

By contrast, the route of antidepressant uptake in invertebrates is likely to vary with taxonomic group. In bivalve mollusks, the route of uptake could be direct internalization via the gills. However, because bivalves filter water, the entire mantle cavity containing the gonads, foot, digestive gland, and adductor muscles, as well as the gills, would be exposed to the water where contact with external receptors would be possible. Matsutani and Nomura [44] have shown that isolated fragments of scallop ovaries will release eggs when treated with 5-HT, suggesting that 5-HT receptors are located directly on the gonad. Isolated mussel siphons and mantle tissues can also be induced to contract and relax with externally applied 5-HT, and these responses can be mimicked by vertebrate 5-HT$_2$ receptor ligands, again suggesting the presence of 5-HT receptors directly on the siphon and mantle [45]. Similar to bivalves, aquatic snails with gills (prosobranchs) or a modified lung (pulmonates) could take up antidepressants via these respiratory surfaces, but the foot and all tissues within the mantle cavities are also available surfaces for uptake.

In crustaceans with a heavy exoskeleton that covers most of their body like crabs, crayfish, and shrimps, antidepressants could become internalized via the branchial cavity and then enter the hemocoelomic cavity; but in others that lack gills antidepressants would have to get across the general body surface. Once in the hemocoelomic cavity they can come directly in contact with thoracic and abdominal ganglia of the ventral nerve cord both receptive to and capable of producing 5-HT [46–48]. In planktonic crustaceans with a thin exoskeleton and a large surface area to volume ratio such as *Daphnia*, uptake could occur via the feeding current into the filtering chamber; but a major site of respiratory gas exchange occurs at the inner wall of the carapace [49]. Marine worms can have elaborate uptake structures, such as parapodia, tentacles, gills, and palps [50]; and uptake could be through those structures, across the general body surface, or via ingestion.

Recently, Karlsson et al. [40] examined the route of uptake of the pharmaceuticals triclosan, diclofen, and fluoxetine into the

aquatic oligochaete *Lumbriculus variegatus*. In this worm, the route of uptake could either be integumental or through the oral cavity, and they cleverly used an oligochaete that regenerates head and tail segments; thus, head removal would inhibit ingestion but not integumental uptake. They found that there was no significant difference in uptake of ^{14}C-labeled fluoxetine between feeding and nonfeeding (headless) worms, although they did find that the antibiotic triclosan was taken up more by feeding worms. Their results indicate that even for an aquatic organism like an oligochaete, there could be multiple routes of uptake and, therefore, the effect of pH on the speed of an antidepressant-induced response depends on the target cells and tissues. The behavioral responses that workers are measuring (e.g., spawning in bivalves, locomotion in snails, phototaxis in amphipods, learning and cognition in cephalopods, fecundity in *Daphnia*) would all be affected by the route of uptake and mode of action.

Thus, how quickly a response to antidepressants occurs is likely to be dependent on not only pH but also whether the drug binds to external receptors or is somehow internalized first, travels through blood vessels, makes its way into a coelomic or hemocoelomic cavity, and then binds to potentially a multitude of molecular targets.

ANTIDEPRESSANTS AND THE READ-ACROSS HYPOTHESIS

The read-across hypothesis [51] suggests that a drug will have an effect in nontarget organisms only if the molecular targets have been conserved, resulting in specific pharmacological effects only if plasma concentrations are similar to human therapeutic concentrations [52]. One of the specific concerns of recent low–concentration antidepressant studies is that effect concentrations do not appear to match the read-across hypothesis for therapeutic dose concentrations for humans [35]. Fluoxetine is generally prescribed over many weeks to allow for brain concentrations to rise enough to a concentration whereby beneficial results are observed in the patients (usually within 1 mo [35]). Therefore, it has been highlighted [34] that the antidepressant concentrations in the water of some of these studies are unlikely to produce a concentration of fluoxetine in the nerve synapses matching the therapeutic dose for humans (50–500 µg/L plasma concentration). A recent study nicely demonstrated that fathead minnows only responded in a tank diving test to measure anxiolytic behaviors when plasma concentrations of fluoxetine were within a concentration range similar to or higher than those of human therapeutic doses [53]. The authors concluded that their study represents the first direct evidence of a measured internal dose-response effect of a pharmaceutical in fish, thereby validating the read-across hypothesis for this compound. This was indeed an eloquent study that clearly demonstrated that the endpoints observed within the fish (fish anxiety tests) matched those close to human therapeutic plasma concentrations. How surprised might we have been if they were very much different? Human therapeutic doses, particularly for antidepressants, are often derived from questionnaires given to patients posttreatment, which have themselves been subject to criticism [54]. Therefore, we must be careful about "what" we are reading across when interpreting the read-across hypothesis, especially when interpreting disparate endpoints. This is especially true when drugs may have multiple targets, different affinities for targets in different organisms, or similar biological targets controlling different biological responses [23].

The evolution of the vertebrates represents a minute time frame in history compared with the biological divergence of the invertebrates and their targets for 5-HT and serotonin-like drugs. There are a number of possible targets for antidepressants such as fluoxetine in both vertebrates and invertebrates other than 5-HT reuptake transporters. Ni and Miledi [55] showed that fluoxetine binds to and blocks 5-HT-2C receptors in frog (*Xenopus*) oocytes. They concluded that fluoxetine is a competitive and reversible receptor antagonist of 5-HT-2C receptors. Garcia-Colunga et al. [56] showed that fluoxetine blocks both muscle and neuronal nicotinic acetylcholine receptors. Indeed, the "selectivity" of selective serotonin reuptake inhibitors and serotonin and norepinephrine reuptake inhibitors has been questioned by clinical psychopharmacologists for many years. These drugs show binding affinity not only to 5-HT-2C receptors but to dopamine reuptake transporters, muscarinic cholinergic receptors, sigma receptors, and enzymes such as nitric oxide synthase and a variety of cytochrome P450s [57]. Recently, studies on 5-HT receptors and 5-HT transporters in the nematode *Caenorhabditis elegans* have suggested that antidepressants such as fluoxetine do not act as selective serotonin reuptake inhibitors. Ranganathan et al. [58] found that fluoxetine induces responses in *C. elegans* that lack a 5-HT transporter (mod-5). They suggest that fluoxetine could be acting independently of 5-HT and any 5-HT transporter. That study confirmed the earlier work by Choy and Thomas [59], who found that fluoxetine induces neuromuscular activity in the anterior region of *C. elegans* in 5-HT–deficient mutants and suggest that drugs like fluoxetine have targets other than 5-HT reuptake transporters. Dempsey [60] showed that fluoxetine stimulates egg laying in *C. elegans* independent of 5-HT and independent of the 5-HT transporter. Kullyev et al. [61] demonstrated that fluoxetine binds directly to G protein–coupled 5-HT receptors in *C. elegans*. It should be noted that 5-HT transporters have been identified in all major invertebrate phyla [62]. The G protein–coupled 5-HT receptors may have evolved over 750 million years ago, whereas mammalian 5-HT receptor subtypes may have differentiated 90 million years ago [63]. Thus, the number and type of potential targets of these drugs and the cellular responses to them are likely to be as diverse as the groups of organisms in which they evolved. Therefore, we must be careful when matching endpoints over large phylogenetic distances even when the biological systems such as the nervous system are relatively conserved, a point made in several studies [17,34,35,51,52]. This is especially true when some endpoints are unfeasible to read across, such as serotonin/dopamine-modulated camouflage or photosensitivity. A recent human-based study has highlighted that a biological response to antidepressants (escitalopram) could be detected following a single dose (20 mg) within several hours using resting-state functional magnetic resonance imaging [64]. The authors observed that the single dose of a serotonin reuptake inhibitor dramatically alters functional connectivity throughout the brain in healthy subjects. Specifically, their analysis suggested a widespread decrease in connectivity in most cortical and subcortical areas of the brain. Therefore, some effects of antidepressants in humans are detectable quite rapidly following antidepressants when measuring more sensitive endpoints. In this instance the plasma concentrations of escitalopram were 25 ± 13 ng/mL, which is not uncommon for this particular selective serotonin reuptake inhibitor; but steady-state concentrations are usually observed following 7 d to 10 d and clinical signs of effects following 1 wk to 2 wk [65,66]. Therefore, biologically detectable endpoints might be quite different from human therapeutic dose concentrations but still have unknown biological

disruption, which is an important distinction in environmental protection.

SUMMARY

Antidepressants are ubiquitous in aquatic environments impacted by sewage effluent. Although the number of studies assessing their potential for environmental impact is increasing, they remain few in number and insufficient to enable us to fully understand the ecological risk posed by these compounds. Those studies that have been published show quite variable effect concentrations, and some have limitations in their experimental designs. There does, however, appear to be mounting evidence that very low concentrations can impact the biological function of multiple aquatic organisms. Several studies have recorded the rapid action of antidepressants on some aquatic species; coupled with this, nonmonotonic concentration–response curves have been observed, which suggests that careful consideration must be taken in experimental design and recording. Given that some aquatic organisms are likely to be exposed either continuously or sporadically throughout their life histories, especially during critical life stages, it will be important to ascertain the long-term impacts of serotonergic drugs on neural development. Although we have provided strong evidence that we must be cautious when applying the read-across hypothesis to distant invertebrates, evidence from mammalian models does point to the fact that long-term exposure to antidepressants may cause damage to neural receptors and architecture. The physiological and behavioral implications of these changes will be a future challenge for environmental toxicologists.

REFERENCES

1. Brooks BW, Foran CM, Richards SM, Weston J, Turner PK, Stanley JK, Solomon KR, Slattery M, La Point TW. 2003. Aquatic ecotoxicology of fluoxetine. *Toxicol Lett* 142:169–183.
2. Johnson DJ, Sanderson H, Brain RA, Wilson CJ, Solomon KR. 2007. Toxicity and hazard of selective serotonin reuptake inhibitor antidepressants fluoxetine, fluvoxamine, and sertraline to algae. *Ecotoxicol Environ Saf* 67:128–139.
3. Minagh E, Hernan R, O'Rourke K, Lyng FM, Davoren M. 2009. Aquatic ecotoxicity of the selective serotonin reuptake inhibitor sertraline hydrochloride in a battery of freshwater test species. *Ecotoxicol Environ Saf* 72:434–440.
4. Guler Y, Ford AF. 2010. Anti-depressants make amphipods see the light. *Aquat Toxicol* 99:397–404.
5. Styrishave B, Halling-Sorensen B, Ingerslev F. 2011. Environmental risk assessment of three selective serotonin reuptake inhibitors in the aquatic environment: A case study including a cocktail scenario. *Environ Toxicol Chem* 30:254–261.
6. Fong PP, Ford AT. 2014. The biological effects of antidepressants on the molluscs and crustaceans: A review. *Aquat Toxicol* 151:4–13.
7. National Centre for Health Statistics. 2014. Special feature on prescription drugs. [cited 2015 May 20]. Available from: http://www.cdc.gov/nchs/hus.htm
8. US Department of Health and Human Services. 2012. Health, United States, 2011: With special feature on socioeconomic status and health. DHHS Publication No. 2012-1232. US Government Printing Office, Washington, DC.
9. American Hospital Formulary Service (AHFS). AHFS Di monographs. [Cited 2015 February 24]. Available from: http://www.drugs.com/monograph
10. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
11. Chen M, Ohman K, Metcalfe C, Ikonomou MG, Amatya P, Wilson J. 2006. Pharmaceuticals and endocrine disruptors in wastewater treatment effluent and in the water supply system of Calgary, Alberta, Canada. *Water Qual Res J Can* 41:351–364.
12. Lajeunesse A, Gagnon C, Sauve S. 2008. Determination of basic antidepressants and their N-desmethyl metabolites in raw sewage and wastewater using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Anal Chem* 80:5325–5333.
13. Schultz MM, Furlong ET. 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. *Anal Chem* 80:1756–1762.
14. Schultz MM, Furlong ET, Kolpin DW, Werner SL, Schoenfuss HL, Barber LB, Blazer VS, Norris DO, Vajda AM. 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: Occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environ Sci Technol* 44:1918–1925.
15. Silva LJG, Lino CM, Meisel LM, Pena A. 2012. Selective serotonin re-uptake inhibitors (SSRIs) in the aquatic environment: An ecopharmacovigilance approach. *Sci Total Environ* 437:185–195.
16. Pait AS, Warner RA, Hartwell SI, Nelson JO, Pacheco PA, Mason AL. 2006. Human use pharmaceuticals in the estuarine environment: A survey of the Chesapeake Bay, Biscayne Bay and Gulf of the Farallones. NOS NCCOS 7. NOAA/NOS/NCCOS/Center for Coastal Monitoring and Assessment, Silver Spring, MD, USA.
17. Brooks BW, Riley TM, Taylor RD. 2006. Water quality of effluent-dominated ecosystems: Ecotoxicological, hydrological, and management considerations. *Hydrobiology* 556:365–379.
18. Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, Russell JL. 2005. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ Toxicol Chem* 24:464–469.
19. Chu S, Metcalfe CD. 2007. Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography–tandem mass spectrometry. *J Chromatogr A* 1163:112–118.
20. Metcalfe CD, Chu S, Judt C, Li H, Oakes KD, Servos MR, Andrews DM. 2010. Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. *Environ Toxicol Chem* 29:79–89.
21. Schultz MM, Painter MM, Bartell SE, Logue A, Furlong ET, Werner SL, Schoenfuss HL. 2011. Selective uptake and biological consequences of environmentally relevant antidepressant pharmaceutical exposures on male fathead minnows. *Aquat Toxicol* 104:38–47.
22. Ford AT. 2014. From gender benders to brain benders (and beyond!). *Aquat Toxicol* 151:1–3.
23. Fong PP. 1998. Zebra mussel spawning is induced in low concentrations of putative serotonin reuptake inhibitors. *Biol Bull* 194:143–149.
24. Lazzara R, Blazquez M, Porte C, Barata C. 2012. Low environmental levels of fluoxetine induce spawning and changes in endogenous estradiol levels in the zebra mussel *Dreissena polymorpha*. *Aquat Toxicol* 106–107:123–130.
25. Franzellitti S, Buratti S, Valbonesi P, Fabbri E. 2013. The mode of action (MOA) approach reveals interactive effects of environmental pharmaceuticals on *Mytilis galloprovincialis*. *Aquat Toxicol* 140–141:249–256.
26. Fong PP, Hoy CM. 2012. Antidepressants (venlafaxine and citalopram) cause foot detachment from the substrate in freshwater snails at environmentally relevant concentrations. *Mar Freshw Behav Physiol* 45:145–153.
27. Di Poi C, Darmaillacq A-S, Dickel L, Boulouard M, Bellanger C. 2013. Effects of perinatal exposure to waterborne fluoxetine on memory processing in the cuttlefish *Sepia officinalis*. *Aquat Toxicol* 132–133:84–91.
28. Di Poi C, Bidel F, Dickel L, Bellanger C. 2014. Cryptic and biochemical responses of young cuttlefish *Sepia officinalis* exposed to environmentally relevant concentrations of fluoxetine. *Aquat Toxicol* 151:36–45.
29. De Lange HJ, Noordoven W, Murk AJ, Lürling MFLLW, Peeters ETHM. 2006. Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. *Aquat Toxicol* 78:209–216.
30. De Lange HJ, Peeters ET, Lürling MFLLW. 2009. Changes in ventilation and locomotion of *Gammarus pulex* (Crustacea, Amphipoda) in response to low concentrations of pharmaceuticals. *Hum Ecol Risk Assess* 15:111–120.

31. Bossus MC, Guler YZ, Short SJ, Morrison ER, Ford AT. 2014. Behavioural and transcriptional changes in the amphipod *Echinogammarus marinus* exposed to two antidepressants, fluoxetine and sertraline. *Aquat Toxicol* 151:46–56.

32. Barry MJ. 2013. Effects of fluoxetine on swimming and behavioural responses of the Arabian killifish. *Ecotoxicology* 22:425–432.

33. Yang M, Qiu W, Chen J, Zhan J, Pan C, Lei X, Wu M. 2014. Growth inhibition and coordinated physiological regulation of zebrafish (*Danio rerio*) embryos upon sublethal exposure to antidepressant amitriptyline. *Aquat Toxicol* 151:68–76.

34. Sumpter JP, Donnachie RL, Johnson AC. 2013. The apparently very variable potency of the anti-depressant fluoxetine. *Aquat Toxicol* 151:57–60.

35. Sumpter JP, Margiotta-Casaluci L. 2013. Are some invertebrates exquisitely sensitive to the human pharmaceutical fluoxetine? *Aquat Toxicol* 146:259–260.

36. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. 2012. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocr Rev* 33:378–455.

37. Nakamura Y, Yamamoto H, Sekizawa J, Kondo T, Hirai N, Tatarazako N. 2008. The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* 70:865–873.

38. Valenti TW, Perez-Hurtado P, Chambliss CK, Brooks BW. 2009. Aquatic toxicity of sertraline to *Pimephales promelas* at environmentally relevant surface water pH. *Environ Toxicol Chem* 28:2685–2694.

39. Carter LJ, Garman CD, Ryan J, Dowle A, Bergstrom E, Thomas-Oates J, Boxall AB. 2014. Fate and uptake of pharmaceuticals in soil-earthworm systems. *Environ Sci Technol* 48:5955–5963.

40. Karlsson MV, Marshall S, Gouin T, Boxall ABA. 2016. Routes of uptake of diclofen, fluoxetine, and triclosan into sediment-dwelling worms. *Environ Toxicol Chem* 35:836−842 (*this issue*).

41. Sundaram R, Smith B, Clark T. 2015. pH-dependent toxicity of serotonin selective reuptake inhibitors in taxonomically diverse freshwater invertebrate species. *Mar Freshw Res* 66:518–525.

42. Bostrom ML, Berglund O. 2015. Influence of pH-dependent aquatic toxicity of ionisable pharmaceuticals on risk assessments over environmental pH ranges. *Water Res* 72:154–161.

43. Brooks BW. 2014. Fish on Prozac (and Zoloft): Ten years later. *Aquat Toxicol* 151:61–67.

44. Matsutani T, Nomura T. 1987. In vitro effects of serotonin and prostaglandins on release of eggs from the ovary of the scallop, *Patinopecten yessoensis*. *Gen Comp Endocrinol* 67:111–118.

45. Ram JL, Moore D, Putchakayala S, Paredes AA, Ma D, Croll RP. 1999. Serotonergic responses of the siphons and adjacent mantle tissue of the zebra mussel, *Dreissena polymorpha*. *Comp Biochem Physiol C Comp Pharmacol* 124:211–220.

46. Beltz BS, Kravitz EA. 1983. Mapping of serotonin-like immunoreactivity in the lobster nervous system. *J Neurosci* 3:585–602.

47. Harzsch S, Waloszek D. 2000. Serotonin-immunoreactive neurons in the ventral nerve cord of Crustacea: A character to study aspects of arthropod phylogeny. *Arthropod Structure & Development* 29:307–322.

48. Sosa MA, Spitzer N, Edwards DH, Baro DJ. 2004. A crustacean serotonin receptor: Cloning and distribution in the thoracic ganglia of crayfish and freshwater prawn. *J Comp Neurol* 473:526–537.

49. Pirow R, Wollinger F, Paul RJ. 1999. The sites of respiratory gas exchange in the planktonic crustacean *Daphnia magna*: An in vivo study employing blood haemoglobin as an internal oxygen probe. *J Exp Biol* 202:3089–3099.

50. Ruppert EE, Fox RS, Barnes RD. 2004. *Invertebrate Zoology: A Functional Evolutionary Approach*, 7th ed. Brooks Cole Thomson, Belmont, CA, USA.

51. Huggett DB, Cook JC, Ericson JF, Williams RT. 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritized potential impacts of human pharmaceuticals to fish. *Hum Ecol Risk Assess* 9:1789–1800.

52. Rand-Weaver M, Margiotta-Casaluci L, Patel A, Panter GH, Owen SF, Sumpter JP. 2013. The read-across hypothesis and environmental risk assessment of pharmaceuticals. *Environ Sci Technol* 47:12297–12304.

53. Margiotta-Casaluci L, Owen SF, Cumming RI, de Polo A, Winter MJ, Panter GH, Rand-Weaver M, Sumpter JP. 2014. Quantitative cross-species extrapolation between humans and fish: The case of the anti-depressant fluoxetine. *PLoS One* 9:e110467.

54. Bagby RM, Ryder AG, Schuller DR, Marshall MB. 2004. The Hamilton Depression Rating Scale: Has the gold standard become a lead weight? *Am J Psychiatr* 161:2163–2177.

55. Ni YG, Miledi R. 1997. Blockage of 5-HT2C receptors by fluoxetine (Prozac). *Proc Natl Acad Sci USA* 94:2036–2040.

56. Garcia-Colunga J, Awad JN, Miledi R. 1997. Blockage of muscle and neuronal nicotinic acetylcholine receptors by fluoxetine (Prozac). *Proc Natl Acad Sci USA* 94:2041–2044.

57. Stahl SM. 1998. Not so selective serotonin reuptake inhibitors. *J Clin Psychiatry* 59:343–344.

58. Ranganathan R, Sawin ER, Trent C, Horvitz HR. 2001. Mutations in the *Caenorhabditis elegans* serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. *J Neurosci* 21:5871–5884.

59. Choy RKM, Thomas JH. 1999. Fluoxetine-resistant mutants in *C. elegans* define a novel family of transmembrane proteins. *Mol Cell* 4:143–152.

60. Dempsey CM. 2005. Serotonin (5HT), fluoxetine, imipramine and dopamine target distinct 5HT receptor signaling to modulate *Caenorhabditis elegans* egg-laying behavior. *Genetics* 169:1425–1436.

61. Kullyev ACM, Dempsey S, Miller C-JK, Hapiak VM, Komuniecki RW, Griffin CT, Sze JY. 2010. A genetic survey of fluoxetine action on synaptic transmission in *Caenorhabditis elegans*. *Genetics* 186:929–941.

62. Caveney S, Cladman W, Verellen L, Donly C. 2006. Ancestry of neuronal monoamine transporters in the Metazoa. *J Exp Biol* 209:4858–4868.

63. Peroutka SJ, Howell TA. 1994. The molecular evolution of G protein-coupled receptors: Focus on 5-hydroxytryptamine receptors. *Neuropharmacology* 33:319–324.

64. Schaefer A, Burmann I, Regenthal R, Arélin K, Barth C, Pampel A, Villringer A, Margulies DS, Sacher J. 2014. Serotonergic modulation of intrinsic functional connectivity. *Curr Biol* 24:2314–2318.

65. Rao N. 2007. The clinical pharmacokinetics of escitalopram. *Clin Pharmacokinet* 46:281–290.

66. Sanchez C, Reines EH, Montgomery SA. 2014. A comparative review of escitalopram, paroxetine, and sertraline: Are they all alike? *Int Clin Psychopharmacol* 29:185–196.