Antidepressants in stream ecosystems: influence of selective serotonin reuptake inhibitors (SSRIs) on algal production and insect emergence

Erinn K. Richmond[1,4], Emma J. Rosi-Marshall[2,5], Sylvia S. Lee[2,6], Ross M. Thompson[3,7], and Michael R. Grace[1,8]

[1]Water Studies Centre, School of Chemistry, Monash University, Clayton Victoria, Australia
[2]Cary Institute of Ecosystem Studies, Millbrook New York 12545 USA
[3]Institute for Applied Ecology, University of Canberra, Australian Capital Territory, Australia

Abstract: The effects of pharmaceuticals on aquatic ecosystems are the subject of increasing environmental concern. Of particular interest are a suite of drugs known as selective serotonin reuptake inhibitors (SSRIs), commonly prescribed to treat depression. SSRIs are now detected in the environment worldwide, but their effects on ecosystems are not well understood. We conducted replicated experiments testing for an ecosystem effect of SSRIs in streams. We used artificial stream mesocosms to expose natural biofilms and aquatic insect communities to concentrations (20 μg/L) of fluoxetine or citalopram or a mix of both (totaling 40 μg/L). These concentrations are the lowest found to have an effect on aquatic invertebrates in other studies. Treatments suppressed gross primary production by 29% and community respiration by ≥43% on rock biofilms but did not affect algal biomass or whole-stream metabolism. A common group of dipteran midges emerged earlier in all SSRI treated streams compared with the controls. Total biomass of emerged adults at day 14 was greater in the SSRI-exposed streams, suggesting that fluoxetine and citalopram may influence developmental processes in some stream insects. Ecosystem function and invertebrate population dynamics are sensitive to pharmaceuticals. Our study demonstrates that chronic exposure to fluoxetine and citalopram has the potential to affect aquatic biota and ecosystem function.

Key words: emergence, ecosystem function, aquatic insects, pharmaceuticals, SSRIs

Pharmaceuticals designed for human and veterinary uses are commonly found in surface waters affected by anthropogenic use (Kolpin et al. 2002, Monteiro and Boxall 2010). Most pharmaceuticals are dispersed into the aquatic environment via sewage systems, incorrect disposal, and urban or agricultural runoff (Daughton and Ternes 1999, Monteiro and Boxall 2010). Wastewater treatment plant outputs, septic tank flows, and leaky infrastructure are potential pathways of ecosystem exposure to pharmaceuticals (Kolpin et al. 2002, Kookana et al. 2014). Detection of pharmaceuticals has occurred across the globe, with levels often measured within the range of ng/L to μg/L (Metcalfe et al. 2010). However, how these chemicals affect key ecological processes is not well known (Rosi-Marshall and Royer 2012).

Pharmaceuticals used to treat depression, such as selective serotonin reuptake inhibitors (SSRIs), are prescribed frequently and are now commonly detected in surface waters (Kolpin et al. 2002, Lajeunesse et al 2008, Schultz and Furlong 2008, Vasskog et al. 2008, Metcalfe et al. 2010, Styrishave et al. 2011), groundwater (Barnes et al. 2008), and drinking water (Snyder 2008, Fick et al. 2009). In humans, SSRIs modify the activity of serotonin, a neurotransmitter (5-HTT) (Schafer 1999), by blocking reuptake by receptors in the brain and thereby increasing serotonin levels (Monteiro and Boxall 2010). The SSRIs fluoxetine (trade name Prozac®) and citalopram (trade name Celexa®) are prescribed frequently to humans, and low concentrations have been detected in surface waters (fluoxetine: 12 [Kolpin et al. 2002] to 46 ng/L [Metcalfe et al. 2003], citalopram: 40 to 90 ng/L [Schultz and Furlong 2008]). Concentrations in pharmaceutical discharge from hospitals and pharmaceutical manufacturing processes are often several orders of magnitude greater (Cardoso et al. 2014, Larsson 2014).

SSRIs can affect aquatic insects, amphibians, and fishes. Fluoxetine altered burrowing behavior of the freshwater

E-mail addresses: [4]erinn.richmond@monash.edu; [5]rosimarshalle@caryinstitute.org; [6]lees@caryinstitute.org; [7]ross.thompson@canberra.edu.au; [8]michael.grace@monash.edu

DOI: 10.1086/687841. Received 30 September 2015; Accepted 15 April 2016; Published online 29 June 2016.
Freshwater Science. 2016. 35(3):845–855.

bivalve *Lampsilis fascicola* at 22.3 μg/L (Hazelton et al. 2014) and induced spawning in zebra mussels (*Dreissena polymorpha*) at low concentrations (Fong 1998). Citalopram induced foot detachment in freshwater gastropods (*Leptoxis carinata* and *Stagnicola elodes*) at very low concentrations (405 pg/L and 4.05 μg/L, respectively) (Fong and Hoy 2012). Fluoxetine and sertraline (another commonly prescribed SSRI) reduced the growth rates of tadpoles at a range of concentrations (Conners et al. 2009). In addition, fluoxetine, sertraline, and their metabolites can bioaccumulate up to 1 μg/kg wet mass of fish in the brain, liver, and muscle tissue in several species (Brooks et al. 2005, Chu and Metcalfe 2007).

SSRIs may affect the life history and reproduction of some organisms, but knowledge of how SSRIs affect other taxonomic groups and ecosystem functions is necessary to understand fully the consequences of SSRIs in the environment. Algae are an important taxonomic group in aquatic ecosystems because they often form the basis of the food web and influence rates of ecosystem function (Mayer and Likens 1987, Stevenson 2014). A commonly studied alga, *Pseudokirchneriella subcapitata*, was more sensitive than a common cladoceran, *Daphnia magna*, to citalopram and fluoxetine (Christensen et al. 2007) and developed cell deformities when exposed to 13.6 and 27.2 μg/L of fluoxetine (Brooks et al. 2003a). Some pharmaceutical and personal care products (PPCPs) have the potential to disrupt stream biofilms (Johnson et al. 2007, Hoppe et al. 2012, Rosi-Marshall et al. 2013, 2015), but the influence of SSRIs on these functionally important components of stream ecosystems has not been investigated. Studies on the effect of SSRIs on ecosystem processes, such as primary production and respiration, are lacking. The exception is one study in which a concentration-dependent decrease in dissolved O_2 concentrations was found in mesocosms with phytoplankton exposed to a mixture of fluvoxamine, fluoxetine, and sertraline (Johnson et al. 2007).

Aquatic insects are critical components of stream ecosystems in terms of biodiversity and provision of ecosystem services (Wallace and Webster 1996) and energetic subsidies to terrestrial ecosystems (Baxter et al. 2005). Most studies of the effects of pharmaceuticals on aquatic macroinvertebrates have been single-species toxicology tests for chronic effects, most often in highly controlled laboratory settings (Brooks et al. 2003a, Nentwig 2007, Minagh et al. 2009). Some doubt exists as to whether single-species ecotoxicological assays are effective indicators of ecosystem effects in the field (Schäfer et al. 2013). Laboratory assays contribute to hazard-assessment models, which have proven useful in predicting toxicological effects of SSRIs on aquatic animals (Sanderson et al. 2004, Johnson et al. 2005), but the effects of SSRIs on aquatic insect communities and how these effects influence ecosystem function have not been investigated.

We used sublethal endpoints to examine the effects of SSRIs on whole-stream production and ecosystem respiration (ER), biofilm gross primary production (GPP), community respiration (CR), and algal biomass. We also measured the effects of SSRIs on aquatic insect size, biomass, emergence, and community composition. We used SSRI concentrations chosen to match the lowest found to cause effects in other studies as described above. To our knowledge, ours is the first study of the sublethal effects of SSRIs on ecosystem structure and function.

METHODS

Artificial streams and aquatic insect colonization

We conducted a 14-d experiment in August–September 2014. We filled 16 artificial streams constructed of composite fiberglass (4 m × 15.5 cm × 15 cm) with 60 L of low-nutrient groundwater without pharmaceutical contaminants. The water in the streams was circulated by stainless steel paddle wheels, and velocity was kept constant by maintaining the paddle wheels at 35 rpm with Dayton DC gear motors and speed controller (Dayton, Niles, Illinois). Artificial streams were housed in a greenhouse at the Cary Institute of Ecosystem Studies, Millbrook, New York. Average daily air temperature was 24.5°C during the experiment.

To provide algal, bacterial, and insect colonists to the artificial streams, we collected boulders and cobbles from a riffle–run section of a nearby creek, East Branch Wappinger Creek, Dutchess County, New York. This stream is a 4^{th}-order woodland stream with very limited sources of pharmaceutical contaminants and excellent biotic metrics of in-stream health (Sinsabaugh et al. 1994). We collected 20 rocks: 5 large (10–15 cm, measured along longest axis), 10 medium (5–10 cm), and 5 small (<5 cm) and added these to each artificial stream. All rocks had well-developed biofilm growth and an associated aquatic insect community. We transported rocks in large buckets (1 bucket/stream) with a small amount of stream water to retain any insects that detached from the rocks. Before adding the rocks to the artificial streams, we removed all visible large predators (e.g., *Plecoptera*, *Zygoptera*, *Megaloptera*) to ensure that the number of predators was consistent among replicate streams. In addition to the 20 stream rocks, we added 1 L of crushed quartz rocks and 5 larger (5–10 cm along longest axis) quartz rocks (Maryland River Rock; Ayers Supply, Clarks Summit, Pennsylvania) to increase substrate complexity in the artificial streams. We placed all rocks in the streams in haphazard clusters, in the same orientation (i.e., biofilm-colonized surface up) as in the field, to mimic natural stream benthic conditions. We allowed biofilm and aquatic insect communities in the artificial streams to acclimate for 2 d before treating with fluoxetine and citalopram. To include

large predators in the experiment, we added to each stream 1 h before treatment 10 premeasured (size range: 8–30 mm) predaceous stoneflies (Plecoptera:Perlidae) that had been maintained in similar flows, water chemistry, and temperature to the experimental conditions. We covered the streams with fine-mesh netting (0.25 mm) to capture emerging insects.

Experimental design

We allocated the 16 artificial streams among 4 experimental groups with 4 replicates/group: control, 20 μg/L fluoxetine, 20 μg/L citalopram, and mixed (20 μg/L fluoxetine + 20 μg/L citalopram). We randomly assigned treatments to the artificial streams. We prepared citalopram and fluoxetine (AK Scientific, Union City, California) stock solutions in ultrapure water. Our target concentrations were 20 μg/L (37.8 nmol/L fluoxetine, 49.4 nmol/L citalopram), which is well below maximum solubility of fluoxetine and citalopram (0.03 mol/L). We added 0.1341 g of fluoxetine·HCl and 0.1499 g of citalopram·HBr to ultrapure water (Barnstead Thermo Scientific, Dubuque, Iowa) to prepare 1-L stock solutions and then added 10 mL to each stream. Fluoxetine and citalopram have relatively high sorption capabilities (Kwon and Armbrust 2008) but are not readily degraded by sunlight (Kwon and Armbrust 2005, 2006). To maintain similar concentrations of all solutes throughout the experiment, we removed 50% of the water in all streams on day 7 and replaced it with fresh groundwater. We then added ½ of the stock solution amount (5 mL) to the treated streams.

We measured biofilm gross primary production (GPP) and community respiration (CR) at the end of the 2-wk exposure to SSRIs with light/dark incubations (Hill et al. 2002). We filled gas-tight glass jars with stream water and 1 biofilm-covered rock from each replicate stream. We filled an additional jar with stream water to isolate biofilm metabolism from water-column metabolism. To measure biofilm GPP, we recorded initial dissolved O_2 (DO) concentrations and water temperature with an optical DO probe (model 550A; Yellow Springs Instruments, Yellow Springs, Ohio). We then recapped jars, ensured that all air bubbles were eliminated, placed jars in their respective streams, and incubated them in sunlight for 3 h. After this time, we recorded final DO and temperature. We measured biofilm CR in the same way after jars were incubated in the dark. After incubations, we scrubbed all biofilm from the rocks and filtered subsamples through 0.7-μm glass-fiber filters (Whatman) to measure biofilm chlorophyll *a* by methanol extraction (Wetzel and Likens 1991) and ash-free dry mass (AFDM) by combustion of filtered samples at 550°C (Hauer and Lamberti 2011). We estimated the area of each rock with the aid of ImageJ software (Rasband 1997) and calculated biofilm GPP and CR per rock surface area (mg O_2 cm^{-2} h^{-1}) and per AFDM (mg O_2 g^{-1} AFDM h^{-1}). On day 14, we measured the total chlorophyll *a* and AFDM in each stream by scrubbing all substrates in each stream and subsampling. We calculated whole-stream chlorophyll *a* and AFDM per total stream area (0.62 m^2).

To estimate whole-stream metabolism (mg O_2 m^{-2} d^{-1}), we deployed 12 sensors (miniDOT; Precision Measurement Engineering, Vista, California) to obtain measurements of DO and temperature every 10 min ($n = 3$ sensors/treatment). We recorded incident photosynthetically active radiation (PAR) at the same interval with an Odyssey Light Logger (DataFlow Systems, Christchurch, New Zealand). We estimated daily rates of whole-stream GPP and ecosystem respiration (ER) via the Bayesian Single-station Estimation (BASE) model (Grace et al. 2015). This model involved nonlinear statistical fitting of these 2 rates plus stream reaeration to the DO, temperature, and PAR (light) data in all 12 mesocosms throughout the 14-d deployment.

We used modified drift nets (200-μm mesh) to measure aquatic insect drift for 4 h immediately after treating streams. Drift was measured in only 12 streams ($n = 3$ nets/treatment) because of equipment limitations. We preserved individuals in 70% ethanol for analysis and identification to family level.

We collected emerged adult insects with a vacuum (Model 315.115710; Craftsman, Hoffman Estates, Illinois) every 24 h at 0800 h. We preserved individuals in 70% ethanol for analysis and identification to order level with the exception of dipterans, which we identified to family. We also measured sex ratios by identifying male and female chironomids (males have obvious plumose antennae; Crane et al. 2002). We measured adult lengths with ImageJ and calculated the mass of individuals with a length–mass regression for Chironomidae ($a = 0.1$, $b = 1.57$; Collins 2014).

On day 14, we collected the entire aquatic insect community in each stream. We brushed all rock and stream surfaces into the stream water, subsampled as above for total algal biomass (chlorophyll *a* and AFDM), and sieved the contents of the stream through a 250-μm-mesh sieve. We preserved aquatic insects in 70% ethanol for enumeration and identification to family level and to genus (when possible) with the aid of taxonomic references (Merritt and Cummins 1996, Wiggins 1996). Filter-feeding caddisflies were abundant in all treatments, so we used them as an indicator of growth of aquatic insects when exposed to the treatments. We photographed individuals in the family Hydropsychidae and measured their length with ImageJ. We used length–mass regressions for Hydropsychidae ($M = aL^b$) to estimate individual biomass ($a = 0.0046$, $b = 2.926$), where M is mass (mg), L is length (mm), and *a* and *b* are constants (Benke et al. 1999). We assessed change in biomass relative to control streams.

Statistical analysis

We used 1-way analysis of variance (ANOVA) to examine SSRI treatment effects on biofilm GPP, CR, AFDM, chlorophyll *a*, and aquatic insect biomass and emergence. When examining the treatment effects on biofilm GPP (mg O_2 m^{-2} h^{-1}), we conducted a Dixon's *Q* test to remove any significant outliers and reduced the degrees of freedom. We used a nonparametric Kruskal–Wallis test to analyze biofilm CR (mg O_2 m^{-2} h^{-1}) because data failed normality and equal variance assumptions for ANOVA. For daily whole-stream GPP (mg O_2 m^{-2} d^{-1}) and ER (mg O_2 m^{-2} d^{-1}), number of emerged insects through time, and emerged adult dipteran sex ratios, we used a linear mixed-effect (LME) model run with the *lme*() function in the *nmle* package in R (version 3.1-127; Pinheiro et al. 2016). We fitted LME models with the restricted maximum likelihood method and a continuous autoregressive temporal correlation structure to have day as the repeated factor. To meet the assumptions of LME, we log(*x*)-transformed all data except ER, which we $\sqrt{(x)}$-transformed. We analyzed differences in aquatic insect populations (mean number of individuals of each taxon) among treatments with 1-way ANOVAs. When an ANOVA resulted in a significant *p*-value, we used a *t*-test for multiple comparisons to identify differences. We used analysis of similarity (ANOSIM) to compare community composition (PRIMER, version 6; Clarke and Gorley 2006). We conducted all other statistical analyses in R (version 3.11; R Project for Statistical Computing, Vienna, Austria).

RESULTS

Effects of fluoxetine and citalopram on basal resources

Biofilms exposed to SSRIs had 43 to 66% lower GPP relative to control biofilms ($F_{3,12} = 5.205$, $p = 0.016$). Biofilms exposed to citalopram ($p = 0.004$) and mix ($p = 0.007$) treatments had lower GPP relative to control biofilms. Biofilms exposed to fluoxetine had 57% lower GPP, but this difference was not statistically significant ($p = 0.051$; Fig. 1A). Biofilm CR was reduced 44 to 67% (mg O_2 mg^{-1} AFDM h^{-1}) in all SSRI treatments relative to the control ($F_{3,12} = 5.60$, $p = 0.012$; fluoxetine: $p = 0.032$, citalopram: $p = 0.003$; mix: $p = 0.006$; Fig. 1B). When expressed on a rock-area basis, biofilm GPP was 29 to 41% lower in SSRI than in control treatments ($F_{3,11} = 5.799$, $p = 0.013$; fluoxetine: $p = 0.0021$, citalopram: $p = 0.003$; mix: $p = 0.005$). On a rock-area basis, biofilm CR was 13 to 30% lower in SSRI than in control treatments, but these differences were not statistically significant ($F_{3,12} = 1.32$, $p = 0.314$). Biofilm and whole-stream chlorophyll *a* concentrations ($F_{3,12} = 0.506$, $p = 0.686$) and AFDM ($F_{3,12} = 0.733$, $p = 0.552$) did not differ significantly between SSRI and control treatments (Fig. 1C, D). Whole-stream GPP rates were not statistically different between SSRI and control treatments ($F_{3,8} = 0.045$, $p = 0.986$). Whole-stream ER also was not significantly different between SSRI and control treatments ($F_{3,8} = 0.511$, $p = 0.686$), although some suppression was observed for fluoxetine (45% suppression relative to control). Autotrophic (g O_2 mg^{-1} chlorophyll *a* d^{-1}) and heterotrophic activity (g O_2 g^{-1} AFDM d^{-1}) did not differ between SSRI and control treatments ($F_{3,8} = 0.524$, $p = 0.678$; $F_{3,8} = 0.331$, $p = 0.803$; respectively).

Effects of fluoxetine and citalopram on aquatic insect communities

Four hours after pharmaceutical addition, we found no significant difference in drifting aquatic insects between SSRI and control treatments ($F_{3,8} = 0.478$, $p = 0.706$).

Aquatic insect community composition was similar in the control and treatment groups at the end of the experiment (Table 1). However, the number of individuals of orthocladiine chironomids differed between SSRI and control treatments ($F_{3,12} = 6.566$, $p = 0.007$). Numbers of individuals were higher for fluoxetine than for control treatments ($p = 0.02$). Dissimilarity in community composition among control and SSRI groups was no greater than dissimilarity within groups (ANOSIM, Global $R = -0.049$). Shannon–Wiener diversity ($F_{3,12} = 1.744$, $p = 0.211$) and average taxon richness ($F_{3,12} = 1.286$, $p = 0.324$) did not differ between SSRI and control treatments.

Total mean biomass ($F_{3,12} = 1.798$, $p = 0.201$; Fig. 2A) and number of Hydropsychidae individuals ($F_{3,12} = 0.621$, $p = 0.615$; Table 2) did not differ between SSRI and control treatments. Mean biomass per Hydropsychidae individual was 24 to 46% greater in SSRI than in control treatments, but this difference was not significant ($F_{3,12} = 0.621$, $p = 0.615$; Fig. 2B).

Over the course of the experiment, the average number of emerged dipterans was 32% higher in SSRI relative to control treatments, but this difference was not statistically significant ($F_{3,12} = 0.718$, $p = 0.56$; Fig. 3). The total number and total mass of emerged aquatic insects over the 14-d experiment were not significantly different among SSRI and control treatments ($F_{3,12} = 0.518$, $p = 0.05$; $F_{3,12} = 0.402$, $p = 0.05$, respectively), but the overall trends suggest that a greater number (Fig. 3) and greater total mass of individuals emerged from SSRI-treated streams. The number of dipteran individuals ($F_{3,12} = 0.389$, $p = 0.763$) and total dipteran mass ($F_{3,12} = 0.367$, $p = 0.778$) emerged at days 7 and 14 ($F_{3,12} = 2.7005$, $p = 0.0925$) did not differ between SSRI and control treatments, but we observed a 22 to 42% increase in the numbers emerged at day 7 and a 16 to 44% increase in numbers emerged at day 14 in SSRI relative to control treatments. Emerged dipteran mass at day 14 was greater in fluoxetine ($p = 0.012$) and mix ($p = 0.010$) ($F_{3,12} = 4.016$, $p = 0.0342$) than in control treatments. However,

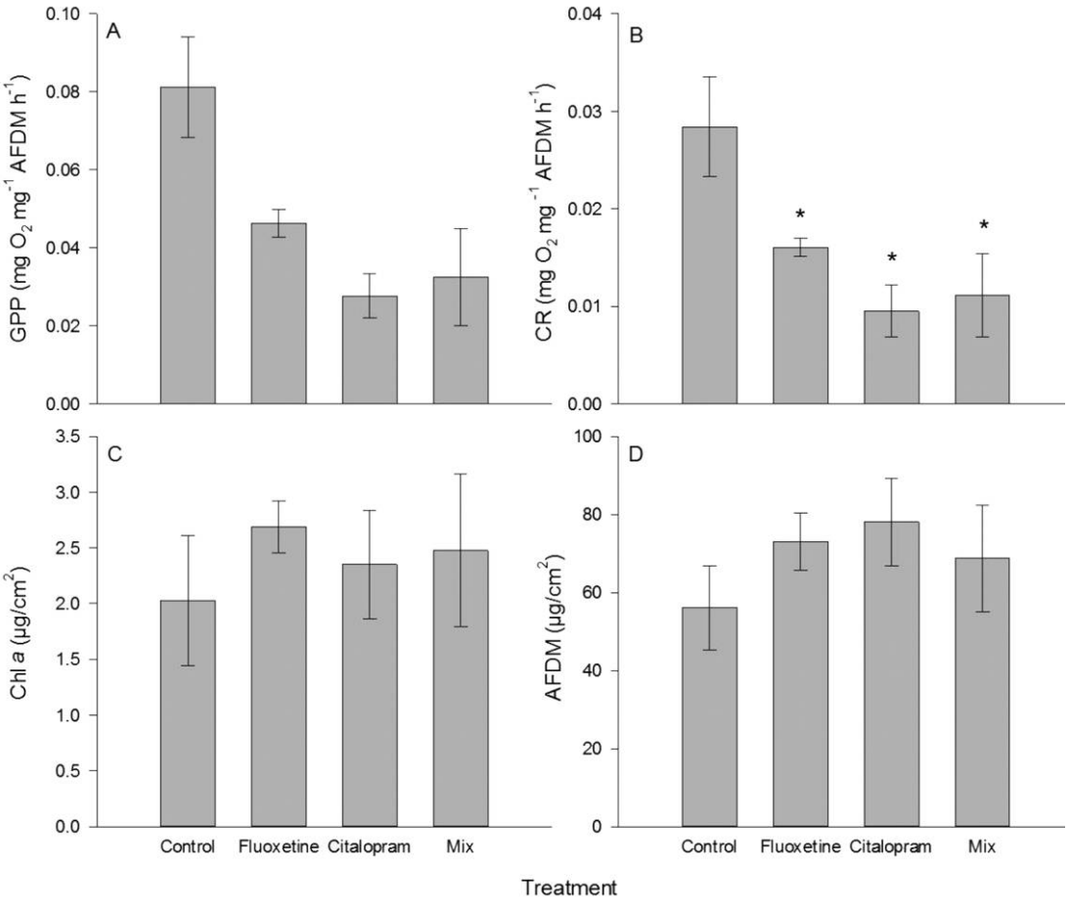

Figure 1. Mean (±1 SE) biofilm gross primary production (GPP) (A), community respiration (CR) (B), chlorophyll *a* (chl *a*) (C), and ash-free dry mass (AFDM) (D) in response to treatment with fluoxetine and citalopram singly and in combination (mix). Asterisks indicate values that differ significantly between treatment and control ($p < 0.05$).

the number of individuals emerged did not differ significantly among SSRI and control treatments at day 7 ($F_{3,12} = 1.76$, $p = 0.28$) or 14 ($F_{3,12} = 0.60$, $p = 0.592$). Sex ratios of emerged dipterans at days 1, 7, and 14 did not differ between SSRI and control treatments ($F_{3,12} = 0.718$, $p = 0.56$).

DISCUSSION

Worldwide, streams receive inputs of human waste with associated pharmaceuticals, and these inputs may have potential ecological effects (Rosi-Marshall and Royer 2012). Our study demonstrates that SSRIs in rivers and streams have the potential to affect both ecosystem processes (biofilm biomass and metabolism) and aquatic insect communities (emergence rates, size at emergence, and biomass). Because SSRIs commonly are detected in streams, the need to understand the effects of SSRIs on ecosystem structure and function and aquatic insect populations is growing. Most of our current knowledge about the effects of SSRIs is limited to single-species toxicity tests that use mortality as an endpoint. Toxicity tests may underestimate sublethal ecological consequences of these compounds, which may occur even at low concentrations (Schäfer et al. 2013). The concentration of SSRIs we used (20 µg/L) was an order of magnitude lower than concentrations reported to lead to mortality (i.e., median lethal concentrations [LC50]), which range from 234 µg/L for *Ceriodaphnia dubia* to 820 µg/L for *Daphnia magna* (Brooks et al. 2003a). However, the concentration we used was comparable to those used in other studies that demonstrated effects on sublethal endpoints (Conners et al. 2009, Fong and Ford 2014). The emerging evidence of the potential ecological effects of SSRIs at low concentrations underscores the need to study sublethal endpoints, particularly at ng/L concentrations (e.g., de Lange et al. 2006).

Pharmaceuticals rarely are found singularly in aquatic systems (Boxall et al. 2012), so the mixed treatment in our study was intended to help build understanding of this additional complexity in real environments and at a lower concentration than those used in toxicology assays.

Table 1. Mean (± SE) number of the 16 most-abundant aquatic insect individuals/treatment (in order of decreasing abundance in the control streams) and results of 1-way analysis of variance (ANOVA). ANOVA analysis was not conducted for taxa with <50% relative abundance. Asterisks indicate significant difference between treatments and control ($p < 0.05$).

Taxon	Control	Fluoxetine	Citalopram	Mix	F	p
Hydropsychidae	47 ± 5	42 ± 12.25	28 ± 5	36 ± 14	0.621	0.615
Chironomidae Orthocladiinae	42 ± 8	80 ± 10	41 ± 7	34 ± 7	6.566	0.007*
Tipulidae *Antocha*	24 ± 6	34 ± 9	25 ± 2	30 ± 10	0.406	0.751
Perlidae	10.8 ± 1.5	9.3 ± 0.9	8 ± 2	10.5 ± 0.9	0.751	0.542
Hydroptilidae	7 ± 2	13 ± 6	4 ± 2	1.3 ± 1.3	2.35	0.109
Chironomidae Chironominae	6 ± 2	12 ± 3	11 ± 5	14 ± 6	0.638	0.605
Chironomidae Tanypodinae	5.0 ± 1.1	8.3 ± 1.5	5 ± 2	5.5 ± 0.6	1.301	0.319
Psephenidae *Psephenus*	5.5 ± 1.3	6 ± 2	9.3 ± 1.1	7.8 ± 0.5	1.86	0.190
Glossosomatidae	4.3 ± 1.4	3 ± 2	3.3 ± 1.0	7 ± 3	0.824	0.506
Elmidae	2.3 ± 1.0	2.5 ± 1.2	4 ± 2	1.5 ± 0.6	0.622	0.614
Brachycentridae	1.8 ± 0.9	1.0 ± 0.7	1.0 ± 0.7	1.0 ± 0.4	0.297	0.827
Heptageniidae	1.0 ± 0.4	3.0 ± 1.0	1.0 ± 0.8	3.0 ± 0.5	1.268	0.329
Limnephilidae	0.5 ± 0.3	3 ± 2	0.5 ± 0.5	0.8 ± 0.5	0.736	0.916
Psephenidae *Ectopria*	0 ± 0	0.5 ± 0.5	2.0 ± 1.0	1.5 ± 0.9	1.82	0.198
Isonychiidae	0 ± 0	0.8 ± 0.5	0.8 ± 0.5	0 ± 0	–	–
Corydalidae *Corydalus*	0	0.5	1.0	0	–	–

Algal and insect responses to the SSRI mixture used in our study were similar to responses observed in other studies based on individual compounds, but we did not observe a synergistic effect with the mixture we used. This result is in contrast to other studies in which mixtures of other SSRIs similar to those we used caused synergistic responses (Christensen et al. 2007, Henry and Black 2007). One possible explanation for the lack of synergistic effects observed in our study may be that pharmaceuticals are designed to be effective at low doses (concentrations), and thus the concentration in our mixture treatment was too high to result in synergistic effects. Other investigators have reported that low concentrations of SSRIs can have greater effects than high concentrations, potentially because of differing or interacting response mechanisms (de Lange et al. 2006).

Biofilms are a key resource in stream food webs, and their function is critical to secondary production of consumers (Wallace and Webster 1996). Adverse effects of compounds on biofilm communities may have direct implications for consumers and stream functions such as nutrient cycling and microbial activity (Rosi-Marshall et al. 2013). We observed a significant reduction in the rate of primary production of biofilms on individual rocks. This result is consistent with the decrease in DO concentrations in phytoplankton communities exposed to fluoxetine reported by Johnson et al. (2007). We did not examine biofilm community structure explicitly, but other investigators have reported that SSRIs have antimicrobial properties with the potential to affect composition of both bacterial and algal communities (Munoz-Bellido et al. 2000). SSRIs can act as antimicrobials by inhibiting serotonin efflux pumps in bacterial cells (Munoz-Bellido et al. 2000, Bohnert et al. 2011) and by producing deformations in algal cells (Brooks et al. 2003a). Algae exposed to fluoxetine also can be smaller than normal (Brooks et al. 2003a), which in combination with cell deformities may reduce photosynthetic performance, leading to lower production and respiration.

We observed a reduction in GPP of biofilms on rocks, but we found no evidence of a reduction in the total algal biomass that grew in the streams during the experiment. SSRIs did not appear to kill algal cells directly but instead reduced activity of the biofilms. In an experiment of longer duration (on the order of months), algal biomass might decrease if photosynthetic efficiency were consistently suppressed over time. We also saw no effect of SSRIs on whole-stream primary production. We attribute this absence of a statistically significant effect to the relatively low level of suppression of biofilm GPP by SSRIs (i.e., a 29% decline as measured in the light–dark chamber incubations). This level of suppression may not be detectable at the whole-stream scale given variability among streams.

Aquatic insect drift is a common phenomenon that can occur unintentionally via dislocation from increased flow regimes or as a behavioral response to predation or food source (Shearer et al. 2002). Drift can also be an indicator of sensitivity to pollutants (Wallace et al. 1989) and often occurs in streams contaminated with heavy metals (Clements 1999). The apparent lack of an effect of SSRIs on aquatic insect community composition and biomass suggests that concentrations were not high

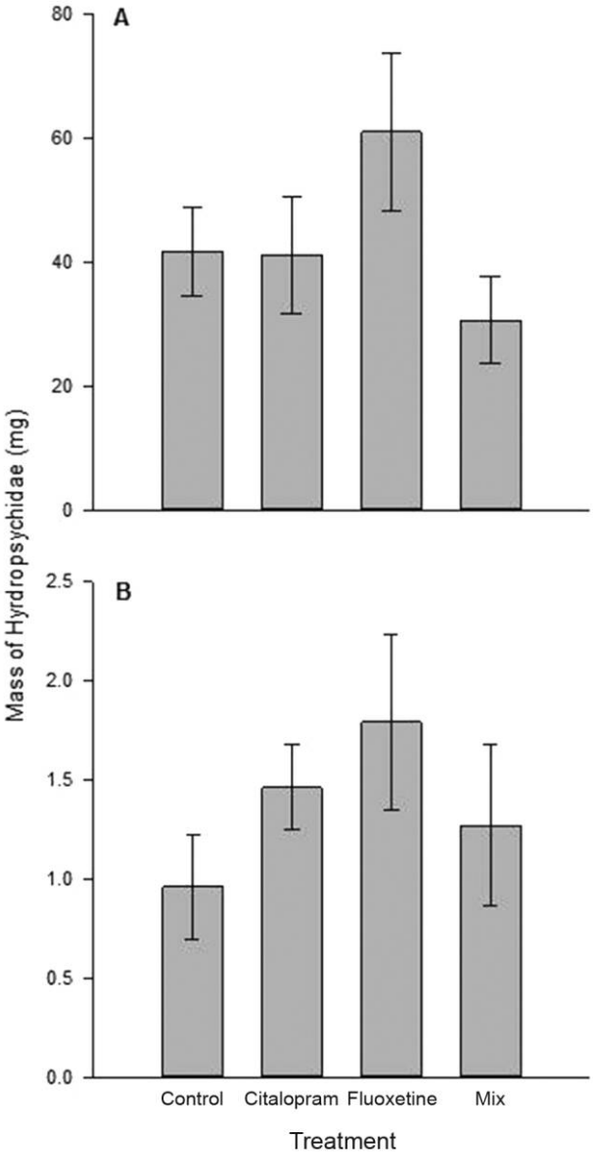

Figure 2. Mean (±1 SE) total Hydropsychidae biomass per treatment (A) and per individual (B) in response to treatment with fluoxetine and citalopram singly and in combination (mix).

enough to cause mortality or create disturbance (e.g., drift) and that SSRIs may act as a different type of stressor than do heavy metals. However, our data suggest that SSRIs could affect aquatic insect life-history dynamics and individual growth rates (e.g., emergence, biomass of emerged adults, and Hydropsychidae biomass) in some situations. The artificial stream communities, which were dominated by hydropsychids from the beginning of the experiment, exhibited a nonsignificant increase in the per capita biomass of Hydropsychidae in SSRI-treated streams. Our findings from the mixed treatment are consistent with those of previous studies that have shown that SSRIs and other PPCPs may have greater effects on organisms at low than at high concentrations (Conners et al. 2009).

Although not statistically significant, the potential increase in insect emergence in response to SSRIs is worth discussion given our observation that dipteran emergence was consistently higher relative to the control treatments on nearly all days of the experiment and given the ecologically important role of emerged aquatic insects as a link between terrestrial and aquatic ecosystems. We pose 3 explanations for the trend toward an increase in dipteran emergence in SSRI-treated streams: 1) serotonin may stimulate insect ecdysteroids, a sex hormone responsible for insect molting (Brooks et al. 2003a), 2) SSRIs may promote growth, so individuals emerged sooner because of increased rates of developmental processes, and 3) pulse releases of SSRIs may create a disturbance, leading to altered rates of emergence and reduced individual size. SSRIs can promote spawning in zebra mussels (Fong and Hoy 2012) and increased the number of *Daphnia magna* produced per brood when they were exposed to 36 µg/L of fluoxetine (Flaherty and Dodson 2005). Other investigators have reported that SSRIs decreased emergence of chironomids (Nentwig 2007, Péry et al. 2008, Sánchez-Argüello et al. 2009). These investigators exposed chironomids to SSRIs by spiking sediments, and uptake of SSRIs by chironomids in our study probably occurred via feeding and direct assimilation of SSRIs from the water column or biofilm rather than via sediment exposure (Brooks et al. 2003b). Further research is necessary to test whether the route of exposure to SSRIs influences their effects on aquatic organisms.

Emerged aquatic adults are an important link between stream and terrestrial environments because they can transport energy (Baxter et al. 2005) and contaminants (Walters et al. 2009) to terrestrial consumers. Chironomids, in particular, are highly productive and have the capacity to transport substantial energy or contaminants to terrestrial ecosystems and their consumers. If contaminant exposure alters rates of emergence and patterns of emergence production, this effect may have consequences for both the aquatic insects and their predators (Kraus et al. 2014). In addition, individuals that emerge sooner typically emerge smaller (Sweeney and Vannote

Table 2. Hydropsychidae total, mean (±SE) number of individuals/treatment, and result of 1-way analysis of variance (ANOVA).

Treatment	Total	Mean	ANOVA
Control	162	41 ± 5	$F_{3,12} = 0.51$, $p = 0.683$
Citalopram	113	28 ± 7	
Fluoxetine	180	45 ± 13	
Mix	125	31 ± 16	

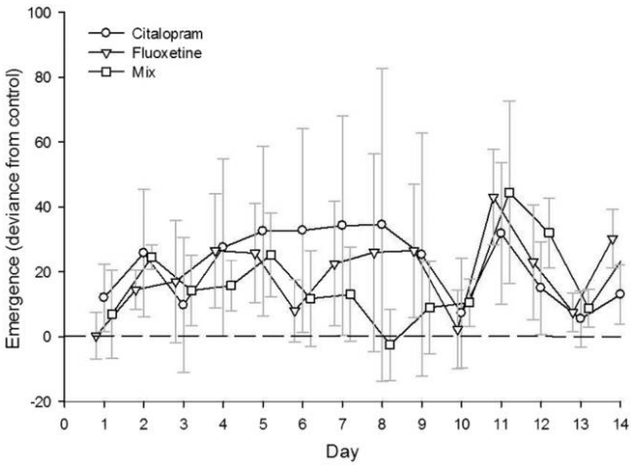

Figure 3. Mean (±1 SE) dipteran individuals emerged in response to treatment with fluoxetine and citalopram singly and in combination (mix) over the 14-d experiment. Treatments are normalized from the control (dashed line at 0).

1978). Thus, they are likely to have decreased caloric value but may have had less time to bioaccumulate contaminants (Kraus et al. 2014). We observed an increase (not statistically significant) in the number of insects emerging from treated streams over time but no difference in dipteran size (although trends at day 14 suggested greater individual size in treated than control streams, but again were not statistically significant). Greater individual size may be linked to higher per capita grazing rate in treated streams, which also could explain the reduction of GPP in treated streams. Overall, our data, although not statistically significant, suggest that SSRIs influenced patterns of aquatic insect emergence, and the potential behavioral or physiological changes underlying the observed patterns may have consequences for adjacent terrestrial consumers and ecosystems receiving the emerged aquatic insects.

We acknowledge that some results in this study were not statistically significant, but because of the lack of previous research on this topic, we have taken a precautionary approach to discuss the potential effects of pharmaceuticals on stream ecosystems (Buhl-Mortensen 1996). The precautionary principle for limiting the risk of type II error, failure to reject the null hypothesis when it is false, has been argued in favor of protecting environmental well-being (e.g., from contaminants) when uncertainties are large and the potential exists for negative effects on a common good (Peterman and M'Gonigal 1992, Buhl-Mortensen 1996). A greater number of replicates or greater sampling effort over time, depending on the research question, probably would decrease the probability of type II error (Underwood and Chapman 2003). In addition, criticisms of over-reliance on p values should be considered when statistical power is low (Colquhoun 2014, Halsey et al. 2015). Our study shows the potential ecological significance of pharmaceuticals on stream ecosystems, but our statistical results were influenced by high variability among replicates within treatments and the low statistical power ($n = 4$ replicates per treatment) that will often be the result of large-scale artificial-stream experiments.

Worldwide use of pharmaceuticals continues to grow, and increased input of these compounds into aquatic systems is anticipated with unknown ecological consequences (Rosi-Marshall and Royer 2012, Rosi-Marshall et al. 2015). Our results demonstrate that pharmaceuticals have the potential to cause direct and indirect effects on ecosystem function and trophic interactions. The next step is to extend the experiments to examine whether similar effects are observed at the SSRI concentrations typically found in many surface waters (typically 10–100 ng/L).

ACKNOWLEDGEMENTS

This research was funded by an Australian Research Council Linkage Grant LP130100040 (MRG, EJR-M) and a Dean's Postgraduate Research Scholarship from the Faculty of Science, Monash University (EKR). We thank Travis Schmidt for assistance with data presentation (Fig. 3).

LITERATURE CITED

Barnes, K. K., D. W. Kolpin, E. T. Furlong, S. D. Zaugg, M. T. Meyer, and L. B. Barber. 2008. A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States—I) Groundwater. Science of the Total Environment 402:192–200.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology 50:201–220.

Benke, A. C., A. H. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18:308–343.

Bohnert, J. A., M. Szymaniak-Vits, S. Schuster, and W. V. Kern. 2011. Efflux inhibition by selective serotonin reuptake inhibitors in *Escherichia coli*. Journal of Antimicrobial Chemotherapy 66:2057–2060.

Boxall, A. B. A., M. A. Rudd, B. W. Brooks, D. J. Caldwell, K. Choi, S. Hickmann, E. Innes, K. Ostapyk, J. P. Staveley, T. Verslycke, G. T. Ankley, K. F. Beazley, S. E. Belanger, J. P. Berninger, P. Carriquiriborde, A. Coors, P. C. DeLeo, S. D. Dyer, J. F. Ericson, F. Gagné, J. P. Giesy, T. Gouin, L. Hallstrom, M. V. Karlsson, D. G. J. Larsson, J. M. Lazorchak, F. Mastrocco, A. McLaughlin, M. E. McMaster, R. D. Meyerhoff, R. Moore, J. L. Parrott, J. R. Snape, R. Murray-Smith, M. R. Servos, P. K. Sibley, J. O. Straub, N. D. Szabo, E. Topp, G. R. Tetreault, V. L. Trudeau, and G. van der Kraak. 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environmental Health Perspectives 120:1221–1229.

Brooks, B. W., C. K. Chambliss, J. K. Stanley, and A. Ramírez. 2005. Determination of select antidepressants in fish from an effluent dominated stream. Environmental Toxicology and Chemistry 24:464–469.

Brooks, B. W., C. M. Foran, S. M. Richards, J. Weston, P. K. Turner, J. K. Stanley, K. R. Solomon, M. Slattery, and T. W. La Point. 2003a. Aquatic ecotoxicology of fluoxetine. Toxicology Letters 142:169–183.

Brooks, B. W., P. K. Turner, J. K. Stanley, J. J. Weston, E. A. Glidewell, C. M. Foran, M. Slattery, T. W. La Point, and D. B. Huggett. 2003b. Waterborne and sediment toxicity of fluoxetine to select organisms. Chemosphere 52:135–142.

Buhl-Mortensen, L. 1996. Type-II statistical errors in environmental science and the precautionary principle. Marine Pollution Bulletin 32:528–531.

Cardoso, O., J.-M. Porcher, and W. Sanchez. 2014. Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: review of evidence and need for knowledge. Chemosphere 115:20–30.

Christensen, A. M., S. Faaborg-Andersen, I. Flemming, and A. Baun. 2007. Mixture and single-substance toxicity of selective serotonin reuptake inhibitors toward algae and crustaceans. Environmental Toxicology and Chemistry 26:85–91.

Chu, S., and C. D. Metcalfe. 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. Journal of Chromatography A 1163:112–118.

Clarke, K., and R. Gorley. 2006. User manual/tutorial. PRIMER-E Ltd., Plymouth, UK.

Clements, W. H. 1999. Metal tolerance and predator–prey interactions in benthic macroinvertebrate stream communities. Ecological Applications 9:1073–1084.

Collins, S. F. 2014. The ecological importance of subsidies from salmon to stream riparian ecosystems: an experimental test and implications for approaches to nutrient mitigation. PhD Thesis, Idaho State University, Pocatello, Idaho.

Colquhoun, D. 2014. An investigation of the false discovery rate and the misinterpretation of *p*-values. Royal Society Open Science 1:140216.

Conners, D. E., E. D. Rogers, K. L. Armbrust, J.-W. Kwon, and M. C. Black. 2009. Growth and development of tadpoles (*Xenopus laevis*) exposed to selective serotonin reuptake inhibitors, fluoxetine and sertraline, throughout metamorphosis. Environmental Toxicology and Chemistry 28:2671–2676.

Crane, M., W. Sildanchandra, R. Kheir, and A. Callaghan. 2002. Relationship between biomarker activity and developmental endpoints in *Chironomus riparius* Meigen exposed to an organophosphate insecticide. Ecotoxicology and Environmental Safety 53:361–369.

Daughton, C. G., and T. A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environmental Health Perspectives 107:907–938.

de Lange, H. J., W. Noordoven, A. J. Murk, M. Lürling, and E. T. H. M. Peeters. 2006. Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquatic Toxicology 78:209–216.

Fick, J., H. Söderström, R. H. Lindberg, C. Phan, M. Tysklind, and D. G. J. Larsson. 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environmental Toxicology and Chemistry 28:2522–2527.

Flaherty, C. M., and S. I. Dodson. 2005. Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction. Chemosphere 61:200–207.

Fong, P. P. 1998. Zebra mussel spawning is induced in low concentrations of putative serotonin reuptake inhibitors. Biological Bulletin 194:143.

Fong, P. P., and A. T. Ford. 2014. The biological effects of antidepressants on the molluscs and crustaceans: a review. Aquatic Toxicology 151:4–13.

Fong, P. P., and C. M. Hoy. 2012. Antidepressants (venlafaxine and citalopram) cause foot detachment from the substrate in freshwater snails at environmentally relevant concentrations. Marine and Freshwater Behaviour and Physiology 45: 145–153.

Grace, M. R., D. P. Giling, S. Hladyz, V. Caron, R. M. Thompson, and R. Mac Nally. 2015. Fast processing of diel oxygen curves: estimating stream metabolism with BASE (BAyesian Single-station Estimation). Limnology and Oceanography: Methods 13:103–114.

Halsey, L. G., D. Curran-Everett, S.L. Vowler, and G. B. Drummond. 2015. The fickle *p* value generates irreproducible results. Nature Methods 12:179–185.

Hauer, F. R., and G. A. Lamberti. 2011. Methods in stream ecology. Academic Press, Burlington, Massachusetts.

Hazelton, P. D., B. Du, S. P. Haddad, A. K. Fritts, C. K. Chambliss, B. W. Brooks, and R. B. Bringolf. 2014. Chronic fluoxetine exposure alters movement and burrowing in adult freshwater mussels. Aquatic Toxicology 151:27–35.

Henry, T. B., and M. C. Black. 2007. Mixture and single-substance acute toxicity of selective serotonin reuptake inhibitors in *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry 26:1751–1755.

Hill, B. H., A. T. Herlihy, and P. R. Kaufmann. 2002. Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern U.S.A. Freshwater Biology 47:185–194.

Hoppe, P. D., E. J. Rosi-Marshall, and H. A. Bechtold. 2012. The antihistamine cimetidine alters invertebrate growth and population dynamics in artificial streams. Freshwater Science 31:379–388.

Johnson, D. J., H. Sanderson, R. A. Brain, C. J. Wilson, K. T. Bestari, and K. R. Solomon. 2005. Exposure assessment and microcosm fate of selected selective serotonin reuptake inhibitors. Regulatory Toxicology and Pharmacology 42:313–323.

Johnson, D. J., H. Sanderson, R. A. Brain, C. J. Wilson, and K. R. Solomon. 2007. Toxicity and hazard of selective serotonin reuptake inhibitor antidepressants fluoxetine, fluvoxamine, and sertraline to algae. Ecotoxicology and Environmental Safety 67:128–139.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environmental Science and Technology 36:1202–1211.

Kookana, R. S., M. Williams, A. B. A. Boxall, D. G. J. Larsson, S. Gaw, K. Choi, H. Yamamoto, H., S. Thatikonda, Y.-G.

Zhu, and P. Carriquiriborde. 2014. Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high-income countries. Philosophical Transactions of the Royal Society of London B: Biological Sciences 369:20130586.

Kraus, J. M., D. M. Walters, J. S. Wesner, C. A. Stricker, T. S. Schmidt, and R. E. Zuellig. 2014. Metamorphosis alters contaminants and chemical tracers in insects: implications for food webs. Environmental Science and Technology 48:10957–10965.

Kwon, J.-W., and K. L. Armbrust. 2005. Degradation of citalopram by stimulated sunlight. Environmental Toxicology and Chemistry 24:618–1623.

Kwon, J.-W., and K. L. Armbrust. 2006. Laboratory persistence and fate of fluoxetine in aquatic environments. Environmental Toxicology and Chemistry 25:2561–2568.

Kwon, J.-W., and K. L. Armbrust. 2008. Aqueous solubility, n-octanol-water partition coefficient, and sorption of five selective serotonin reuptake inhibitors to sediments and soils. Bulletin of Environmental Contamination and Toxicology 81:128–135.

Lajeunesse, A., C. Gagnon, and S. Sauvé. 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. Analytical Chemistry 80:5325–5333.

Larsson, D. G. J. 2014. Pollution from drug manufacturing: review and perspectives. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 369:20130571

Mayer, M. S., and G. E. Likens. 1987. The importance of algae in a shaded headwater stream as food for an abundant caddisfly (Trichoptera). Journal of the North American Benthological Society 6:262–269.

Merritt, R. W., and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt, Dubuque, Iowa.

Metcalfe, C. D., S. Chu, C. Judt, H. Li., K. D. Oakes, M. R. Servos, and D. M. Andrews. 2010. Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. Environmental Toxicology and Chemistry 29:79–89.

Metcalfe, C. D., X.-S. Miao, B. G. Koenig, and J. Struger. 2003. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada. Environmental Toxicology and Chemistry 22:2881–2889.

Minagh, E., R. Hernan, K. O'Rourke, F. M. Lyng, and M. Davoren. 2009. Aquatic ecotoxicity of the selective serotonin reuptake inhibitor sertraline hydrochloride in a battery of freshwater test species. Ecotoxicology and Environmental Safety 72:434–440.

Monteiro, S., and A. A. Boxall. 2010. Occurrence and fate of human pharmaceuticals in the environment. Reviews of Environmental Contamination and Toxicology 202:53–154.

Munoz-Bellido, J. L., S. Munoz-Criado, and J. A. Garcıa-Rodrıguez. 2000. Antimicrobial activity of psychotropic drugs: selective serotonin reuptake inhibitors. International Journal of Antimicrobial Agents 14:177–180.

Nentwig, G. 2007. Effects of pharmaceuticals on aquatic invertebrates. Part II: The antidepressant drug fluoxetine. Archives of Environmental Contamination and Toxicology 52:163–170.

Péry, A. R. R., M. Gust, B. Vollat, R. Mons, M. Ramil, G. Fink, T. Ternes, and J. Garric. 2008. Fluoxetine effects assessment on the life cycle of aquatic invertebrates. Chemosphere 73:300–304.

Peterman, R. M., and M. M'Gonigle. 1992. Statistical power analysis and the precautionary principle. Marine Pollution Bulletin 24:231–234.

Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2016. nlme: linear and nonlinear mixed effects models. Version 3.1-127. R Project for Statistical Computing, Vienna, Austria. (Available from: http://CRAN.R-project.org/package=nlme)

Rasband, W. 1997. ImageJ. Version 1.48. US National Institutes of Health, Bethesda, Maryland. (Available from: http://imagej.nih.gov/ij/)

Rosi-Marshall, E. J., and T. Royer. 2012. Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. Ecosystems 15:867–880.

Rosi-Marshall, E. J., D. W. Kincaid, H. A. Bechtold, T. V. Royer, M. Rojas, and J. J. Kelly. 2013. Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. Ecological Applications 23: 583–593.

Rosi-Marshall, E. J., D. Snow, S. L. Bartelt-Hunt, A. Paspalof, and J. L. Tank. 2015. A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. Journal of Hazardous Materials 282:18–25.

Sánchez-Argüello, P., C. Fernández, and J. V. Tarazona. 2009. Assessing the effects of fluoxetine on *Physa acuta* (Gastropoda, Pulmonata) and *Chironomus riparius* (Insecta, Diptera) using a two-species water–sediment test. Science of the Total Environment 407:1937–1946.

Sanderson, H., D. J. Johnson, T. Reitsma, R. A. Brain, C. J. Wilson, and K. R. Solomon. 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regulatory Toxicology and Pharmacology 39:158–183.

Schäfer, R. B., N. Gerner, B. J. Kefford, J. J. Rasmussen, M. A. Beketov, D. de Zwart, M. Liess, and P. C. von der Ohe. 2013. How to characterize chemical exposure to predict ecologic effects on aquatic communities. Environmental Science and Technology 47:7996–8004.

Schafer, W. R. 1999. How do antidepressants work? Prospects for genetic analysis of drug mechanisms. Cell 98:551–554.

Schultz, M. M., and E. T. Furlong. 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. Analytical Chemistry 80:1756–1762.

Shearer, K. A., J. W. Hayes, and J. D. Stark. 2002. Temporal and spatial quantification of aquatic invertebrate drift in the Maruia River, South Island, New Zealand. New Zealand Journal of Marine and Freshwater Research 36:529–536.

Sinsabaugh, R. L., M. P. Osgood, and S. Findlay. 1994. Enzymatic models for estimating decomposition rates of particulate detritus. Journal of the North American Benthological Society 13:160–169.

Snyder, S. A. 2008. Occurrence, treatment, and toxicological relevance of EDCs and pharmaceuticals in water. Ozone: Science and Engineering 30:65–69.

Stevenson, R. J. 2014. Ecological assessments with algae: a review and synthesis. Journal of Phycology 50:437–461.

Styrishave, B., B. Halling-Sørensen, and F. Ingerslev. 2011. Environmental risk assessment of three selective serotonin reuptake inhibitors in the aquatic environment: a case study including a cocktail scenario. Environmental Toxicology and Chemistry 30:254–261.

Sweeney, B. W., and R. L. Vannote. 1978. Size variation and the distribution of hemimetabolous aquatic insects: two thermal equilibrium hypotheses. Science 200:444–446.

Underwood, A. J., and M. G. Chapman. 2003. Power, precaution, type II error and sampling design in assessment of environmental impacts. Journal of Experimental Marine Biology and Ecology 296:49–70.

Vasskog, T., T. Anderssen, S. Pedersen-Bjergaard, P. Kallenborn, and E. Jensen. 2008. Occurrence of selective serotonin reuptake inhibitors in sewage and receiving waters at Spitsbergen and in Norway. Journal of Chromatography A 1185:194–205.

Wallace, J. B., G. J. Lugthart, T. Cuffney, and G. Schurr. 1989. The impact of repeated insecticidal treatments on drift and benthos of a headwater stream. Hydrobiologia 179:135–147.

Wallace, J. B., and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41:115–139.

Walters, D. M., M. A. Mills, K. M. Fritz, and D. F. Raikow. 2009. Spider-mediated flux of PCBs from contaminated sediments to terrestrial ecosystems and potential risks to arachnivorous birds. Environmental Science and Technology 44:2849–2856.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analyses. Springer Verlag, New York.

Wiggins, G. B. 1996. Larvae of the North American caddisfly genera (Trichoptera). University of Toronto Press, Toronto, Ontario.