



## Corbicula fluminea rapidly accumulate pharmaceuticals from an effluent dependent urban stream



S. Rebekah Burket<sup>a</sup>, Mendie White<sup>b</sup>, Alejandro J. Ramirez<sup>a, c</sup>, Jacob K. Stanley<sup>a</sup>, Kenneth E. Banks<sup>d</sup>, W. Thomas Waller<sup>b</sup>, C. Kevin Chambliss<sup>a, c</sup>, Bryan W. Brooks<sup>a, e, \*</sup>

<sup>a</sup> Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX, USA

<sup>b</sup> Institute of Applied Science, University of North Texas, 704 W Mulberry St, Denton, TX, USA

<sup>c</sup> Department of Chemistry and Biochemistry, Baylor University, Waco, TX, USA

<sup>d</sup> City of Denton, 215 E. McKinney St., Denton, TX, USA

<sup>e</sup> School of Environment, Jinan University, Guangzhou, China

### HIGHLIGHTS

- Pharmaceutical bioaccumulation by bivalves is poorly understood in urban ecosystems.
- Rapid uptake observed in clams caged incrementally downstream from effluent discharge.
- Spatiotemporal uptake by clams and bioaccumulation factors differed among chemicals.
- Exposure route influences on uptake and elimination kinetics require further study.

### ARTICLE INFO

#### Article history:

Received 11 November 2018

Received in revised form

28 February 2019

Accepted 3 March 2019

Available online 4 March 2019

Handling Editor: Klaus Kümmerer

#### Keywords:

Urbanization

Wastewater

Bioaccumulation

Bivalve

Mollusca

### ABSTRACT

Freshwater bivalve populations are stressed by watershed development at the global scale. Though pharmaceuticals released from wastewater treatment plant effluent discharges are increasingly reported to bioaccumulate in fish, an understanding of bioaccumulation in bivalves is less defined. In the present study, we examined accumulation of 12 target pharmaceuticals in *C. fluminea* during a 42 day *in situ* study in Pecan Creek, an effluent dependent wadeable stream in north central Texas, USA. Caged clams were placed at increasing distances (5 m, 643 m, 1762 m) downstream from a municipal effluent discharge and then subsampled on study days 7, 14, 28 and 42. Acetaminophen, caffeine, carbamazepine, diltiazem, diphenhydramine, fluoxetine, norfluoxetine, sertraline, desmethylsertraline, and methylphenidate were identified in *C. fluminea* whole body tissue homogenates via isotope dilution liquid chromatography-tandem mass spectrometry. Tissue concentrations ranged from low  $\mu\text{g}/\text{kg}$  (methylphenidate) to 341  $\mu\text{g}/\text{kg}$  (sertraline). By study day 7, rapid and apparent pseudo-steady state accumulation of study compounds was observed in clams; this observation continued throughout the 42 d study. Notably, elevated bioaccumulation factors (L/kg) for sertraline were observed between 3361 and 6845, which highlights the importance of developing predictive bioaccumulation models for ionizable contaminants with bivalves. Future research is also necessary to understand different routes of exposure and elimination kinetics for pharmaceutical accumulation in bivalves.

© 2019 Elsevier Ltd. All rights reserved.

### 1. Introduction

By 2050, the World Health Organization (WHO) anticipates greater than 70 percent of the world population will live in urban

environments (UN, 2014, 2017). Increasing urbanization results in more densely populated cities with concentrated use and disposal of chemicals, including pharmaceuticals and other contaminants of emerging concern (CEC), for many of which ecological effects are not well understood (Brooks, 2018). Additional side effects from urbanization, such as increased contaminant releases to aquatic environments, are known to alter population trajectories in receiving waters and contribute to habitat loss (Ricciardi and

\* Corresponding author. Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX, USA.

E-mail address: [bryan\\_brooks@baylor.edu](mailto:bryan_brooks@baylor.edu) (B.W. Brooks).

Rasmussen, 1999). For example, municipal wastewater treatment plants (WWTPs) discharge effluent of diverse quality directly to surface waters, and as a result, total stream flow in arid urban regions is often dominated by or even dependent on wastewater effluent (Brooks et al., 2003). Arid and semi-arid regions often represent worst-case exposure scenarios for exposure to pharmaceuticals and other CECs in developed countries because effluent discharge receives minimal dilution (Brooks et al., 2006), which increases effective exposure duration to aquatic life (Ankley et al., 2007). This has contributed to a rapidly developing literature for environmental assessment, monitoring, and reporting of CECs in water and aquatic life (Daughton and Ternes, 1999; Kolpin et al., 2002; Du et al., 2012, 2014; McEneff et al., 2014; Burket et al., 2018). Understanding factors influencing environmental disposition, including bioaccumulation, has been identified as a critical research need for pharmaceuticals in the environment (Boxall et al., 2012; Rudd et al., 2014).

Though reports of bioaccumulation of ionizable CECs have increased over the last decade, most reported tissue residues in wildlife are derived from fish (Ramirez et al., 2009; Daughton and Brooks, 2011; Petrie et al., 2015). Field studies focused on bioaccumulation of pharmaceuticals by freshwater bivalves are lacking (Bringolf et al., 2010; Du et al., 2014; Hazelton et al., 2014; de Solla et al., 2016). Bivalves, including oysters, mussels and clams, are priorities for both conservation and aquaculture, and provide ecosystem services in freshwater streams. Additionally, a number of freshwater mussels are protected species in multiple regions, while representing important aquaculture products. Clams and mussels filter large quantities of water, with some species clearing up to 267 mL/h per individual and ~95% of the stream water column (Way et al., 1990; Gerritsen et al., 1994). However, global populations of native freshwater mussels are rapidly declining (Bogan, 1993). In North America, nearly 70% of freshwater bivalves are threatened, endangered, or extinct (Bogan, 1993; Williams et al., 1993; Ricciardi and Rasmussen, 1999; Lopes-Lima et al., 2018). Aquatic contaminants, among other stressors, have contributed to declining populations in urban ecosystems, but factors influencing bioaccumulation of pharmaceuticals by bivalves are not well understood (de Solla et al., 2016).

In one recent study, bivalves accumulated some of the highest levels of antidepressants and antibiotics, when compared to fish in the same system (Du et al., 2014). In this effluent dependent river, fish accumulated up to  $14 \pm 4.6$   $\mu\text{g}/\text{kg}$  of the selective serotonin reuptake inhibitor (SSRI) antidepressant sertraline, whereas tissue concentrations in unionid mussels were an order of magnitude higher ( $140 \pm 21$   $\mu\text{g}/\text{kg}$ ) (Du et al., 2014). Similar differences in accumulation were observed in field studies by Bringolf et al. (2010), in which fluoxetine concentrations in freshwater mussels, *Elliptio complanata*, which were caged downstream of a municipal WWTP effluent discharge, were higher than similarly exposed fish (9.8–79.1 ng/g ww compared to 0.1–1.6 ng/g ww). More recently, over 40 different pharmaceuticals and personal care products were detected in both caged and native *Lasmigona costata* in a river receiving wastewater effluent, where seasonal lower flow periods influenced accumulation (de Solla et al., 2016). However, spatial and temporal influences on bioaccumulation by freshwater bivalves remains poorly understood.

Previous reports from Pecan Creek, an effluent-dependent wadeable stream in north central Texas, USA, have indicated continuous release of antidepressants from the WWTP (Schultz and Furlong, 2008) and accumulation of antidepressants and other pharmaceuticals in several fish species (Brooks et al., 2005; Ramirez et al., 2007). The primary objective of the present study was to explore potential influences of distance and time on pharmaceutical bioaccumulation by *Corbicula fluminea* during a 42 day

*in situ* study in Pecan Creek. We selected *C. fluminea*, an invasive freshwater bivalve, for this study because this species is ubiquitous, easy to collect and transport, and has been successfully utilized for biomonitoring of legacy contaminants (McMahon, 1999; Ilarri and Sousa, 2011). We specifically hypothesized that accumulation of pharmaceuticals in *C. fluminea* would increase over time, but decrease with distance downstream from an effluent discharge. The present study provides an initial report of differential accumulation of pharmaceuticals by *C. fluminea* from a municipal effluent dependent stream.

## 2. Material and methods

### 2.1. Literature review

Building from a previous review of pharmaceutical bioaccumulation in aquatic life by Daughton and Brooks (2011), a literature search was performed of refereed publications using Scopus and Web of Science to characterize existing information on pharmaceutical bioaccumulation in bivalves from the field. Search terms included combinations of 'pharmaceutical' with different key words for bivalves including: bivalve, clam, mollusk, mussel, oyster, bivalvia, Mollusca, heterodonta, palaeoheterodonta, protobranchia, and pteriomorpha.

### 2.2. Study location and sample collection

We selected Pecan Creek (Denton County, Texas, USA) for a field study during in late summer 2005. *C. fluminea* were collected from Clear Creek, which is also located in Denton County, does not receive municipal effluent discharges and has previously been employed by our research team as a regional reference stream (Brooks et al., 2005; Ramirez et al., 2007). Pecan Creek was selected as the primary study site because instream flow is often dominated by or dependent on treated wastewater effluent from the Pecan Creek Water Reclamation Plant (PCWRP, Brooks et al., 2005). In fact, the region experienced abnormal to extreme drought during the 2005 study period (<http://droughtmonitor.unl.edu/>) and stream flows were composed entirely of treated effluent discharge, with no water flowing upstream from the effluent discharge, no other confluences and no other point sources of pharmaceuticals. A detailed description of PCWRP is provided elsewhere (Brooks et al., 2005).

*C. fluminea* specimens were hand collected from Clear Creek on 10 August 2005. Clam length ( $16.1 \pm 2.8$  mm) and weight ( $2.5 \pm 1.2$  g) were measured on site. Clams were labeled and transferred to coolers filled with Clear Creek water and equipped with portable aerators. Clams were transported to the study site, where they were placed in PVC cages and immediately deployed in Pecan Creek at three incremental locations downstream from the PCWRP discharge: 5, 643, and 1762 m. 12 cages were stationed at each location downstream. Each cage contained 7 clams. Clams were subsampled from each location on four different occasions: 18 August, 25 August, 8 September, and 22 September. On individual sampling dates, three entire cages were pulled from each sample site ( $n = 3$ ). Sampled clams were frozen and transported on ice to Baylor University. Samples were stored at  $-80$  °C prior to analysis.

Though surface water samples could not be analyzed for every target analyte, our collaborators previously reported water concentrations of several antidepressants, including three compounds examined (fluoxetine, norfluoxetine, and sertraline) (Schultz and Furlong, 2008). Water samples were collected at the same sampling locations and sampling times as those reported in the present study. Therefore, field derived bioaccumulation factors (BAFs) were calculated for fluoxetine, norfluoxetine, and sertraline and were

calculated for each site downstream as the mean tissue concentration of these analytes divided by reported water concentration (Arnot and Gobas, 2006).

### 2.3. Tissue analysis

Target analytes were selected based on previously published characteristics including human use and previous detections in fish and invertebrate tissues (Kolpin et al., 2002; Brooks et al., 2005; Ramirez et al., 2007; Du et al., 2012). Acetaminophen, acetaminophen-d4, caffeine, carbamazepine, carbamazepine-d10, diclofenac, diltiazem, diphenhydramine, diphenhydramine-d3, fluoxetine, fluoxetine-d6, methylphenidate, methylphenidate-d9, norfluoxetine, norfluoxetine-d6, and sertraline were purchased as certified analytical standards from Cerilliant (Round Rock, TX, USA). Caffeine-d9, desmethylsertraline, desmethylsertraline-d4, diclofenac-d4, diltiazem-d3, and sertraline-d3 were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). All chemicals were reagent grade and used as received. HPLC grade methanol (MeOH) was obtained from Fisher Scientific (Fair Lawn, NJ, USA), formic acid was purchased from VWR Scientific (Radnor, PA, USA), and a Thermo Barnstead Nanopure (Dubuque, IA, USA) Diamond UV water purification system was used throughout sample analysis to provide 18 M $\Omega$  water.

Tissue samples were processed following previously reported methods (Du et al., 2012, 2014; Burket et al., 2018). Thawed clam soft tissue was separated from the shell and homogenized with a handheld blender. Individual specimens from a single cage were pooled to achieve 500 mg to 1 g tissue wet weight for pharmaceutical extraction (n = 3). Homogenized tissue was placed in a 20 mL borosilicate glass vial (Wheaton; VWR Scientific, Rockwood, TN, USA). Next, 50  $\mu$ L of 2000  $\mu$ g/L internal standard solution was spiked in each sample, followed by 4 mL of MeOH and 4 mL of 0.1 M acetic acid (pH 4.0). Vials were mixed on a rotating table at 150 rpm for 25 min at room temperature prior to centrifugation at 7500 rpm for 45 min at 4 °C. Supernatant was collected and blown to dryness under a gentle stream of nitrogen in a Turbovap set to 40 °C. Samples were then reconstituted to 1 mL with 5:95 MeOH:0.1% formic acid. Reconstituted samples were syringe filtered (Pall Acrodisc hydrophobic Teflon Supor membrane syringe filters, 13-mm diameter, 0.2- $\mu$ m pore size) and placed in 2 mL analytical vials (Agilent Technologies, Santa Clara, CA, USA) for analysis. Samples were analyzed using isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS). Instrumentation parameters, separation strategy, detection limits of target analytes, and calibration methods have been previously reported (Bean et al., 2018). We did not examine lipid levels in bivalves because, as previously demonstrated by our laboratory, lipid normalization of tissue levels for these ionizable pharmaceuticals is not appropriate (Ramirez et al., 2009; Haddad et al., 2018).

### 2.4. Statistical analysis

Detected analyte concentrations below method detection limits (MDLs) were substituted with  $\frac{1}{2}$  MDL for statistical analysis (Antweiler and Taylor, 2008). Normality and homoscedasticity were evaluated prior to further analysis, and analytes, except for acetaminophen and diphenhydramine, were normally distributed. We therefore employed a two factor generalized linear model to examine whether tissue concentrations of target analytes significantly differed ( $\alpha = 0.05$ ) with distance and time (SPSS, IBM Software).

## 3. Results

Literature searches using Scopus and Web of Science on the occurrence of pharmaceuticals in bivalves prior to August 2018 returned 277 relevant publications from 593 total manuscripts. Manuscripts were identified as potentially relevant if they described toxicity or accumulation of pharmaceuticals in mollusks. Of the relevant articles, 183 focused on marine species, 6 focused on estuarine species, and 94 focused on freshwater species. Of the freshwater manuscripts, 14 described field experiments. Additional evaluation of these peer-reviewed publications refined this list to a total of only 4 unique manuscripts reporting pharmaceutical accumulation in freshwater bivalves from field studies (Table 1). The remaining freshwater studies were either laboratory experiments or toxicity studies, where biomarker responses were measured but tissue concentrations were not reported. Whereas only 4 studies have been performed in inland waters, 20 manuscripts have examined bivalve accumulation in coastal and marine systems (Table SI). Such observations support relatively limited reports of bioaccumulation of pharmaceuticals by marine fish (Gaw et al., 2014). Though three previous manuscripts included a spatial component no studies have examined differential bioaccumulation of pharmaceuticals by bivalves through time with increasing distance from a WWTP discharge (Table S1).

In the present study, we observed ten of 12 target analytes in *C. fluminea* tissue samples from Pecan Creek at low  $\mu$ g/kg levels (mean  $\pm$  std. dev., range): acetaminophen ( $30.8 \pm 34.2$   $\mu$ g/kg, 6.3–347  $\mu$ g/kg); caffeine ( $2.8 \pm 2.2$   $\mu$ g/kg, <0.5–7.8  $\mu$ g/kg); carbamazepine ( $2.5 \pm 0.5$   $\mu$ g/kg, 1.3–5.1  $\mu$ g/kg); diltiazem ( $1.3 \pm 0.4$   $\mu$ g/kg, 0.5–2.8  $\mu$ g/kg); diphenhydramine ( $7.8 \pm 3.5$   $\mu$ g/kg, 2.9–22.1  $\mu$ g/kg); and methylphenidate ( $0.08 \pm 0.03$   $\mu$ g/kg, <0.06–0.18  $\mu$ g/kg) (Fig. 1). Only the nonsteroidal anti-inflammatory drug diclofenac and the artificial sweetener sucralose, which is considered an ideal effluent tracer (Soh et al., 2011), were not detected in clam tissue. Sertraline and its primary active metabolite, desmethylsertraline, were consistently detected at elevated levels in *C. fluminea* tissue when compared to other detected analytes, ranging from 97 to 341  $\mu$ g/kg and 45–160  $\mu$ g/kg, respectively (Fig. 2). These observations were an order of magnitude higher than another SSRI, fluoxetine ( $6.7 \pm 1.6$   $\mu$ g/kg, 4.1–12  $\mu$ g/kg), and its primary active metabolite norfluoxetine ( $0.8 \pm 0.3$   $\mu$ g/kg, <0.7–2.7  $\mu$ g/kg) (Fig. 2).

Influences of distance and time on pharmaceutical accumulation yielded inconsistent results (Fig. 1). For example, tissue concentrations generally decreased over time for acetaminophen and caffeine across all sampling sites, with highest tissue concentrations observed on the first sampling event (day 7; Fig. 1;  $p < 0.05$ ). Tissue levels of carbamazepine, methylphenidate, and norfluoxetine remained relatively consistent across all sampling locations and did not change over time ( $p > 0.05$ ). However, significant site specific differences were observed for diphenhydramine, fluoxetine, sertraline and desmethylsertraline, most often with highest observed tissue concentrations 1762 m downstream from the effluent discharge by study day 42 (Figs. 1 and 2;  $p < 0.05$ ). To our knowledge, here we report accumulation of acetaminophen, caffeine, and methylphenidate in freshwater bivalves for the first time.

Field derived BAFs from this *in situ* study are presented in Table 2. BAFs were specifically calculated for fluoxetine, norfluoxetine and sertraline. BAFs were highest for sertraline (3361–6845), followed by norfluoxetine (780–1090) and then fluoxetine (313–702). For all of these compounds, BAFs were highest at the farthest downstream location.

**Table 1**  
 Refereed journal articles reporting accumulation of pharmaceuticals and personal care products in freshwater bivalves from field studies. Searches with SCOPUS and Web of Science were performed through August 2018, and included combinations of "pharmaceutical" and each of the following terms: bivalve, clam, mollusk, mussel, oyster, bivalvia, Mollusca, heterodonta, palaeoheterdonta, protobranchia, and pteriomorpha. WWTP: wastewater treatment plant. ND: not detected. <: denotes lower than method detection limit.

Authors	Year	Location	Organism	Compound	Mean tissue concentration ( $\mu\text{g}/\text{kg}$ )	Exposure duration (days)	Distance from WWTP (m)	Caged/ Wild			
Bringolf et al.	2010	North America	<i>Elliptio complanata</i>	Fluoxetine	79.1	14	0	caged			
					18.0	14	50	caged			
					9.80	14	100	caged			
					0.30	14	–100	caged			
Du et al.	2014	North America	<i>Corbicula fluminea</i>	Carbamazepine	1.20			wild			
				Celecoxib	26.0			wild			
				Desmethylsertraline	86.0			wild			
				Diclofenac	19.0			wild			
				Diltiazem	<0.08			wild			
				Diphenhydramine	2.80			wild			
				Fluoxetine	10.0			wild			
				Norfluoxetine	<3.0			wild			
				Sertraline	140			wild			
				<i>Unio meris tetralasmus</i>	Carbamazepine	1.40			wild		
					Celecoxib	<8.5			wild		
					Desmethylsertraline	88.0			wild		
			Diclofenac		11.0			wild			
			Diltiazem		ND			wild			
			Diphenhydramine		1.20			wild			
			Fluoxetine		15.0			wild			
			Norfluoxetine		<3.0			wild			
			Sertraline		130			wild			
			<i>Utterbackia imbecillis</i>		Carbamazepine	1.10			wild		
					Celecoxib	<8.5			wild		
					Desmethylsertraline	78.0			wild		
					Diclofenac	15.0			wild		
					Diltiazem	0.27			wild		
					Diphenhydramine	4.40			wild		
					Fluoxetine	22.0			wild		
				Norfluoxetine	<3.0			wild			
			Xie et al.	2015	Asia	<i>Corbiculidae/ Anodonta</i>	Propranolol	10.7			wild
de Solla et al.	2016	North America	<i>Lasmigona costata</i>	1,7-dimethylxanthine	641	28	–300	caged			
				1,7-dimethylxanthine	<91.2	28	500	caged			
				10-HO-amitriptyline	0.20	28	–300	caged			
				10-HO-amitriptyline	0.40	28	500	caged			
				Amitriptyline	9.50	28	–300	caged			
				Amitriptyline	30.6	28	500	caged			
				Amlodipine	<0.70	28	–300	caged			
				Amlodipine	2.00	28	500	caged			
				Amphetamine	2.30	28	–300	caged			
				Amphetamine	2.80	28	500	caged			
				Anhydrotetracycline	<1.49	28	–300	caged			
				Anhydrotetracycline	<1.49	28	500	caged			
				Azithromycin	<1.23	28	–300	caged			
				Azithromycin	2.30	28	500	caged			
				Betamethasone	0.30	28	–300	caged			
				Cefotaxime	4.20	28	–300	caged			
				Cefotaxime	<16.4	28	500	caged			
				Citalopram	8.20	28	–300	caged			
				Citalopram	15.3	28	500	caged			
				Clarithromycin	0.80	28	–300	caged			
				Clarithromycin	0.60	28	500	caged			
				Clotrimazole	<0.91	28	–300	caged			
				Clotrimazole	2.10	28	500	caged			
				Cocaine	0.40	28	–300	caged			
				Cocaine	0.90	28	500	caged			
Codeine	1.60	28	–300	caged							
Codeine	6.10	28	500	caged							
DEET	1.30	28	–300	caged							
DEET	0.90	28	500	caged							
Desmethyldiltiazem	0.04	28	–300	caged							
Desmethyldiltiazem	0.30	28	500	caged							
Diltiazem	0.10	28	–300	caged							
Diltiazem	0.80	28	500	caged							
Diphenhydramine	12.3	28	–300	caged							
Diphenhydramine	32.1	28	500	caged							

Table 1 (continued)

Authors	Year	Location	Organism	Compound	Mean tissue concentration ( $\mu\text{g}/\text{kg}$ )	Exposure duration (days)	Distance from WWTP (m)	Caged/Wild
				Enrofloxacin	0.70	28	–300	caged
				Enrofloxacin	<1.66	28	500	caged
				Erythromycin	<1.05	28	–300	caged
				Erythromycin	<1.05	28	500	caged
				Fluoxetine	1.10	28	–300	caged
				Gemfibrozil	<0.78	28	–300	caged
				Gemfibrozil	0.10	28	500	caged
				Glyburide	<0.98	28	–300	caged
				Glyburide	0.40	28	500	caged
				Hydrocortisone	<27.4	28	–300	caged
				Hydrocortisone	17.0	28	500	caged
				Iopamidol	86.1	28	–300	caged
				Iopamidol	79.4	28	500	caged
				Metformin	<2.8	28	–300	caged
				Metformin	1.90	28	500	caged
				Miconazole	<1.25	28	–300	caged
				Miconazole	1.00	28	500	caged
				Minocycline	<27.4	28	–300	caged
				Minocycline	<27.4	28	500	caged
				Naproxen	<1.71	28	–300	caged
				Naproxen	0.70	28	500	caged
				Norfluoxetine	0.60	28	–300	caged
				Norfluoxetine	1.10	28	500	caged
				Norverapamil	<0.08	28	–300	caged
				Norverapamil	<0.08	28	500	caged
				Oxolinic Acid	0.30	28	–300	caged
				Oxolinic Acid	<1.11	28	500	caged
				Oxycodone	<1.79	28	–300	caged
				Oxycodone	0.10	28	500	caged
				Paroxetine	<1.83	28	–300	caged
				Paroxetine	4.10	28	500	caged
				Propranolol	3.10	28	–300	caged
				Propranolol	5.40	28	500	caged
				Sarafloxacin	1.90	28	–300	caged
				Sarafloxacin	<8.3	28	500	caged
				Sertraline	8.20	28	–300	caged
				Sertraline	20.4	28	500	caged
				Theophylline	111	28	–300	caged
				Theophylline	<27.4	28	500	caged
				Triclocarban	5.70	28	–300	caged
				Triclocarban	27.4	28	500	caged
				Triclosan	48.4	28	–300	caged
				Triclosan	23.0	28	500	caged
				Venlafaxine	<1.83	28	–300	caged
				Venlafaxine	<1.83	28	500	caged
				Verapamil	<0.07	28	–300	caged
				Verapamil	0.03	28	500	caged
				Warfarin	<0.69	28	–300	caged
				Warfarin	<0.69	28	500	caged
de Solla et al.	2016	North America	<i>Lasmigona costata</i>	1,7-dimethylxanthine	<91.2		–500	wild
				1,7-dimethylxanthine	<91.2		7500	wild
				10-HO-amitriptyline	0.80		–500	wild
				10-HO-amitriptyline	1.20		7500	wild
				Amitriptyline	9.40		–500	wild
				Amitriptyline	30.1		7500	wild
				Amlodipine	<0.70		–500	wild
				Amlodipine	<0.70		7500	wild
				Amphetamine	2.30		–500	wild
				Amphetamine	4.70		7500	wild
				Anhydrotetracycline	5.40		–500	wild
				Anhydrotetracycline	15.9		7500	wild
				Azithromycin	1.80		–500	wild
				Azithromycin	10.0		7500	wild
				Betamethasone	<0.68		–500	wild
				Betamethasone	<0.68		7500	wild
				Cefotaxime	<16.4		–500	wild
				Cefotaxime	<16.4		7500	wild
				Citalopram	10.2		–500	wild
				Citalopram	33.3		7500	wild
				Clarithromycin	0.90		–500	wild
				Clarithromycin	7.80		7500	wild

(continued on next page)

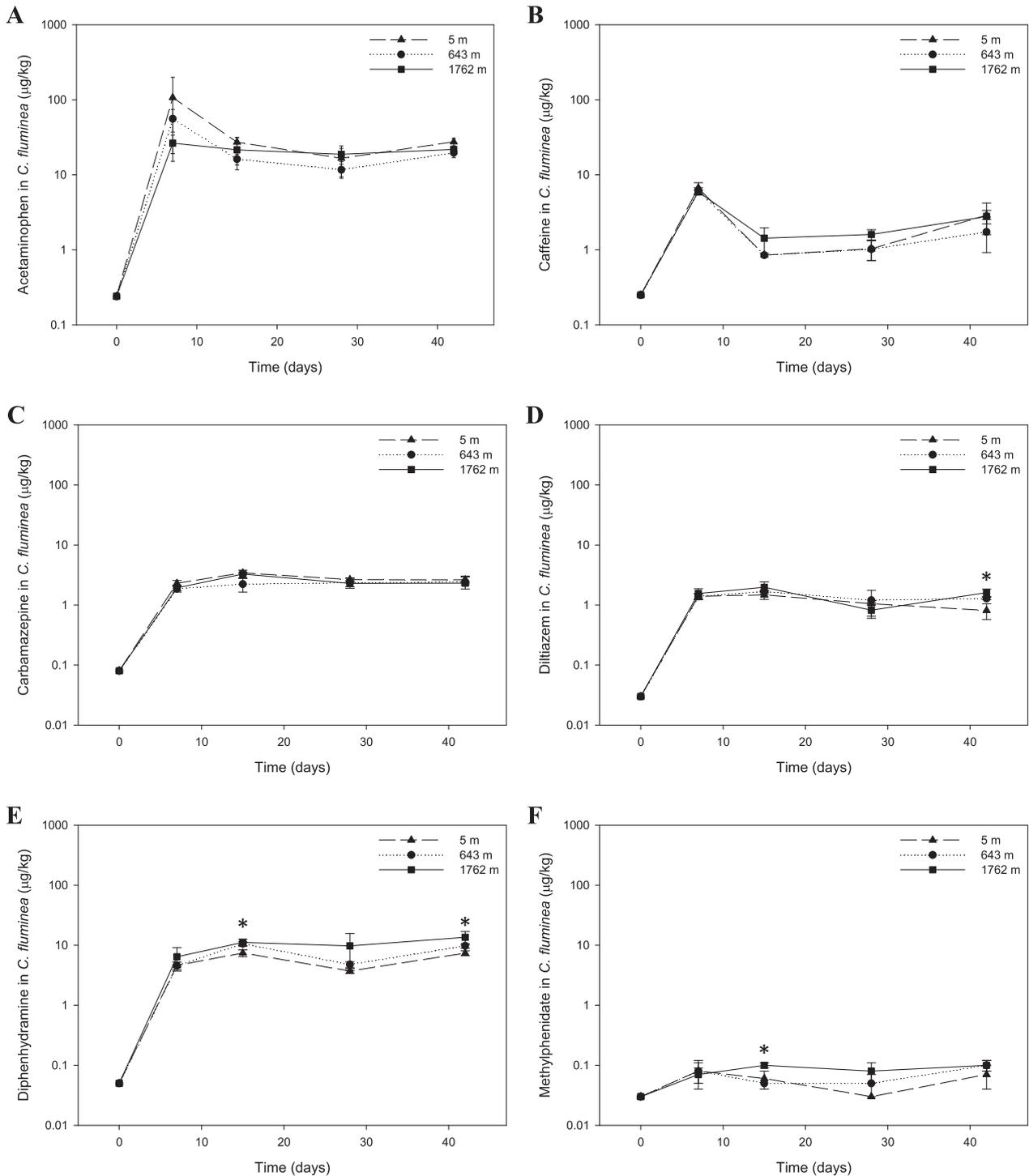
Table 1 (continued)

Authors	Year	Location	Organism	Compound	Mean tissue concentration ( $\mu\text{g}/\text{kg}$ )	Exposure duration (days)	Distance from WWTP (m)	Caged/Wild
				Clotrimazole	<0.91		–500	wild
				Clotrimazole	0.60		7500	wild
				Cocaine	0.10		–500	wild
				Cocaine	0.50		7500	wild
				Codeine	<4.10		–500	wild
				Codeine	8.20		7500	wild
				DEET	0.50		–500	wild
				DEET	0.70		7500	wild
				Desmethyldiltiazem	<0.07		–500	wild
				Desmethyldiltiazem	0.20		7500	wild
				Diltiazem	0.40		–500	wild
				Diltiazem	1.80		7500	wild
				Diphenhydramine	10.4		–500	wild
				Diphenhydramine	52.2		7500	wild
				Enrofloxacin	0.40		–500	wild
				Enrofloxacin	<1.66		7500	wild
				Erythromycin	0.30		–500	wild
				Erythromycin	0.80		7500	wild
				Fluoxetine	1.30		–500	wild
				Fluoxetine	7.20		7500	wild
				Gemfibrozil	<0.78		–500	wild
				Gemfibrozil	<0.78		7500	wild
				Glyburide	<0.98		–500	wild
				Glyburide	<0.98		7500	wild
				Hydrocortisone	<27.4		–500	wild
				Hydrocortisone	<27.4		7500	wild
				lopamidol	74.3		–500	wild
				lopamidol	110		7500	wild
				Metformin	<2.80		–500	wild
				Metformin	<2.80		7500	wild
				Miconazole	0.10		–500	wild
				Miconazole	0.20		7500	wild
				Minocycline	<27.4		–500	wild
				Minocycline	11.4		7500	wild
				Naproxen	<1.71		–500	wild
				Naproxen	<1.71		7500	wild
				Norfluoxetine	0.10		–500	wild
				Norfluoxetine	1.50		7500	wild
				Norverapamil	<0.08		–500	wild
				Norverapamil	0.09		7500	wild
				Oxolinic Acid	<1.11		–500	wild
				Oxolinic Acid	<1.11		7500	wild
				Oxycodone	0.10		–500	wild
				Oxycodone	5.60		7500	wild
				Paroxetine	1.30		–500	wild
				Paroxetine	2.40		7500	wild
				Propranolol	3.40		–500	wild
				Propranolol	8.30		7500	wild
				Sarafloxacin	<8.30		–500	wild
				Sarafloxacin	<8.30		7500	wild
				Sertraline	34.2		–500	wild
				Sertraline	58.7		7500	wild
				Theophylline	<27.4		–500	wild
				Theophylline	<27.4		7500	wild
				Triclocarban	5.30		–500	wild
				Triclocarban	4.40		7500	wild
				Triclosan	96.3		–500	wild
				Triclosan	12.5		7500	wild
				Venlafaxine	5.10		–500	wild
				Venlafaxine	20.1		7500	wild
				Verapamil	<0.07		–500	wild
				Verapamil	0.30		7500	wild
				Warfarin	0.20		–500	wild
				Warfarin	<0.69		7500	wild

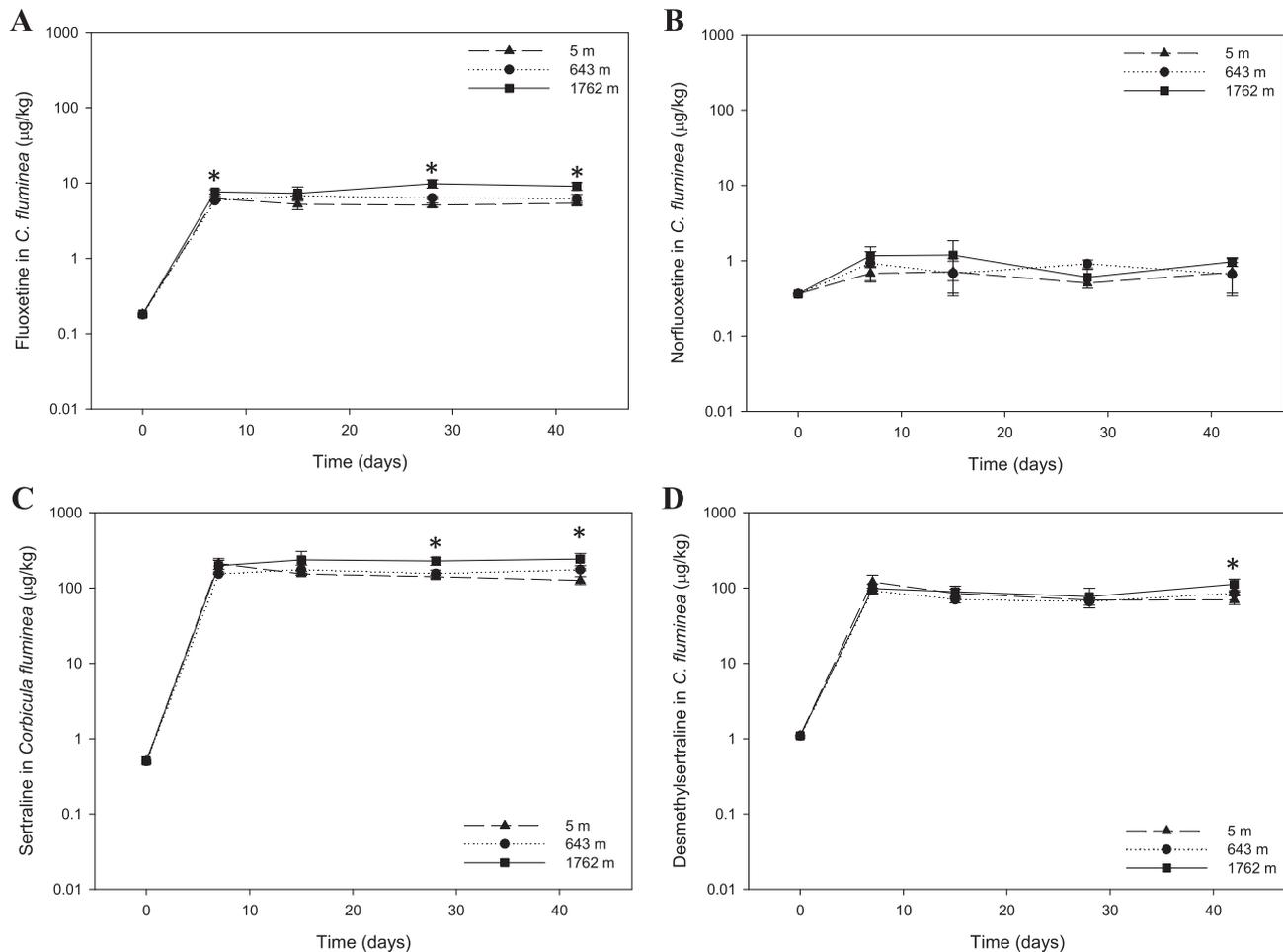
#### 4. Discussion

Pharmaceutical bioaccumulation has been increasingly reported in aquatic life (Boxall et al., 2012; Liu and Wong, 2013; Petrie et al., 2015; Wang et al., 2015; Fabbri and Franzellitti, 2016; Gaw and Brooks, 2016; Lagesson et al., 2016; Kristofco and Brooks, 2017; Saari et al., 2017; Bean et al., 2018). In fact, Boxall et al. (2012)

identified several priority research questions necessary to define risks of pharmaceuticals in the environment, including the importance of understanding uptake of pharmaceuticals by non-target species. Many field studies have focused on bioaccumulation in fish (Brooks et al., 2005; Ramirez et al., 2009; Du et al., 2012; Garcia et al., 2012; Haddad et al., 2018), while others have focused on trophic transfer from invertebrates to fish (Du



**Fig. 1.** Concentrations of target analytes detected in *Corbicula fluminea* tissue, including an analgesic (acetaminophen (A)), stimulants (caffeine (B) and methylphenidate (F)), mood stabilizer (carbamazepine (C)), antihypertensive (diltiazem (D)), and antihistamine (diphenhydramine (E)), from Pecan Creek, an effluent dependent Wadeable Stream in north central Texas, USA. Clams were caged at three increasing distances from a municipal wastewater treatment plant effluent discharge (5 m, 643 m, 1762 m). Asterisks indicate statistically significant differences ( $p < 0.05$ ,  $n = 3$ ,  $\pm\text{SD}$ ) among sites.



**Fig. 2.** Concentrations of fluoxetine and sertraline, two selective serotonin reuptake inhibitors, detected in *Corbicula fluminea* tissue from Pecan Creek, an effluent dependent wadeable stream in north central Texas, USA. The primary metabolites norfluoxetine and desmethylsertraline were also detected. Clams were caged at three increasing distances from a municipal wastewater treatment plant effluent discharge (5 m, 643 m, 1762 m). Asterisks indicate statistically significant differences ( $p < 0.05$ ,  $n = 3$ ,  $\pm\text{SD}$ ) among sites.

**Table 2**

Field derived bioaccumulation factors (BAF) for the selective serotonin reuptake inhibitors, fluoxetine (FLU) and sertraline (SER), and a primary metabolite, norfluoxetine (NOR), from soft tissues of *Corbicula fluminea* caged for 42 d at increasing distances downstream from a municipal wastewater treatment plant discharge to Pecan Creek, an effluent dependent wadeable stream in north central Texas, USA.

Distance (m)	Water <sup>a</sup> (ng/L)			Bivalve Tissue (ng/kg)			BAF ( $\text{L kg}^{-1}$ )		
	FLU	NOR	SER	FLU	NOR	SER	FLU	NOR	SER
5	12	0.83	36	5447	647	157,562	454	780	4377
643	20	1	49	6268	792	164,694	313	792	3361
1762	12	0.9	33	8423	981	225,887	702	1090	6845

<sup>a</sup> Schultz and Furlong (2008).

et al., 2014; Lazarus et al., 2015; Xie et al., 2015; Lagesson et al., 2016; Bean et al., 2018; Haddad et al., 2018). Recent efforts have also developed mechanistic uptake modeling approaches for ionizable pharmaceuticals in fish (Nichols et al., 2015). However, compared to these laboratory and field observations with fish, research on bioaccumulation in bivalves is lacking. For example, we identified 20 manuscripts that evaluated bioaccumulation in bivalves from marine and coastal settings (Table S1), but only 4 manuscripts describing bioaccumulation in freshwater bivalves (Table 1). In the present study, we aimed to initially understand uptake kinetics of pharmaceuticals in the common freshwater clam, *C. fluminea*, in an effluent-dependent urban stream.

Previous studies conducted by our research team in Pecan Creek indicated accumulation of antidepressants in three different fish species (*L. macrochirus*, *I. punctatus*, and *P. nigromaculatus*) (Brooks et al., 2005; Ramirez et al., 2007). The antidepressants fluoxetine and sertraline, along with their primary active metabolites, norfluoxetine and desmethylsertraline, were detected in fish brain, liver and muscle tissues from fish, providing the first report of human pharmaceuticals bioaccumulation in field collected wildlife (Brooks et al., 2005). In fish, the highest concentrations of SSRIs were detected in brain tissue (mean  $\pm$  standard deviation: fluoxetine,  $1.58 \pm 0.74 \mu\text{g/kg}$ ; norfluoxetine,  $8.86 \pm 5.9 \mu\text{g/kg}$ ; sertraline,  $4.27 \pm 1.4 \mu\text{g/kg}$ ; desmethylsertraline,  $15.6 \pm 1.4 \mu\text{g/kg}$ ; Brooks et al., 2005). In the present study, detected concentrations of sertraline in *C. fluminea* tissue were two orders of magnitude higher than those previously reported in fish collected from the same stream under similar effluent dependent conditions (mean  $\pm$  standard deviation: sertraline,  $182 \pm 24 \mu\text{g/kg}$  in clams compared to  $4.27 \pm 1.4 \mu\text{g/kg}$  in fish brain tissue).

Du et al. (2014) previously reported similar observations of elevated levels of SSRIs in bivalves compared to fish from the North Bosque River, another effluent dependent river in central Texas, USA. We also observed higher levels of the SSRI fluoxetine in marine mussels than predicted using traditional BCF models, which are derived from empirical partitioning relationships with fish (Franzellitti et al., 2014). Such observations of higher SSRI

accumulation in bivalves from the present study and in these previous efforts may have resulted from accumulation in lysosomes (Daniel and Wojcikowski, 1997), which are abundant in bivalves, and warrants future study. It is also important to note that mean tissue concentrations for the SSRI sertraline in bivalves of the present study were similar to those recently reported in *C. fluminea* collected from the North Bosque River (Du et al., 2014), another effluent-dominated river in central Texas, USA. In the North Bosque River, 9 pharmaceuticals, including sertraline, desmethylsertraline, fluoxetine and norfluoxetine, were detected in three bivalve species (*C. fluminea*, and the unionid mussels *Unio meris tetralasmus* and *Utterbackia imbecillis*). Similar to the current study, detected tissue concentrations were highest for sertraline and desmethylsertraline in the North Bosque where *C. fluminea* accumulated mean tissue levels of  $140 \pm 21$   $\mu\text{g}/\text{kg}$  of sertraline and  $86 \pm 20$   $\mu\text{g}/\text{kg}$  of desmethylsertraline (Du et al., 2014). These sertraline levels were very similar to observations in the present study (sertraline,  $182 \pm 24$   $\mu\text{g}/\text{kg}$ ; desmethylsertraline,  $85.9 \pm 12$   $\mu\text{g}/\text{kg}$ ). However, mean sertraline concentrations in the present study (mean  $\pm$  std. dev., range:  $182 \pm 24$   $\mu\text{g}/\text{kg}$ ,  $97$ – $341$   $\mu\text{g}/\text{kg}$ ) were an order of magnitude higher than those reported in tissues of freshwater mussels (*L. costata*) caged downstream from an effluent discharge in the Grand River in Ontario, Canada ( $20.4 \pm 5.1$   $\mu\text{g}/\text{kg}$ ,  $15.7$ – $26.3$   $\mu\text{g}/\text{kg}$ ) (de Solla et al., 2016). Such differential observations could have resulted from increased dilution in the Grand River (i.e. effluent contributing 4.8% to instream flow (de Solla et al., 2016)), consumer pharmaceutical use and/or WWTP treatment effectiveness. Bivalve spawning, lure display reproductive behaviors, feeding rates and burrowing behavior can change after exposure to and accumulation of antidepressants at aqueous concentrations as low as 20 ng/L (Fong and Molnar, 2008; Bringolf et al., 2010; Lazzara et al., 2012; Hazelton et al., 2014). Whether tissue residues observed in the present study result in ecologically important adverse behavioral or reproduction outcomes in bivalves is not known, and thus represents an important research need.

In Pecan Creek, we observed rapid accumulation for each detected analyte by day 7 (Figs. 1 and 2). Such rapid accumulation of ionizable pharmaceuticals has been demonstrated in both laboratory and field experiments (Chang et al., 2015; Nichols et al., 2015; Lagesson et al., 2016). For example, fathead minnows (*Pimephales promelas*) exposed to nicotine during a 24-h laboratory uptake study, reached apparent steady state internal concentrations within the first 12 h (Chang et al., 2015). In another recent field study, several invertebrate species (Zygoptera, *Asellus aquaticus* and Planorbidae) accumulated diphenhydramine and hydroxyzine over 7 days of exposure and subsequently exhibited decreasing tissue concentrations as measured pharmaceutical concentrations decreased in the water (Lagesson et al., 2016). In fish, accumulation of ionizable pharmaceuticals appears to primarily occur via inhalation (Du et al., 2014; Nichols et al., 2015; Haddad et al., 2018). During a laboratory inhalational study with the ionizable base diphenhydramine, fish tissue levels reached steady-state conditions within 96 h (Nichols et al., 2015).

Recent studies have identified pharmaceutical sorption to suspended particulate matter (Baker and Kasprzyk-Hordern, 2011) and reported higher concentrations of some pharmaceuticals in periphytic biofilms (Haddad et al., 2018). Therefore, diet may be an underrepresented exposure pathway for ionizable pharmaceuticals in filter feeding bivalves. Detected concentrations for carbamazepine, diphenhydramine, desmethylsertraline, fluoxetine and sertraline were similar to tissue concentrations of bivalves sampled from the North Bosque River in Central Texas. Tissue levels of diltiazem (mean  $\pm$  std. dev., range:  $1.3 \pm 0.4$   $\mu\text{g}/\text{kg}$ ,  $0.5$ – $2.8$   $\mu\text{g}/\text{kg}$ ) were similar to those recently reported in caged mussels downstream from an effluent discharge in Canada (de Solla et al., 2016).

In the present study, consistent with our previous work in the North Bosque river (Du et al., 2014) and a Grand River study (de Solla et al., 2016), antidepressants were detected at the highest levels in bivalve tissue when compared to other target analytes. Further, in each of these studies, sertraline was detected at higher tissue concentrations in bivalves when compared to fish collected under similar experimental conditions from the same lotic system (Brooks et al., 2005; Du et al., 2014; de Solla et al., 2016). Our results potentially indicate different accumulation patterns for antidepressants compared to other pharmaceuticals. However, whether increased dietary exposure to sorbed SSRIs contributed to these observations is not known, but deserves future attention.

During the present study, surface water samples collected simultaneously from the same sampling locations demonstrated consistent water concentrations of fluoxetine, norfluoxetine, and sertraline at each of the sampling sites in Pecan Creek (Schultz and Furlong, 2008). Such surface water and BAF observations across the stream reach examined here likely resulted from increased effective exposure duration (Ankley et al., 2007) because introduction rates of these down the drain chemicals exceed degradation rates in effluent-dominated and dependent receiving systems. In the present study, calculated BAFs for fluoxetine (Table 2) were similar to previously reported 14-day BAFs for freshwater mussels (*E. complanata*) caged at an effluent discharge site in North Carolina (Bringolf et al., 2010), but BAFs for sertraline were lower than values recently reported for another freshwater mussel species (*L. costata*) in Ontario (de Solla et al., 2016), which may have resulted from lower exposure through suspended particulates, species specific differences or other factors. Still, we observed BAFs for sertraline to reach 6845 at the most downstream study location (1762 m).

Previous efforts from our research team have investigated relationships between predicted and observed BAFs in *Planorbis* sp. (Du et al., 2015). Predicted BAFs based on the octanol-water partitioning coefficient ( $\log K_{ow}$ ), octanol-water distribution coefficient ( $\log D_{ow}$ ), and the liposome-water distribution coefficient ( $\log D_{lipw}$ ) differed from observed BAFs in the present study (Table 2). Specifically, these BAF values were over-predicted by  $\log K_{ow}$ , but under-predicted when using  $\log D_{ow}$  or  $\log D_{lipw}$  ( $\text{pH} = 8$ ). It is important to note that these common predictive BAF modeling approaches were initially derived from empirical studies with fish (Arnot and Gobas, 2006; Du et al., 2015). Thus, such differences highlight the need for future development of empirical models for predicting bioaccumulation of pharmaceuticals in aquatic invertebrates, including bivalves (Boxall et al., 2012; Meredith-Williams et al., 2012; Du et al., 2015). However, field BAFs calculated from water concentrations alone do not account for potential uptake via dietary routes of exposure because water samples were prefiltered prior to extraction (Schultz and Furlong, 2008) and thus surface water levels of SSRIs employed for field BAF calculations reflected dissolved concentrations of pharmaceuticals in Pecan Creek. Relative influences of waterborne and dietary exposure routes on accumulation of pharmaceuticals remains poorly understood, particularly among bivalve species.

## 5. Conclusions

In the present study, *C. fluminea* rapidly accumulated pharmaceuticals within the first 7 days of effluent exposure and appeared to reach pseudo-steady state conditions within this period. In urban streams in arid and semi-arid environments of developed countries, effluent dependent streams represent worst case exposure scenarios for down the drain chemicals. Often, as was the case in Pecan Creek, when introduction rates exceed pharmaceutical half-lives in a receiving system, organisms encounter increased effective exposure durations (Ankley et al., 2007). However, this

study was not designed to examine uptake kinetics with the first 7 days, depuration kinetics, or metabolism, including conjugated metabolites. Future studies are needed to understand how rapidly uptake and depuration of CECs with diverse physicochemical properties occurs in bivalves. Bioaccumulation differences among fish and bivalves have not been adequately studied and potentially result from different routes of exposure, disposition and metabolism. Recent reports have suggested that uptake of dissolved ionizable pharmaceuticals across fish gills represents a primary route of exposure (Nichols et al., 2015; Kristofco et al., 2018). However, differences among fish and bivalve tissue bioaccumulation suggest that dietary exposure to sorbed pharmaceuticals on particulate matter could be important for bivalves. Future studies should investigate uptake kinetics of pharmaceuticals, and the relative importance of inhalational and dietary routes of exposure on bivalve accumulation.

## Acknowledgements

This research was supported by Baylor University.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2019.03.014>.

## References

- Ankley, G.T., Brooks, B.W., Huggett, D.B., Sumpter, J.P., 2007. Repeating history: pharmaceuticals in the environment. *Environ. Sci. Technol.* 41, 8211–8217.
- Antweiler, R.C., Taylor, H.E., 2008. Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. summary statistics. *Environ. Sci. Technol.* 42, 3732–3738.
- Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ. Rev.* 14, 257–297.
- Baker, D.R., Kasprzyk-Hordern, B., 2011. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. *J. Chromatogr. A* 1218, 7901–7913.
- Bean, T.G., Rattner, B.A., Lazarus, R.S., Day, D.D., Burket, S.R., Brooks, B.W., Haddad, S.P., Bowerman, W.W., 2018. Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay. *Environ. Pollut.* 232, 533–545.
- Bogan, A.E., 1993. Freshwater bivalve extinctions (Mollusca: unionoida): a search for causes. *Am. Zool.* 33, 599–609.
- Boxall, A.B.A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Bezley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., DeLeo, P.C., Dyer, S.D., Ericson, J.F., Gagné, François, Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M.V., Larsson, D.G.J., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray-Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.R., Trudeau, V.L., Kraak, G.V.D., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? *Environ. Health Perspect.* 120, 1221–1229.
- Bringolf, R.B., Heltsley, R.M., Newton, T.J., Eads, C.B., Fraley, S.J., Shea, D., Cope, W.G., 2010. Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. *Environ. Toxicol. Chem.* 29, 1311–1318.
- Brooks, B.W., 2018. Urbanization, environment and pharmaceuticals: advancing comparative physiology, pharmacology and toxicology. *Conserv. Physiol.* 6, cox079.
- Brooks, B.W., Riley, T.M., Taylor, R.D., 2006. Water quality of effluent-dominated ecosystems: ecotoxicological, hydrological, and management considerations. *Hydrobiologia* 556, 365–379.
- Brooks, B.W., Chambliss, C.K., Stanley, J.K., Ramirez, A., Banks, K.E., Johnson, R.D., Lewis, R.J., 2005. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ. Toxicol. Chem.* 24, 464–469.
- Brooks, B.W., Foran, C.M., Peterson, B.N., Weston, J., La Point, T.W., Huggett, D.B., 2003. Linkages between population demographics and municipal effluent estrogenicity. *Bull. Environ. Contam. Toxicol.* 71, 504–511.
- Burket, S.R., Sapozhnikova, Y., Zheng, J.S., Chung, S.S., Brooks, B.W., 2018. At the intersection of urbanization, water, and food security: determination of select contaminants of emerging concern in mussels and oysters from Hong Kong. *J. Agric. Food Chem.* 66, 5009–5017.
- Chang, Y.W., Nguyen, H.P., Chang, M., Burket, S.R., Brooks, B.W., Schug, K.A., 2015. Determination of nicotine and its metabolites accumulated in fish tissue using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry. *J. Sep. Sci.* 38, 2414–2422.
- Daniel, W.A., Wojcikowski, J., 1997. Contribution of lysosomal trapping to the total tissue uptake of psychotropic drugs. *Pharmacol. Toxicol.* 62–68.
- Daughton, C.G., Brooks, B.W., 2011. Active pharmaceuticals ingredients and aquatic organisms. In: Meador, J., Beyer, N. (Eds.), *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Taylor and Francis, New York, pp. 281–341.
- Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.* 107, 907–938.
- de Solla, S.R., Gilroy, È.A.M., Klinck, J.S., King, L.E., McInnis, R., Struger, J., Backus, S.M., Gillis, P.L., 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel *Lasmigona costata* in a river receiving wastewater effluent. *Chemosphere* 146, 486–496.
- Du, B., Haddad, S.P., Scott, W.C., Chambliss, C.K., Brooks, B.W., 2015. Pharmaceutical bioaccumulation by periphyton and snails in an effluent-dependent stream during an extreme drought. *Chemosphere* 119, 927–934.
- Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A., Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., Brooks, B.W., 2014. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. *Phil. Trans. R. Soc. B* 369.
- Du, B., Perez-Hurtado, P., Brooks, B.W., Chambliss, C.K., 2012. Evaluation of an isotope dilution liquid chromatography tandem mass spectrometry method for pharmaceuticals in fish. *J. Chromatogr. A* 1253, 177–183.
- Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: focus on exposure and biological effects in animal species. *Environ. Toxicol. Chem.* 35, 799–812.
- Fong, P.P., Molnar, N., 2008. Norfluoxetine induces spawning and parturition in estuarine and freshwater bivalves. *Bull. Environ. Contam. Toxicol.* 81, 535–538.
- Franzellitti, S., Buratti, S., Capolupo, M., Du, B., Haddad, S.P., Chambliss, C.K., Brooks, B.W., Fabbri, E., 2014. An exploratory investigation of various modes of action and potential adverse outcomes of fluoxetine in marine mussels. *Aquat. Toxicol.* 151, 14–26.
- Garcia, S.N., Foster, M., Constantine, L.A., Huggett, D.B., 2012. Field and laboratory fish tissue accumulation of the anti-convulsant drug carbamazepine. *Ecotoxicol. Environ. Saf.* 84, 207–211.
- Gaw, S., Brooks, B.W., 2016. Changing tides: adaptive monitoring, assessment, and management of pharmaceutical hazards in the environment through time. *Environ. Toxicol. Chem.* 35, 1037–1042.
- Gaw, S., Thomas, K.V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. *Phil. Trans. R. Soc. B* 369.
- Gerritsen, J., Holland, A.F., Irvine, D.E., 1994. Suspension-feeding bivalves and the fate of primary production: an estuarine model applied to Chesapeake Bay. *Estuaries* 17, 403–416.
- Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Burket, S.R., Kristofco, L., Corrales, J., Rasmussen, J.B., Chambliss, C.K., Luers, M., Rogers, C., Brooks, B.W., 2018. Spatio-temporal bioaccumulation and trophic transfer of ionizable pharmaceuticals in a semi-arid urban river influenced by snowmelt. *J. Hazard Mater.* 359, 231–240.
- Hazelton, P.D., Du, B., Haddad, S.P., Fritts, A.K., Chambliss, C.K., Brooks, B.W., Bringolf, R.B., 2014. Chronic fluoxetine exposure alters movement and burrowing in adult freshwater mussels. *Aquat. Toxicol.* 151, 27–35.
- Ilarri, M., Sousa, R., 2011. Corbicula Fluminea Müller (Asian Clam), pp. 173–183. *Aquatic Invertebrates*.
- Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environ. Sci. Technol.* 36, 1202–1211.
- Kristofco, L.A., Haddad, S.P., Chambliss, C.K., Brooks, B.W., 2018. Differential uptake of and sensitivity to diphenhydramine in embryonic and larval zebrafish. *Environ. Toxicol. Chem.* 37, 1175–1181.
- Kristofco, L.A., Brooks, B.W., 2017. Global scanning of antihistamines in the environment: analysis of occurrence and hazards in aquatic systems. *Sci. Total Environ.* 592, 477–487.
- Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., Klaminder, J., 2016. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web - insights from a field experiment. *Sci. Total Environ.* 568, 208–215.
- Lazzara, R., Blázquez, 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.
- Lazarus, R.S., Rattner, B.A., Brooks, B.W., Du, B., McGowan, P.C., Blazer, V.S., Ottinger, M.A., 2015. Exposure and food web transfer of pharmaceuticals in ospreys (*Pandion haliaetus*): predictive model and empirical data. *Integr. Environ. Assess. Manag.* 11, 118–129.
- Liu, J.-L., Wong, M.-H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. *Environ. Int.* 59, 208–224.
- Lopes-Lima, M., Burlakova, L.E., Karatayev, A.Y., Mehler, K., Seddon, M., Sousa, R., 2018. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. *Hydrobiologia* 810, 1–14.
- McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. *Sci. Total Environ.* 476–477, 317–326.

- McMahon, R.F., 1999. Invasive Characteristics of the Freshwater Bivalve *Corbicula fluminea*. Nonindigenous Freshwater Organisms: Vectors, Biology, and Impacts. CRC Press LLC, pp. 315–343.
- Meredith-Williams, M., Carter, L.J., Fussell, R., Raffaelli, D., Ashauer, R., Boxall, A.B., 2012. Uptake and depuration of pharmaceuticals in aquatic invertebrates. *Environ. Pollut.* 165, 250–258.
- Nichols, J.W., Du, B., Berninger, J.P., Connors, K.A., Chambliss, C.K., Erickson, R.J., Hoffman, A.D., Brooks, B.W., 2015. Observed and modeled effects of pH on bioconcentration of diphenhydramine, a weakly basic pharmaceutical, in fathead minnows. *Environ. Toxicol. Chem.* 34, 1425–1435.
- Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* 72, 3–27.
- Ramirez, A.J., Brain, R.A., Usenko, S., Mottaleb, M.A., O'Donnell, J.G., Stahl, L.L., Wathen, J.B., Snyder, B.D., Pitt, J.L., Perez-Hurtado, P., Dobbins, L.L., Brooks, B.W., Chambliss, C.K., 2009. Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. *Environ. Toxicol. Chem.* 28, 2587–2597.
- Ramirez, A.J., Mottaleb, M.A., Brooks, B.W., Chambliss, C.K., 2007. Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. *Anal. Chem.* 79, 3155–3163.
- Ricciardi, A., Rasmussen, J.B., 1999. Extinction rates of north American freshwater fauna. *Conserv. Biol.* 13, 1220–1222.
- Rudd, M.A., Ankley, G.T., Boxall, A.B.A., Brooks, B.W., 2014. International scientists' research priorities for pharmaceuticals and personal care products in the environment. *Integr. Environ. Assess. Manag.* 10, 576–587.
- Saari, G.N., Scott, W.C., Brooks, B.W., 2017. Global scanning assessment of calcium channel blockers in the environment: review and analysis of occurrence, ecotoxicology and hazards in aquatic systems. *Chemosphere* 189, 466–478.
- Schultz, M.M., Furlong, E.T., 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. *Anal. Chem.* 80, 1756–1762.
- Soh, L., Connors, K.A., Brooks, B.W., Zimmerman, J., 2011. Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. *Environ. Sci. Technol.* 45, 1363–1369.
- UN, 2014. World Urbanization Prospects: 2014 Revision. New York.
- UN, 2017. World Population Prospects. The 2017 Revision: Key Findings & Advance Tables. New York.
- Wang, Z., Zhang, X.-H., Huang, Y., Wang, H., 2015. Comprehensive evaluation of pharmaceuticals and personal care products (PPCPs) in typical highly urbanized regions across China. *Environ. Pollut.* 204, 223–232.
- Way, C.M., Hornbach, D.J., Miller-Way, C.A., Payne, B.S., Miller, A.C., 1990. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: corbiculidae). *Can. J. Zool.* 68, 115–120.
- Williams, J.D., Warren, M.L., Cummings, K.S., Harris, J.L., Neves, R.J., 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* 18, 6–22.
- Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., Chen, W., 2015. Occurrence, bioaccumulation, and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. *Chemosphere* 138, 140–147.