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Pharmaceutical uptake kinetics in rainbow trout: In situ bioaccumulation in an effluent-dominated river influenced by snowmelt

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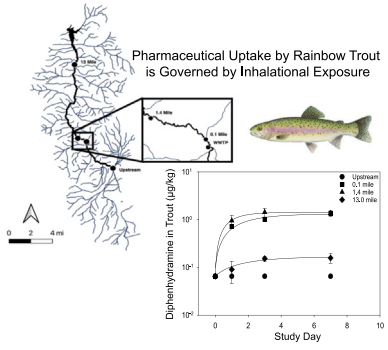
HIGHLIGHTS

- Spatio-temporal uptake studies by rainbow trout were performed with pharmaceuticals.
- Tissue levels of pharmaceuticals corresponded with surface water concentrations.
- Uptake kinetics for individual pharmaceuticals did not vary among sites or seasons.
- Inhalational exposure from water governed accumulation of ionizable pharmaceuticals.

GRAPHICAL ABSTRACT



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ABSTRACT

Whether seasonal instream flow dynamics influence bioaccumulation of pharmaceuticals by fish is not well understood, specifically for urban lotic systems in semi-arid regions when flows are influenced by snowmelt. We examined uptake of select pharmaceuticals in rainbow trout (*Oncorhynchus mykiss*) caged in situ upstream and at incremental distances downstream (0.1, 1.4, 13 miles) from a municipal effluent discharge to East Canyon Creek in Park City, Utah, USA during summer and fall of 2018. Fish were sampled over 7-d to examine if uptake occurred, and to define uptake kinetics. Water and fish tissues were analyzed via isotope dilution liquid chromatography tandem mass spectrometry. Several pharmaceuticals were consistently detected in water, fish tissue and plasma, including carbamazepine, diphenhydramine, diltiazem, and fluoxetine. Pharmaceutical levels in water ranged up to 151 ng/L for carbamazepine, whereas the effluent tracer sucralose was consistently observed at low µg/L levels. During both summer and fall experiments at each of three downstream locations from effluent discharge, rainbow trout rapidly accumulated these pharmaceuticals; tissue levels reached steady state conditions within 24–96 h. Spatial and temporal differences for pharmaceutical levels in rainbow trout directly corresponded with surface water exposure concentrations, and uptake kinetics for individual pharmaceuticals did not vary among sites or seasons. Such observations are consistent with recent laboratory bioconcentration studies, which collectively indicate inhalational exposure from water governs rapid accumulation of ionizable base pharmaceuticals by fish in inland surface waters.

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1. Introduction

By 2050, approximately 70% of the global population will live in urban areas. Currently, 82% of the population in North America already live in urban regions (United Nations, 2018), following transitions from a predominantly agricultural society with more disperse populations in rural areas (Rhind, 2009). Such concentration of human populations concomitantly results in concentration of resource (e.g., food, energy, water) consumption, including consumer chemical use, in cities (Brooks, 2018). For example, reclaimed sewage effluents of diverse quality are returned to urban surface waters, and in some watersheds result in effluent-dominated and dependent systems, in which instream flows are mostly or entirely composed of effluent discharges, respectively (Brooks et al., 2006). When these surface waters are subsequently extracted for various uses, an urban water cycle emerges (Brooks, 2014, 2018).

Effluent-dominated systems occur across arid to humid regions (Rice and Westerhoff, 2017), and present important water management opportunities in the face of climate change (Luthy et al., 2015). In North America, the 100th meridian has historically denoted a transition from arid to more humid climate but has effectively moved east as the Western USA experiences less precipitation (Seager et al., 2018). In some urban watersheds, decreased snowpack has also occurred in response to climate change, which subsequently alters instream flows and further stresses management of water quantity and quality (Mankin et al., 2015). These effluent-dominated urban surface waters can represent worst case scenarios for aquatic exposure to consumer chemicals, including pharmaceuticals and other contaminants of emerging concern (CECs; Brooks et al., 2006) because effective exposure duration increases with decreased instream dilution (Ankley et al., 2007). Unlike historically studied persistent and bioaccumulative compounds, such as organochlorines, pharmaceuticals are more water soluble, are less bioaccumulative in aquatic life, and are found in surface waters at low concentrations (Daughton and Brooks, 2011). However, with increasing urbanization and population growth, pharmaceutical consumption and introduction to the environment is expected to continue to increase (Brooks, 2018; United Nations, 2018). Subsequently, a number of priority research questions have been identified to understand risks of pharmaceuticals in the environment, including bioaccumulation in aquatic organisms (Boxall et al., 2012). Therefore, understanding environmental fate and bioaccumulation of ionizable contaminants remain important environmental quality research needs (Boxall et al., 2012; Brooks, 2019; Fairbrother et al., 2019).

Though traditional bioaccumulation models for organic contaminants were developed to address nonionizable chemicals, surface water pH influences bioavailability, bioaccumulation and toxicity of ionizable contaminants (Valenti et al., 2009; Erickson et al., 2006a, 2006b; Nichols et al., 2015; Armitage et al., 2017), including the majority of pharmaceuticals (Manallack, 2007). Unfortunately, empirical laboratory and field bioaccumulation information is lacking for most ionizable CECs, including many common use pharmaceuticals. In the case of bioaccumulation of ionizable bases by fish, uptake appears primarily driven by partitioning across the gill, compared to the dietary route of exposure, which is important for non-ionizable contaminants (Arnot and Gobas, 2006; Armitage et al., 2017). For example, Du et al. (2014) initially reported dilution of diphenhydramine across trophic positions, not biomagnification, in an effluent-dependent river in Texas, USA. Building on previous efforts to model gill uptake of ionizable organic acid contaminants in rainbow trout (Erickson et al., 2006a, 2006b), Nichols et al. (2015) developed a novel gill uptake model for ionizable organic bases with the fathead minnow, again using diphenhydramine as an experimental compound for empirical studies across pH gradients. However, mechanistic accumulation models for ionizable bases are not available for trout and other fish (Nichols et al., 2015; Scott et al., 2019). More recently, Haddad et al. (2018) extended the efforts by Du et al. (2014) to examine trophic transfer of other ionizable base pharmaceuticals in East Canyon Creek, an effluent-dominated river that is seasonally influenced by snowmelt, in Utah, USA. Haddad et al. (2018) consistently observed trophic dilution of diphenhydramine and several other ionizable pharmaceuticals, regardless of season or sampling site in this popular location for recreational brown trout angling, which further highlights the importance of understanding ionizable contaminant uptake dynamics by trout.

In previous laboratory studies, uptake of ionizable base pharmaceuticals by fish is rapid, reaching apparent steady state conditions within a few days, and uptake is altered by pH (Nichols et al., 2015; Kristofco et al., 2018; Scott et al., 2019). Whether such observations extend to fish exposures in the field is not well studied for ionizable contaminants. Thus, in the present study we aimed to examine if uptake occurred and then to define uptake kinetics of pharmaceuticals in trout under field conditions, and we specifically hypothesized that bioaccumulation dynamics with trout in the field would not differ among study locations or seasons. We selected East Canyon Creek in Utah, USA for in situ uptake studies with rainbow trout caged along a longitudinal gradient downstream from an effluent discharge. We performed these experiments when effluent-dominated instream flow conditions existed to varying extents during the summer and fall. Targeted pharmaceuticals were quantified in water and fish caged at an upstream reference site, in the effluent discharge and at three downstream locations.

2. Methods and materials

2.1. Study system

The East Canyon Creek watershed is located in north central Utah, approximately 20 miles east of Salt Lake City, Utah, USA, flowing through both Summit and Morgan counties (Fig. 1). This semi-arid river experiences fluctuating instream flows because of seasonal snowmelt. The East Canyon Water Reclamation Facility (ECWRF) contains a wastewater treatment plant (WWTP) that provides service to the residents of Park City and Snyderville Basin area of Summit County. This treatment plant has a design capacity supporting a maximum monthly flow of 5 million gallons per day (mgd) and a mean monthly flow of 4 mgd. The treatment processes of ECWRF include primary, secondary, and tertiary treatment. Tertiary treatment involves a two-step process including Biological Nutrient Removal (BNR) and Chemical Nutrient Removal (CNR) which involves the addition of alum to coagulate remaining solids, and sand filters, and UV radiation for disinfection prior to being discharged to East Canyon Creek, which subsequently is impounded downstream to form East Canyon Reservoir.

2.2. Study chemicals

Study pharmaceuticals were selected to be consistent with our recent field sampling efforts in East Canyon Creek (Haddad et al., 2018). All pharmaceuticals and their corresponding isotopically labeled analogs were commercially acquired, reagent-grade and used as they were received. Acetaminophen (ACE), acetaminophen-*d*4, amitriptyline (AMI), amitriptylen-*d*3, aripiprazole (ARI), aripiprazole-*d*8, benzoylecgonine (BEN), benzoylecgonine-*d*3, buprenorphine (BUP), buprenorphine-*d*4, caffeine (CAF), carbamazepine (CAR), carbamazepine-*d*10, diclofenac (DIC), diltiazem (DIL), diphenhydramine (DIP), diphenhydramine-*d*3, duloxetine (DUL), duloxetine-*d*3 fluoxetine (FLU), fluoxetine-*d*6, methylphenidate (MET), methylphenidate-*d*9, norfluoxetine (NOR), norfluoxetine-*d*6, norsertraline (NORS), promethazine (PROM), promethazine-*d*3 and sertraline (SER) were purchased as certified analytical standards from Cerilliant (Round Rock, TX, USA). Amlodipine, amlodipine-*d*4 (AML), caffeine-*d*9, desmethylsertraline (DES), desmethylsertraline-*d*4, diclofenac-*d*4, diltiazem-*d*3, and sertraline-*d*3 were acquired from Toronto Research Chemicals (Toronto, Ontario, Canada). Sucralose (SUC) was purchased from Sigma-Aldrich (St.

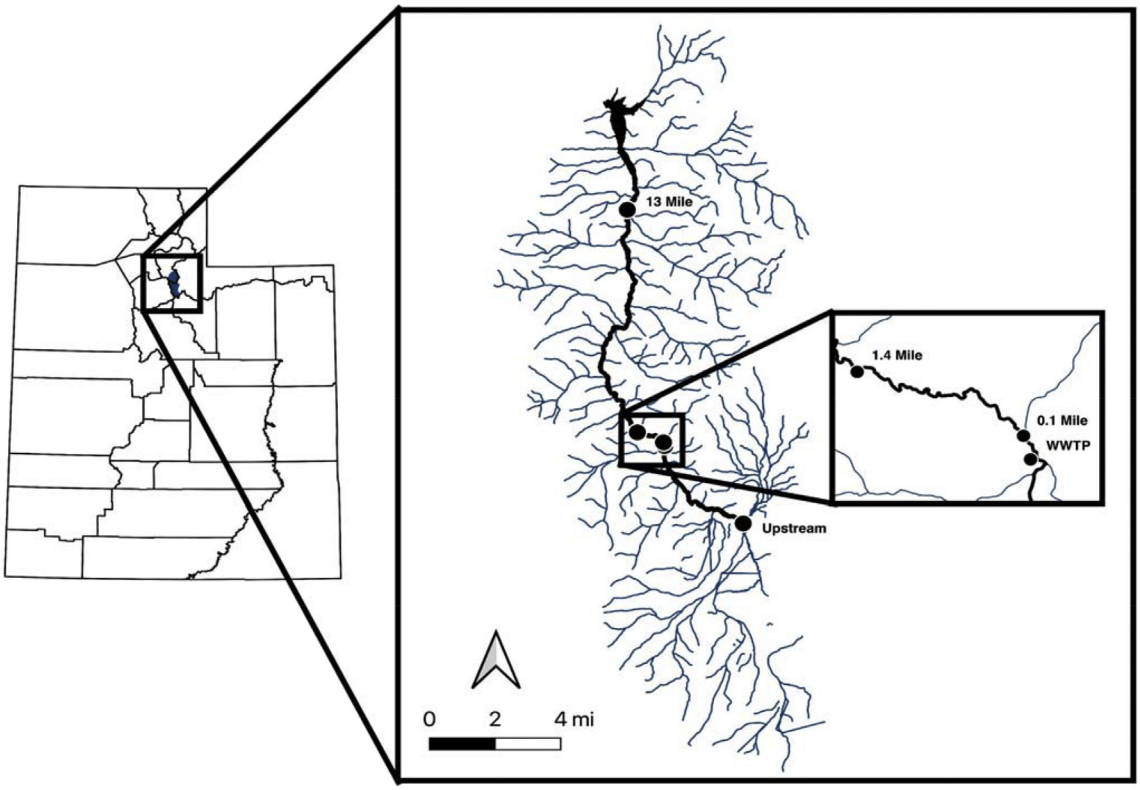

Fig. 1. Sampling sites in East Canyon Creek, Utah, USA for uptake experiments, performed during July and October 2018. WWTP: wastewater treatment plant.

Louis, MO, USA) and sucralose-d6 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. Study design

We performed two 7-day studies in East Canyon Creek during low flow conditions in the Summer (14–21 July 2018) and Fall (13–20 October 2018) seasons (Fig. 2). We initially reported accumulation of pharmaceuticals in fish from East Canyon Creek, where a physical barrier exists upstream from the ECWRF discharge, which limits fish movement upstream (Du et al., 2012). Study sites consisted of an upstream reference site, and three locations at incremental distances downstream (0.1, 1.4, 13 miles) from the municipal discharge at ECWRF. During both studies, water quality parameters, including pH, temperature, dissolved oxygen, and specific conductivity were measured at each site every 15 min for 24 h/day using pre- and post-deployment calibrated multiparameter datasondes (Exo 2, 7-Port Multiparameter Water Quality Sonde, YSI Incorporated, Yellow Springs, Ohio, USA). On study days 0, 1, 3 and 7, discharge (ft^3/s) was determined using a Marsh McBirney Flow Mate and standard methods at the upstream and 1.4-mile locations, while discharge was recorded at the 0.1- and 13-mile by United States Geological Survey (USGS) gauges (# 10133800 and 10133980, respectively). Also, on study days 0, 1, 3 and 7, total and dissolved nitrogen and phosphorus were determined at each site and from the effluent discharge.

Juvenile rainbow trout (mean weight = 25.18 ± 5.75 g; mean length = 13.52 ± 1.49 cm) were acquired from and delivered by a local hatchery (Cold Springs Trout Farm, North Ogden, Utah). Three fish were collected from the hatchery delivery before each exposure period for tissue analyses. At the juvenile stage, rainbow trout are difficult to sex and thus sex was described as indeterminate. Fish were acclimated to simulate stream conditions for 24 h before deployment and were not fed before or during the field campaign. Trout were caged in 25.4 cm PVC tubing with a diameter of 10.2 cm and mesh fiberglass fixed to each end. Mesh pore size allowed small aquatic invertebrates to enter cages. On study day 0, cages were deployed at each site with one fish per cage. At each site, triplicate samples were collected days 1, 3, and 7 (n = 3). Length, weight, and blood were collected on site immediately after anesthetization by immersion in 2–4 °C water following an approved Institutional Animal Care and Use Protocol at Baylor University. Water and tissue samples were stored on wet ice in the field and transferred to a −20°C freezer until sample preparation and analysis. Plasma samples were stored in dry ice in the field and stored at −80 °C prior to analysis.

2.4. Water extraction

Water samples (N = 24) were collected at all sites on each sampling day (n = 6) using 4-l amber glass bottles that were pre-cleaned with analytical-grade methanol (MeOH). On each study day, one site was randomly selected for duplicate collection. After collection, water samples were immediately filtered in sequence to remove particulate: a glass fiber prefilter (1.0-μm pore size, 47 mm, Pall Corporation, Port Washington, NY, USA), a nitrocellulose filter (0.45-μm pore size, 47 mm, GE Healthcare, Little Chalfont, BUX, UK), and a Nylaflo filter (0.2-μm pore size, 47 mm, Pall Corporation, Port Washington, NY, USA). Water samples were then concentrated on-site using solid phase extraction (SPE) cartridges. 1 L was separated into 2–500 mL volumetric flasks for extraction with either an Oasis HLB cartridge (6 mL, 200 mg, Water corporations, Milford, MA, USA) or Strata SCX cartridge (6 mL, 200 mg, Phenomex, Torrance, CA, USA). Each water sample was spiked with 50 μL of a 2000 μg/L deuterated internal standard (ISS) mix prior to extractions. The Oasis HLB sample cartridges were pre-treated with 5 mL of methyl tert-butyl ether (MTBE), 5 mL of methanol (MeOH), and 5 mL of nanopure water, respectively. Strata SCX samples were also spiked with 5 mL of MeOH and 100 μL of 85% phosphoric acid for acidification. Strata SCX cartridges were pre-treated with 4 mL of MeOH followed by 8 mL of nanopure water. Samples

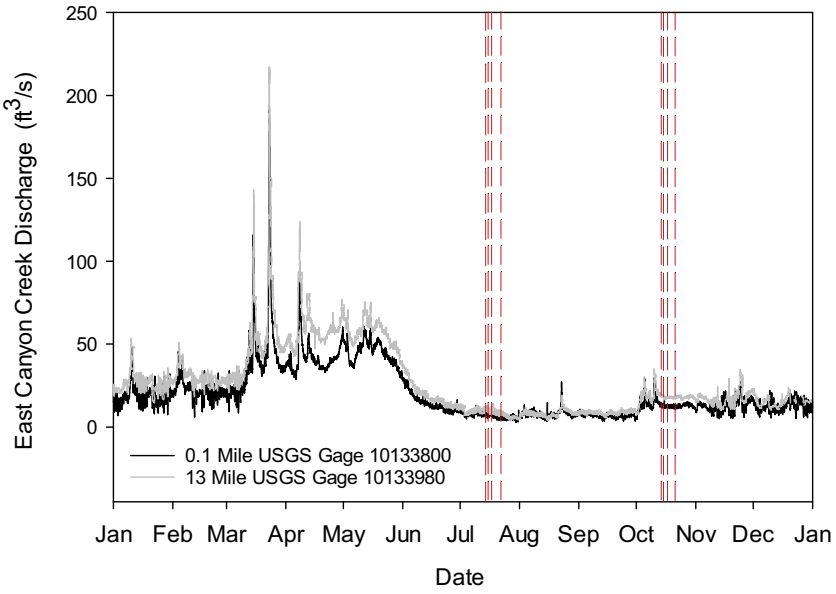

Fig. 2. Discharge (ft3/s) of East Canyon Creek, Utah, USA during, 2018. The red dashed lines indicate sampling events in July and October 2018. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were extracted via a 24-port Visiprep vacuum manifold (Supelco Inc., Bellefonte, PA, USA) with a flow rate of approximately 9 mL/min. SPE cartridges were stored in a freezer at −20 °C on-site then transferred on dry ice to Baylor University for further analysis. Oasis HLB cartridges were eluted with 5 mL MeOH and 5 mL 10:90 (v/v) MeOH:MTBE. Strata SCX cartridges were first washed with 4 mL of aqueous 0.1% HCl solution and 5 mL MeOH, respectively, then eluted with 6 mL 5:95 (v/v) ammonium hydroxide (NH_4OH): MeOH. Eluates were blown to dryness under a gentle stream of nitrogen, that ramps up to 2.5 L/min, in a Turbovap (Zynmark, Hopkinton, MA, USA) set to 45 °C, then reconstituted to 1 mL with 5:95 (v/v) MeOH:aqueous 0.1% formic acid (Bean et al., 2018; Du et al., 2014; Haddad et al., 2018; Lajeunesse et al., 2011; Vanderford and Snyder, 2006).

2.5. Tissue and plasma extraction

Whole body homogenates, minus the liver, were prepared for fish samples. Livers were used in other experiments and results will be reported elsewhere. After homogenization, 1 g of tissue was separated into a borosilicate glass vial, and 50 μL of 2000 μg/L ISS was spiked into the vial. Next, 4 mL of MeOH and 4 mL of 0.1 M acetic acid were added into the mixture and vials were rotated at 15 rpm for 25 min. After rotation, the contents in the vial were poured into plastic round bottom centrifuge tubes. The tubes were balanced with MeOH prior to centrifugation (25,000 rpm × 45 min at 10 °C). The supernatant was transferred to a glass culture tube and blown to dryness under a gentle stream of nitrogen in a TurboVap (Zynmark, Hopkinton, MA) set to 45 °C. Samples were reconstituted to 1 mL with 5:95 (v/v) MeOH:aqueous 0.1% formic acid (Bean et al., 2018; Du et al., 2014; Haddad et al., 2018; Vanderford and Snyder, 2006).

Blood samples were collected from the caudal artery of each rainbow trout with heparin-rinsed syringes. Immediately following, samples were centrifuged at 1000 ×g for 10 min. The separated plasma was transferred to another 1 mL collection tube. As noted above, plasma samples were stored on dry ice and transferred to a −80 °C freezer at Baylor University. Next, 5 mL of 0.1% formic acid and 50 μL of ISS were spiked into each sample. The samples were then loaded onto Oasis HLB cartridges that were preconditioned with 5 mL of MeOH and 5 mL nanopure water, respectively. After extraction, cartridges were eluted with 5 mL of MeOH into 20 mL glass culture tubes. The eluate of each cartridge was evaporated to dryness under a stream of nitrogen and reconstituted to 1 mL 5:95 (v/v) MeOH:aqueous 0.1% formic acid. Prior to LC-MS/MS analysis, all reconstituted samples were filtered using a BD 1 mL TB syringe (BD, Franklin Lakes, NJ, USA) and Acrodisc hydrophobic Teflon Supor membrane syringe filters (13-mm diameter, 0.2 μm pore size, Pall Corporation, Port Washington, NY, USA) and placed in 2 mL analytical vials (Agilent Technologies, Santa Clara, CA, USA) for analysis (Bean et al., 2018; Du et al., 2014; Haddad et al., 2018; Vanderford and Snyder, 2006).

2.6. Instrumental analyses

Water, plasma, and tissue samples were analyzed using isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) with previously reported instrumental and quality assurance and quality control parameters (Bean et al., 2018; Haddad et al., 2018) using Agilent Infinity 1260 autosampler and a model 6420 quadrupole mass analyzer (Agilent Technologies, Santa Clara, USA). A binary gradient of 0.1% formic acid and MeOH was used as mobile phase. A 2.1 × 100 mm poroshell 120 SB-C18 column was used for separation of analytes, with a 5 × 2.1 mm poroshell SB-C18 guard column (Agilent Technologies, Santa Clara, CA, USA). The flow rate was held constant at 0.5 mL/min.

To perform the calibration method, eight vials were prepared with 50 μL of deuterated analogs combined with varying concentrations of target analyte standards in 5:95 (v/v) MeOH:aqueous 0.1% formic acid. The linear ranges for water, tissue and plasma are reported in the supplemental information (Table S3). Linear regressions were performed on the calibration data of each analyte, and correlation coefficients, r^2, for each analyte were ≥0.995. Continuous calibration verification (CCV) samples were run every 8 samples to ensure calibration method was valid. An acceptable CCV concentration was ±20% of target concentration. Quality assurance and quality control included fish that were deployed in an unrelated reference site, field blanks for water, and matrix spikes with duplicates that were included into each sample batch. Method detection limits (MDLs) (lowest concentration of an analyte with 99% confidence) were calculated for water, tissue, and plasma, and were generated by following EPA guideline (40 CFR Part 136, Appendix B).

2.7. Predicted and observed bioaccumulation analysis

Bioaccumulation factors (BAFs) were calculated as the ratio of analyte concentration in the tissue to the analyte concentration in the surrounding environment (water) for mean values by site and day:

$$BAF = \frac{C_{tissue}}{C_{water}}.$$

Predicted BAFs and bioconcentration factors (BCFs) were calculated using a previously reported models, respectively (Arnot and Gobas, 2006):

$$logBAF = (0.86 * logD_{OW}) + 0.12 \text{and } logBCF = (0.60 * log D_{OW}) - 0.23.$$

Here, we replaced the log K_{ow} of each ionizable study compound with a site-specific log D_{ow} to account for the influence of pH on uptake (Nichols et al., 2015; Scott et al., 2016). Site-specific log Dow values were calculated based on median pH measurements (Kah and Brown, 2008). Predicted tissue levels were estimated using predicted log BAFs or BCFs and observed water concentrations of target analytes. When plasma levels of these target analytes were observed in fish, we calculated the blood – water partitioning coefficient (P_{BW}) and estimated apparent volume of distribution (V_D; Nichols et al., 2015).

2.8. Statistical analyses

Two-way ANOVAs were performed using SigmaPlot 13 Systat Software (San Jose, CA, USA), with $\alpha = 0.05$ to examine spatial and temporal (i.e. season and site) influences on accumulation of individual analytes in fish tissue, with Tukey's pair-wise post-hoc test. Only tissue samples from the end of each sampling event (day 7) were evaluated because analyte concentrations appeared to reach steady state by day 7. Two-way ANOVA was also performed on BAFs, with season and site being the experimental factors. Values below MDLs were not used to estimate BAFs or for statistical analyses.

Regression analyses were performed to examine potential relationships between predicted and observed tissue levels (IBM SPSS Statistics 25 Software, Armonk, New York, USA). Uptake rates were modeled with non-linear regression for select pharmaceuticals in fish tissue using GraphPad Prism (Version 5.00 for Windows, GraphPad Software, San Diego, California, USA).

3. Results and discussion

Though our recent field studies have reported accumulation of diverse pharmaceuticals in fish (Du et al., 2012, 2014; Haddad et al., 2018) and laboratory experiments have examined uptake kinetics of ionizable pharmaceuticals in several fish species (Nichols et al., 2015; Kristofco et al., 2018; Scott et al., 2019), in the current study we performed 7-day uptake studies with rainbow trout caged in situ in a semi-arid river that is seasonally influenced by snowmelt. However, we selected two time periods in summer and fall seasons for study when instream flows of the river were dominated by municipal effluent (Fig. 2). Such conditions represent important consideration for future water management efforts in watersheds like East Canyon Creek, which may be stressed by the intersections of population growth and climate change. In addition to measuring routine water quality parameters (Table S1), we examined targeted pharmaceuticals and other wastewater indicators in water, trout tissue and plasma samples (Table S2). These targeted analytes were not detected in rainbow trout acquired from a hatchery at the beginning of the study or in caged fish at an upstream reference site, but low levels of several analytes, most notably sucralose and caffeine, were detected in water at the upstream reference site (Table S2).

In July and October, fourteen and thirteen pharmaceuticals were detected in water at one or more of the study locations, respectively. Pharmaceutical levels in surface water ranged up to 150 ng/L for the antiepileptic carbamazepine, 10 μg/L for the effluent tracer sucralose, and 19 ng/L for caffeine (Table S2). Analyte concentrations were consistently higher in the effluent and decreased in surface waters with increasing distance from the effluent discharge during both summer and fall study periods. Such observations are thus similar to our previous observations, and likely can be attributed to instream dilution from groundwater and smaller tributaries or instream attenuation (Acuña et al., 2015; Haddad et al., 2018). For example, Haddad et al. (2018) identified that diphenhydramine levels in surface water were ~16× higher at the sampling location immediately downstream from the effluent discharge (0.1-mile site) than at the 13-mile site during in the summer and ~38× higher in the fall. In the current study, diphenhydramine levels at the 0.1 mile site were ~19× and 12× higher than the 13-mile sampling location during the summer and fall studies, respectively (Table S2). Though effluent levels of diphenhydramine, diltiazem and fluoxetine did not differ between seasons ($p > 0.05$) in the current study, levels of these pharmaceuticals in surface waters were significantly higher ($p < 0.05$) in July at the 0.1-, 1.4- and 13-mile sites,

Table 1
pH from 24 h datasondes, and pharmaceutical levels in water and rainbow trout caged at sampling locations upstream and downstream (0.1 miles, 1.4 miles, 13 miles) of or effluent from the East Canyon Water Reclamation Facility discharge to East Canyon Creek, Park City, Utah, USA during July and October 2018. SD = standard deviation; BAF = bioaccumulation factor; m = mile.

Analyte	Season	Site (m)	Median pH (n)	Concentration in water (ng/L; n)	Concentration in whole-body fish[a] (μg/kg; SD, n)	BAF[a] (L/kg; SD, n)
Diphenhydramine	July	0.1	7.52 (96)	34.95 (1)	1.35 (0.19, 3)	38.58 (4.35, 3)
		1.4	7.71 (1)	32.05 (2)	1.37 (0, 2)	42.65 (0, 2)
		13	8.41 (96)	1.79 (1)	0.16 (0.04, 3)	88.15 (16.03, 3)
	October	0.1	7.87 (96)	11.48 (2)	0.62 (0.17, 3)	54.29 (11.89, 3)
		1.4	8.31 (96)	19.99 (1)	0.64 (0.10, 3)	32.07 (4.09, 3)
		13	8.2 (96)	0.95 (1)	–	–
Diltiazem	July	0.1	7.52 (96)	12.45 (1)	0.22 (0.01, 3)	17.32 (0.57, 3)
		1.4	7.71 (1)	25.80 (2)	0.26 (0, 2)	10.13 (0, 2)
		13	8.41 (96)	0.88 (1)	0.04 (0.02, 3)	72.57 (0, 1)
	October	0.1	7.87 (96)	4.99 (2)	0.08 (0.02, 3)	16.86 (3.38, 3)
		1.4	8.31 (96)	10.87 (1)	0.23 (0.18, 3)	20.72 (16.92, 3)
		13	8.2 (96)	2.86 (1)	0.06 (0.03, 3)	28.04 (0, 2)
Fluoxetine	July	0.1	7.52 (96)	11.18 (1)	4.76 (0.37, 3)	425.78 (32.75, 3)
		1.4	7.71 (1)	13.50 (2)	5.50 (0, 2)	407.59 (0, 2)
		13	8.41 (96)	<MDL (1)	–	–
	October	0.1	7.87 (96)	3.42 (2)	0.61 (0.32, 3)	284.77 (0, 1)
		1.4	8.31 (96)	5.74 (1)	0.76 (0.59, 3)	250.87 (0, 1)
		13	8.2 (96)	<MDL (1)	–	–

[a] Mean ± SD values of the 7 day study period.

compared to October. These elevated surface water concentrations in July appear to have resulted from lower instream dilution, because discharge was 12–14 ft^3/s in October compared to 5.8–8.9 ft^3/s in July (Fig. 2). It is also important to note that ECWRF receives wastewater from Park City, which experiences extreme population fluxes from tourism during each year. Additional studies are needed in locations like Park City to examine how pharmaceuticals and other CECs in effluent discharges respond to dramatic temporal population dynamics (Gaw and Brooks, 2016).

In the current study, we aimed to identify whether pharmaceuticals accumulated in rainbow trout, and if so, to examine uptake kinetics under field conditions. Diphenhydramine (antihistamine), fluoxetine

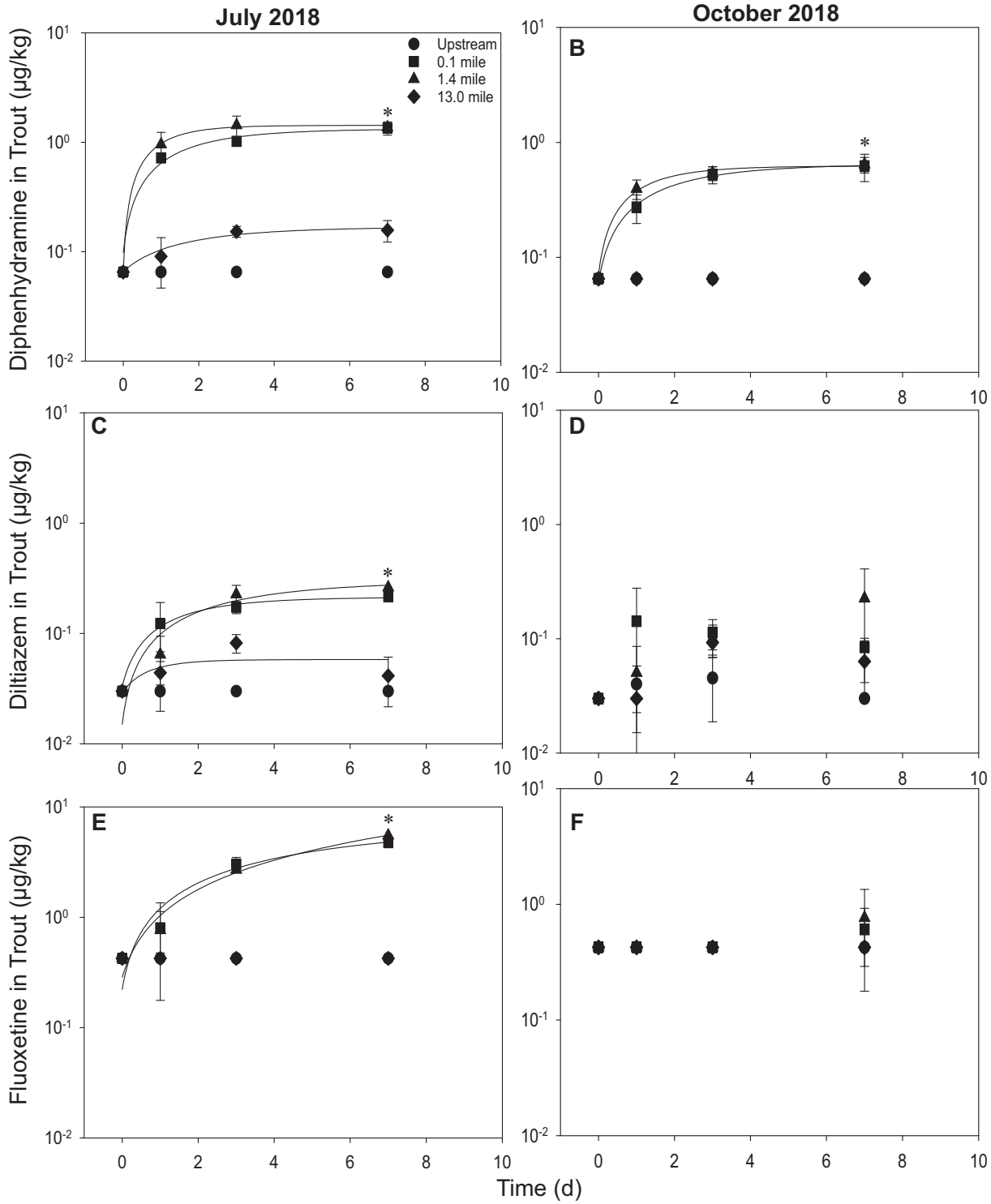

Fig. 3. Diphenhydramine (A and B), diltiazem (C and D), and fluoxetine (E and F) accumulation and uptake over 7-, day exposure period at sites in East Canyon Creek, Utah, USA, in July and October 2018, respectively. K1 and R2 for, (A) were 0.59 ± 0.13 µg/Kg d^{-1} and 0.9498; 1.10 ± 0.35 µg/Kg d^{-1} and 0.9027; 0.47 ± 0.30 µg/Kg d^{-1} and 0.7051 at the, 0.1, 1.4 and 13 mile sites, respectively. K1 and R2 for (B) were 0.49 ± 0.18 µg/Kg d^{-1} and 0.8716; 0.81 ± 0.21 µg/Kg d^{-1}, and 0.9199 at the 0.1 and 1.4 mile sites, respectively. K1 and R2 for (C) were 0.61 ± 0.25 µg/Kg d^{-1} and 0.8389; 0.34 ±, 0.20 µg/Kg d^{-1} and 0.8229; 1.21 ± 1.93 µg/Kg d^{-1} and 0.2531 at the 0.1, 1.4 and 13 mile sites, respectively. K1 and R2, for (E) were 0.15 ± 0.08 µg/Kg d^{-1} and 0.9544; 0.75 ± 0.04 µg/Kg d^{-1} and 0.9718 at the 0.1 and 1.4 mile sites, respectively.

(selective serotonin reuptake inhibitor), and diltiazem (calcium channel blocker) were detected in fish in both July and October (Table 1). Similar to water observations (Table S2) where concentrations were elevated downstream from the effluent discharge, trout at 0.1 and 1.4 m study locations accumulated diltiazem and diphenhydramine to significantly higher levels ($p < 0.05$) than fish located at the upstream or 13 m sites (Fig. 3). Only diphenhydramine was detected in fish plasma samples, and it was only observed in trout from the 0.1- and 1.4- mile sites. Fluoxetine was detected in fish tissue up to 5.6 μg/kg on day 7 in July. However, this analyte had the lowest detection frequency of the three pharmaceuticals measured in rainbow trout tissue, which precluded more detailed study. Diphenhydramine levels in rainbow trout tissue, which ranged from 0.07–1.66 and 0.07–0.81 μg/kg in July and October, respectively, were significantly higher ($p < 0.05$) during the summer study than in fall. Further, diphenhydramine levels in fish plasma were higher in July (up to 0.76 ng/mL) than in October (up to 0.38 ng/mL) (Table 2).

We (Haddad et al., 2018) previously observed trophic dilution in East Canyon Creek for diphenhydramine and other weak ionizable bases, regardless of site or season, indicating that these pharmaceuticals do not biomagnify to trout in this system. In fact. Haddad et al. (2018) identified tissue levels in fish that corresponded to concentrations in surface water, suggesting that waterborne exposure of fish to pharmaceuticals governed accumulation. In the present study, spatial (distance from discharge) and temporal (between seasons) differences for pharmaceutical levels in rainbow trout tissue also corresponded directly with surface water exposure concentrations. Such spatiotemporal experimental findings support previous observations from trophic transfer studies in the field that inhalational uptake represents the primary route of exposure for ionizable base pharmaceuticals in aquatic systems (Du et al., 2014; Haddad et al., 2018).

BAFs were calculated using measured whole-body tissue and water concentrations (Fig. 4). After one week, diphenhydramine, diltiazem and fluoxetine BAFs ranged up to 104.63, 72.57 and 449.71 L/kg in July, and up to 70.42, 39.92 and 284.77 L/kg, in October, respectively (Table 1). Diltiazem and diphenhydramine BAFs were elevated in July at the 13-mile site ($p < 0.05$), and fluoxetine BAFs were elevated in July compared to October ($p < 0.05$). These observations may have resulted from three-fold higher surface water temperatures in July compared to October (Table S1) and elevated pH at the 13-mile site (Table 1 and Table S4). At higher temperatures, oxygen saturation in water decreases and ventilation rates in fish respond accordingly to increase oxygen uptake, which can result in increased contaminant uptake due to these metabolic changes (Blewett et al., 2013; Saari et al., 2020).

Diphenhydramine, diltiazem and fluoxetine are ionizable base pharmaceuticals, and representative of ~70% of human pharmaceuticals that are also bases (Manallack, 2007). Diphenhydramine has subsequently been employed as a model base; a number of studies have examined its bioaccumulation and effects with aquatic species (Berninger et al., 2011; Nichols et al., 2015; Tanoue et al., 2015; Xie et al., 2016; Kristofco et al., 2018). Here, we observed a positive, though not significant, relationship between trout BAFs and median 24 h pH for diltiazem ($R^2 = 0.468$, $p > 0.05$) and diphenhydramine ($R^2 = 0.259$, $p > 0.05$) (Fig. S1). Such observations are not surprising because greater accumulation of weakly basic pharmaceuticals is expected with increasing pH due to a higher proportion of the non-ionized, and more lipophilic species (Nichols et al., 2015). However, it is important to note that previous laboratory studies (Nichols et al., 2015; Kristofco et al., 2018; Scott et al., 2019) carefully controlled pH and exposure concentrations of the compounds, in contrast to exposure conditions in the current in situ field studies, in which surface water levels of these ionizable pharmaceuticals and surface water pH varied temporally throughout the study period in East Canyon Creek (Table 1, Table S2).

Previous laboratory uptake studies with diphenhydramine (Nichols et al., 2015; Scott et al., 2019) and diltiazem (Scott et al., 2019) observed increasing pH to significantly increase fish BCFs. In the current study, field based BAFs (Table 1) for diphenhydramine in rainbow trout were comparable to BCFs reported by (Nichols et al., 2015) with fathead minnows when uptake studies were performed at pH 6.7, 7.7 and 8.7, though increasing pH strongly increased BCF. Field based BAFs for diphenhydramine and diltiazem observed here were also similar to BCF values identified by Scott et al. (2019), who performed a similar study examining pH influences on uptake of diphenhydramine and diltiazem by Gulf killifish. Here again, higher pH (8.3) resulted in increased uptake of both ionizable pharmaceuticals (Scott et al., 2019). However, field based BAFs for diphenhydramine in the current study and previous BCF observations by Nichols et al. (2015) and Scott et al. (2019), which were made at comparable pH levels to those we measured in East Canyon Creek, were higher than uptake studies with embryonic and juvenile zebrafish (Kristofco et al., 2018). These differences also appear related to pH influences on bioavailability; for example, pH in the zebrafish uptake experiments by Kristofco et al. (2018) were maintained ~7, and their diphenhydramine BCF values were similar those reported by Nichols et al. (2015) at pH 6.7. Thus, BAF observations in the current in situ study in the field and previous laboratory studies examining whole-body homogenate BCFs for diphenhydramine appear consistent regardless of the fish models employed. Because BAFs account for exposure from waterborne and dietary routes of exposure, but BCFs focus on chemical uptake from water only, such similarities between previous laboratory BCFs and the current field BAFs further supports previous indications that inhalational exposure governs rapid accumulation of these ionizable pharmaceuticals by fish in inland surface waters (Du et al., 2014; Haddad et al., 2018; Kristofco et al., 2018; Nichols et al., 2015; Scott et al., 2019).

Here, we used a common BAF and a BCF model to predict fish tissue levels of these ionizable pharmaceuticals (Arnot and Gobas, 2006), but modified it by substituting log D_{ow} for log K_{ow}, using the pKa of each study compound and median pH measures in East Canyon Creek. We did so as an attempt to account for pH influences on bioavailability of these ionizable pharmaceuticals and examine whether predicted tissue concentrations differed between models (Du et al., 2015; Kah and

Table 2
Diphenhydramine (DPH) levels in water and plasma of rainbow trout caged at sampling locations downstream (0.1 miles, 1.4 miles, 13 miles) from the East Canyon Water Reclamation Facility discharge to East Canyon Creek, Park City, Utah, USA during July and October 2018. SD = standard deviation; P_{BW} = blood-water partitioning coefficient; V_D = apparent volume distribution.

Season	Site (mi)	Concentration in water (ng/L; n)	DPH in fish plasma[a] (ng/mL; SD, n)	P_{BW}[a, b] (unitless; SD, n)	V_D[a, b, c] (L/kg; SD, n)
July	0.1	34.95 (1)	0.57 (0.17, 3)	16.31 (4.78, 3)	2.55 (0.92, 3)
	1.4	32.05 (2)	0.19 (0, 1)	5.95 (0, 1)	7.38 (0, 1)
	13	1.79 (1)	–	–	–
October	0.1	11.48 (2)	0.34 (0.06, 3)	29.21 (5.26, 3)	1.88 (0.47, 3)
	1.4	19.99 (1)	0.17 (0.03, 3)	8.29 (1.67, 3)	3.95 (0.84, 3)
	13	0.95 (1)	–	–	–

[a] Mean ± SD values of the 7 day study period.
[b] Calculated as the ratio of diphenhydramine in plasma and surface water.
[c] Calculated as ratio of diphenhydramine in tissue and plasma.

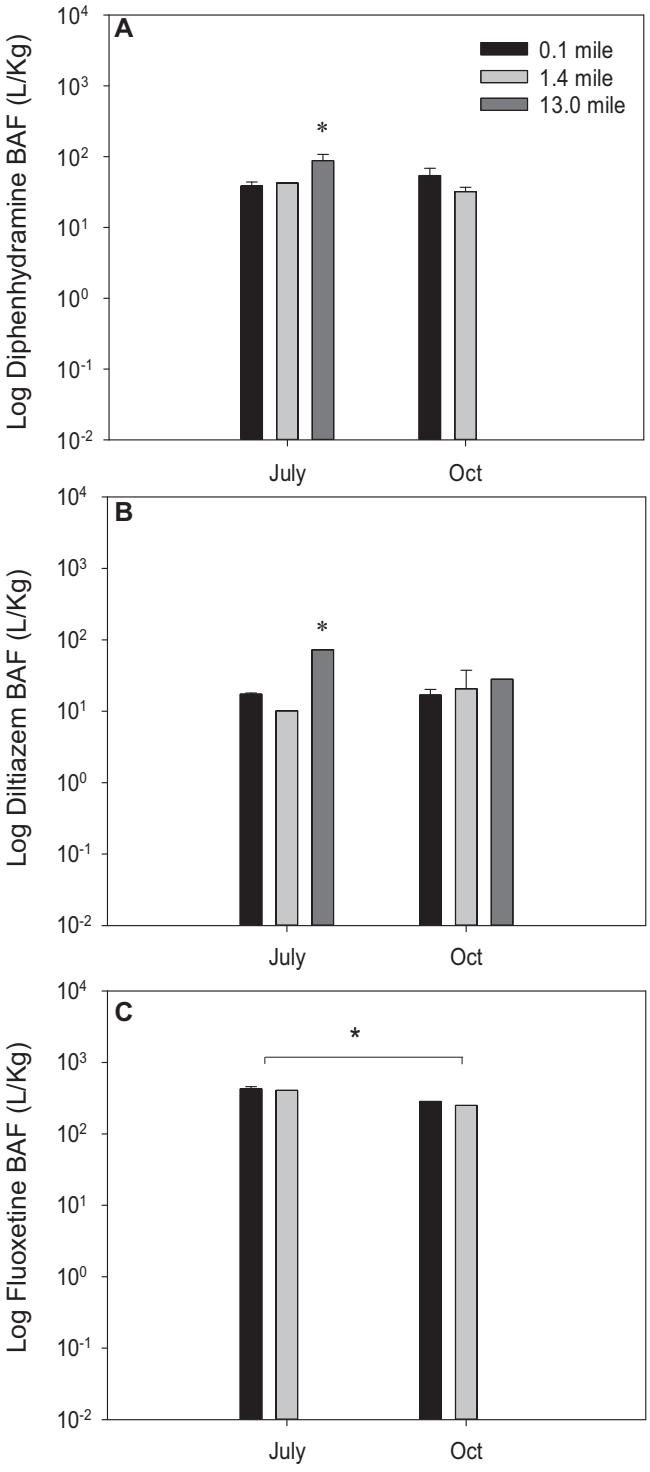

Fig. 4. Bioaccumulation factors (BAFs; mean ± SD) for diphenhydramine (A.), diltiazem, (B.) and fluoxetine (C.) following 7 day in situ studies with rainbow trout in East Canyon, Creek, Utah, USA, during July and October 2018. *: $p < 0.05$.

Brown, 2008). Positive relationships were observed between measured tissue concentrations and predicted tissue levels, and these relationships were significant for diphenhydramine ($R^2 = 0.832$, $p < 0.05$) and fluoxetine ($R^2 = 0.940$, $p < 0.05$), but not diltiazem ($R^2 = 0.620$, $p > 0.05$) when examining the BAF model (Fig. S2). Similar observations occurred when the BCF model was employed, in which significant relationships were observed between predicted and observed tissue concentrations for diphenhydramine ($R^2 = 0.890$, $p < 0.05$), diltiazem ($R^2 = 0.662$, $p < 0.05$) and fluoxetine ($R^2 = 0.976$, $p < 0.05$) (Fig. S2). Thus, for both models significant relationships were observed between predicted and observed pharmaceutical levels in fish. However, predicted tissue levels from the BAF model were up to several orders of magnitude higher than their observed counterparts, while predicted levels from the BCF model were only up to one order of magnitude higher. Such observations may have been influenced by not examining chemical levels in liver tissue, which we did not account for in the current work, because livers were removed from trout for other analyses and thus were not included in our whole-body tissue analyses. For example, previous research has demonstrated that pharmaceuticals reach elevated levels in fish liver tissue relative to muscle (Brooks et al., 2005). It is important to note that the BAF and BCF models used to predict tissue levels do not account for elimination, which highlights the importance of the need for future work in this area. We also did not perform more advanced uptake modeling because a gill model has not yet been developed for weakly basic contaminants (Nichols et al., 2015) in trout, but appears necessary to improve predictions of ionizable accumulation in these ecologically and commercially important fish.

In the current study, we identified rainbow trout to rapidly accumulate diphenhydramine and diltiazem to apparent steady-state conditions within 7 days (Fig. 3). Though standard methods for laboratory bioconcentration studies often recommend 28 days for the uptake phase (OECD, 2012), our previous observations with these compounds in the laboratory also indicate apparent steady-state conditions within a few days of exposure. For example, diphenhydramine accumulated to steady-state conditions in fathead minnows (Nichols et al., 2015), in zebrafish (Kristofco et al., 2018) and in Gulf killifish (Scott et al., 2019) within 96 h. When sufficient data was available, we modeled uptake rate (k_1) for diphenhydramine, diltiazem and fluoxetine in rainbow trout by study site and study period. For example, the k_1 values for diphenhydramine were 0.59, 1.10 and 0.47 μg/Kg d^{-1} at the 0.1, 1.4 and 13 mile sites, respectively, in July, while k_1 values were 0.49 and 0.81 μg/Kg d^{-1} at the 0.1 and 1.4 mile sites, respectively, in October. It is interesting to note that uptake rates for diphenhydramine were not significantly different between season or study sites ($p > 0.05$). Thus, as noted above, even though water and tissue concentrations differed among sites and were typically higher in July than October (Fig. 4), uptake rates for diphenhydramine by trout were similar between study periods, which suggest that uptake rate is independent of water concentration of these ionizable pharmaceuticals (Fig. 3). Future studies should further examine uptake and elimination kinetics of ionizable pharmaceuticals and other contaminants to improve risk characterization.

As noted above, we only detected diphenhydramine in rainbow trout plasma. Subsequently, P_{BW} values for diphenhydramine on study day 7 ranged from 4.05–28.18 and 6.81–33.04 (unitless) during the July and October studies, respectively (Table 2). These partition coefficients are similar to P_{BW} values that were initially reported in fathead minnows by Nichols et al. (2015), who observed mean P_{BW}s for diphenhydramine of 26.6 and 53.3 (unitless) at pH 7.7 and 8.7, respectively. However, P_{BW} observations for diphenhydramine in the present study are lower than P_{BW} values in Gulf killifish (Scott et al., 2019), for which diphenhydramine P_{BW} were up to markedly higher (~ 112) than rainbow trout (Table 2) and fathead minnows (Nichols et al., 2015). We further examined the distribution of diphenhydramine between blood and tissues, and estimated apparent V_D values for rainbow trout, based on this field-based study. These apparent V_D values for diphenhydramine (Table 2) were comparable to observations in fathead minnow, another common freshwater fish model, by Nichols et al. (2015), but were markedly higher than apparent V_D values reported by Scott et al. (2019) in the Gulf killifish, a euryhaline estuarine fish model. One possible explanation for such marked interspecies differences observed for diphenhydramine distribution among rainbow trout, fathead minnows and Gulf killifish may be plasma binding among these species. Ionizable organics such as diphenhydramine

bind to α1-acid glycoproteins; however, little information is known about the plasma protein binding of ionizable contaminants (Armitage et al., 2017) and different levels of important plasma binding proteins in fish. Here again, such observations identify the importance of developing a predictive understanding of ionizable chemical bioaccumulation, including internal partitioning, among fish species.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139603.

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