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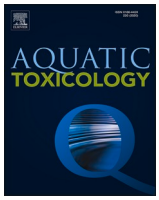

Differential tissue distribution of pharmaceuticals in a wild subtropical marine fish

N.A. Castillo [a,*], R.O. Santos [b], W.R. James [a,b], R. Rezek [c], D. Cerveny [d,e], R.E. Boucek [f], A.J. Adams [f,g], J. Fick [h], T. Brodin [d], J.S. Rehage [a]

[a] Earth and Environment Department, Florida International University, Miami, FL, USA
[b] Department of Biology, Florida International University, Miami, FL, USA
[c] Department of Marine Science, Coastal Carolina University, Conway, SC, USA
[d] Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden
[e] Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in Ceske Budejovice, Vodňany, Czech Republic
[f] Bonefish and Tarpon Trust, Miami, FL, USA
[g] Florida Atlantic University Harbor Branch Oceanographic Institute, Fort Pierce, FL, USA
[h] Department of Chemistry, Umeå University, Umeå, Sweden

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ABSTRACT

To date, the presence of pharmaceuticals has been extensively documented across a wide range of aquatic systems and biota. Further, substantial progress has been made in transitioning from laboratory assessments of pharmaceutical fate and effects in fish to *in situ* assessments of exposure and effects; however, certain research areas remain understudied. Among these is investigation of differential accumulation across multiple internal tissues in wild marine fish beyond the species commonly sampled in laboratory and freshwater field settings. This study examined the presence of pharmaceuticals across four tissues (plasma, muscle, brain, and liver) in a wild marine fish, bonefish (*Albula vulpes*), throughout coastal South Florida, USA. Differential accumulation across tissues was assessed for the number and concentration, identity, and composition of accumulated pharmaceuticals by sampling 25 bonefish and analyzing them for 91 pharmaceuticals. The concentration of pharmaceuticals was highest in plasma > liver > brain > muscle, while the number of pharmaceuticals was highest in liver > brain > plasma > muscle. The identity of detected pharmaceuticals was tissue specific, and there was an inverse relationship between the number of detections for each pharmaceutical and its log K_{ow}. The composition of pharmaceuticals was tissue specific for both pharmaceutical presence/absence and concentration. Across all tissues, the greatest similarity was between brain and liver, which were more similar to plasma than to muscle, and muscle was the most distinct tissue. For tissue compositional variability, muscle was the most diverse in accumulated pharmaceuticals, while plasma, brain, and liver were similarly variable. With the highest concentrations in plasma and highest number in liver, and documented variability in accumulated pharmaceuticals across tissues, our results highlight the importance of tissue selection when surveying exposure in wild fish, suggesting that multi-tissue analysis would allow for a more comprehensive assessment of exposure diversity and risk of adverse effects.

1. Introduction

The presence of pharmaceuticals throughout aquatic environments has been well established over the last few decades. This is of concern since pharmaceuticals are physiologically active at low environmentally relevant concentrations (Hernández-Tenorio et al., 2022) and have the ability to elicit adverse effects in exposed biota (Brodin et al., 2017; Saaristo et al., 2018). Until recently, the majority of studies examining the presence of pharmaceutical contaminants in aquatic environments have focused on surveys of freshwater and riverine systems (Miller et al., 2021; Świacka et al., 2022). Yet, the persistence of pharmaceuticals across aquatic ecosystems, their uptake in exposed biota, and their

* Corresponding author at: Florida International University, 11200 SW 8th St., OE-148, Miami, Florida, 33199, USA.
E-mail address: ncast169@fiu.edu (N.A. Castillo).

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ability to elicit effects can vary widely across environments, hydrological regimes, and among biota of differing physiology (Gómez-Regalado et al., 2023). In fishes, a review of 451 studies investigating bioconcentration across multiple tissues from 1979-2020 found that 86% were performed in freshwater taxa, and only 11.3% were done in brackish or marine species (Duarte et al., 2022). Further, when sampling fish as a means to detect pharmaceutical exposure, most studies collect one type of tissue or analyze whole body homogenates (Heynen et al., 2016). In spite of this, previous work has established that pharmaceuticals differentially accumulate across internal tissues (Armitage et al., 2017; Duarte et al., 2023; McCallum et al., 2017). Last, investigation of pharmaceutical uptake is often done in a controlled laboratory setting, and when compared to *in situ* studies of wild fish, results are frequently inconsistent (Gómez-Regalado et al., 2023; McCallum et al., 2019). The goals of laboratory studies are often different to field studies, as such there exists the need to establish the behavior of pharmaceutical accumulation across tissues in wild fish. Additionally, a recent review found that only 37% of bioconcentration/bioaccumulation studies involved field sampling, while 63% were laboratory based (Gómez-Regalado et al., 2023). Thus, there is a need to expand research to a greater diversity of wild fish species and compare pharmaceutical accumulation across multiple tissues (Armitage et al., 2017; Liu et al., 2018).

A compound's lipophilicity is of particular importance in the extent to which it accumulates in an organism. It is generally understood that bioconcentration/bioaccumulation increases as log K_{ow} increases (Arnot and Gobas, 2006; Mackay et al., 2018), with compounds having a log $K_{ow} > 3$ considered to have a high potential to bioconcentrate/bioaccumulate in aquatic organisms (Organization for Economic Co-operation Development Guidline, 2005). However, pharmaceutical accumulation in fish frequently diverges from this accepted metric (Matthee et al., 2023). For example, some pharmaceuticals with no predicted bioconcentration/bioaccumulation potential (e.g., log $K_{ow} < 3$) have been found to extensively accumulate in fish (Duarte et al., 2022). Despite the growing evidence for the unreliability of log K_{ow} in predicting bioaccumulation of pharmaceuticals, there remains the need to evaluate log K_{ow} from a more nuanced perspective beyond measuring only pharmaceutical number and concentration of accumulated pharmaceuticals, such as assessment of pharmaceutical composition and specificity in accumulated pharmaceuticals across tissues. Accordingly, additional investigation into the accuracy of log K_{ow} as a predictor of bioconcentration/bioaccumulation potential across multiple tissues in fish is necessary.

Studies inconsistently examine a breadth of internal tissues, evidenced by a recent review of over 100 bioconcentration/bioaccumulation studies finding that muscle was the most frequently analyzed tissue (35% of studies), followed by brain (18%), liver (15%), whole body homogenates (9%), and other organs such as gill, gonad, kidney, or bile (20%; Gómez-Regalado et al., 2023). Further, the majority of bioconcentration/bioaccumulation studies focus on four freshwater fish species, the crucian carp (*Carassius auratus*), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and European perch (*Perca fluviatilis*; Gómez-Regalado et al., 2023). Differences in tissue accumulation between wild freshwater and marine fish have been documented. For example, an examination of eight freshwater fish species from an urbanized riverine system found no clear pattern in number of pharmaceuticals across tissues, but found that concentrations were highest in liver > plasma > bile > muscle (Zhao et al., 2015). Meanwhile, Liu et al., (2018) examined tissue specific uptake in seven wild marine fish, finding the highest number of pharmaceuticals detected in kidney > liver > muscle > gill, with the highest concentrations in liver and lowest in muscle. The researchers concluded that pharmaceutical physiochemical properties (e.g., liposome-water distribution coefficient) related to an increase in liver concentrations but did not correlate with muscle concentrations.

This study aimed to determine if pharmaceuticals differentially accumulated across blood plasma and internal tissues (muscle, brain, and liver) in a wild marine subtropical mesoconsumer fish. This study addressed two questions: 1) Do pharmaceuticals differentially accumulate across tissues, considering both the number of pharmaceuticals and their concentrations? and 2) Does the identity and composition of accumulated pharmaceuticals differ between tissues? To address these questions, we sampled bonefish across four coastal regions, expanding 250 km of the South Florida (USA) coastline. We hypothesized that: 1) The accumulation of pharmaceuticals would be tissue specific in pharmaceutical number and concentration; and 2) Variability would be present across tissues in the identity and composition of pharmaceuticals.

2. Materials and methods

2.1. Study species

To understand *in situ* pharmaceutical uptake and tissue distribution in a marine mesoconsumer fish, we selected bonefish (*Albula vulpes*), an important recreational fishery, as their ecology makes them particularly susceptible to exposure of pharmaceutical contaminants. Recent literature has documented widespread pharmaceutical contamination of bonefish throughout South Florida and the Caribbean Basin (Castillo et al., 2024a), accumulating to concentrations capable of pharmacological effects. Bonefish diet consists of benthic vertebrates and invertebrates (Crabtree et al., 1998), including bivalves, gastropods, and polychaetes (Campbell et al., 2022), all of which have been shown to bioaccumulate pharmaceuticals (Almeida et al., 2020; Du et al., 2014). Bonefish utilize shallow nearshore habitats consisting of seagrass beds, intertidal sand flats, mangroves, and hardbottom, which can be in close proximity to urbanized coastal areas and anthropogenic influence (Larkin, 2011).

2.2. Sampling regions

Bonefish collection was distributed across four distinct regions of coastal South Florida, USA (Fig. 1). Regions were selected based on management zones designated by the Florida Keys National Marine Sanctuary (FKNMS; National Oceanic and Atmospheric Administration, 1996), and regions of importance to the bonefish fishery (Boucek et al., 2022). The four regions were: Biscayne Bay, Upper Keys, Lower Keys, and Key West (Fig. 1, Table 1). Biscayne Bay spans the length of Miami-Dade county, the most populous county in Florida (Browder et al., 2005), and contains Biscayne National Park (BNP; Browder et al., 2005). The Upper Keys, Lower Keys, and Key West (Monroe County) have a resident population of 82,000 total, but experience substantial tourism with over 5 million visitors annually (Shifflet and Schutz, 2019; Thomas et al., 2021).

2.3. Sample collection

We collected 25 bonefish throughout the four regions using hook and line angling between January and November 2019 ($n = 16$), and between May and September 2020 ($n = 9$; Fig. 1, Table 1). Collection was distributed across regions as follows: 8 bonefish from Biscayne Bay, 6 from Upper Keys, 5 from Lower Keys, and 6 from Key West (Table 1). All bonefish were captured from shallow, nearshore habitats (<10 m to 15 km from a shoreline with human presence). A total of 3 mL of blood for bonefish greater than 50 cm total length (1-2 mL for bonefish smaller than 50 cm) was collected from the ventral caudal vein using a sterile 18-gauge needle (BD PrecisionGlide™ Sterile Single-use Needles) and a sterile 5 mL syringe (BD Syringe). Blood samples were placed in 5 mL Lithium Heparin tubes (Greiner Bio-One), shielded from sunlight using aluminum foil, and stored on ice. Within 6 hours of collection, samples were centrifuged for 15 min at 3500 rpm (LW Scientific USA E8 Portable Centrifuge) to separate plasma. Plasma was aliquoted using sterile polyethylene transfer pipets (Corning Scientific™), placed in 2 mL

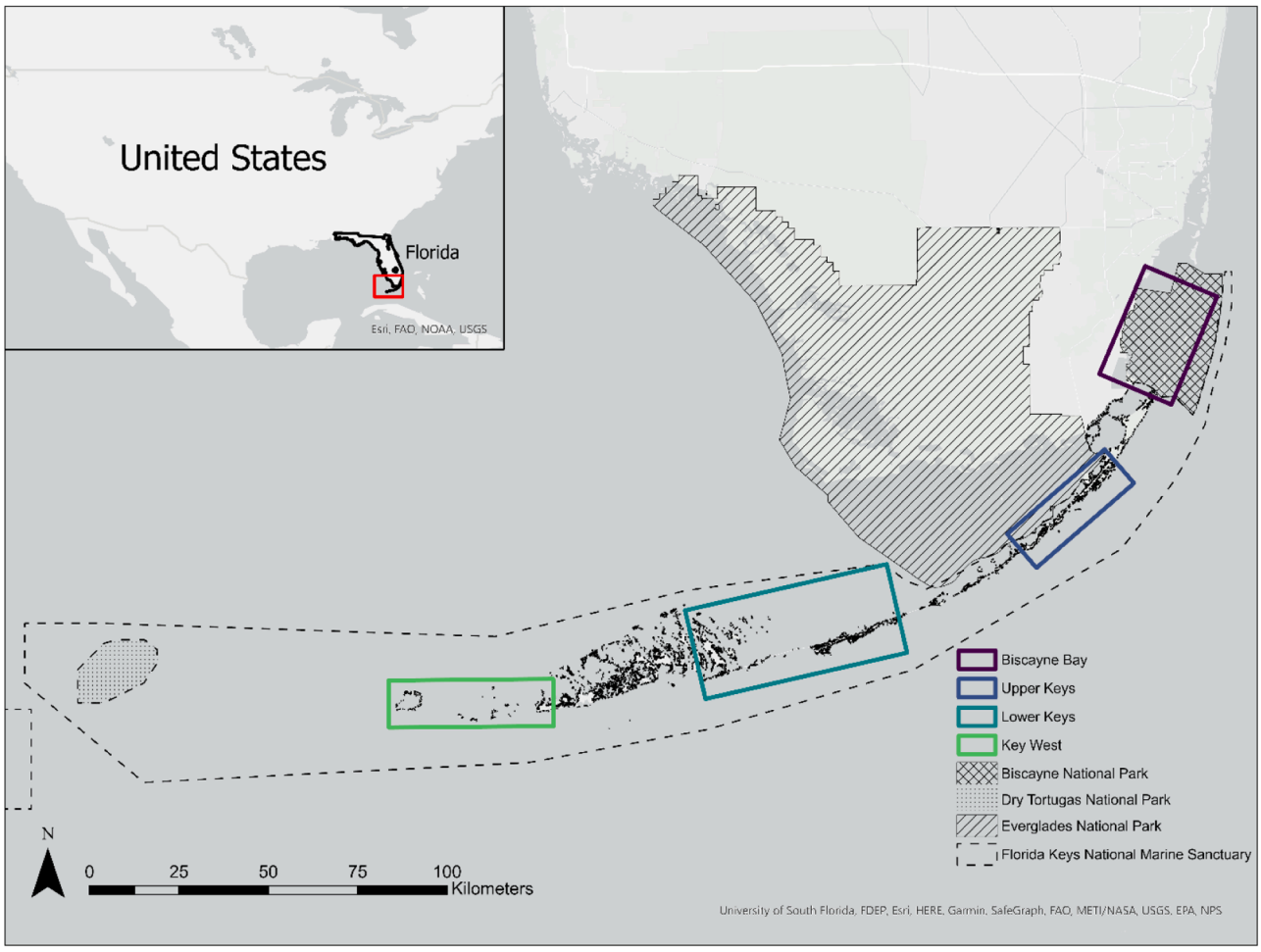

Fig. 1. Map of the four South Florida sampling regions. Exact bonefish sampling locations are omitted due to their status as a prohibited and protected species and the sensitive nature of the fishing locations.

Table 1
Sampling effort, summary of pharmaceutical findings, and regional characteristics. Shown are the number of samples per region, pharmaceutical detections (total, mean, max, min, and median) for each tissue, resource management (jurisdiction), population summaries, and land area summaries. FKNMS = Florida Keys National Marine Sanctuary.

Region	Sample Type	Total Samples	Total Detections	Mean	Max	Min	Median	Jurisdiction	Human Pop.	Annual Visitation	sq/km	People per sq/km
Biscayne Bay	Plasma	8	55	6.9	12	3	7	Biscayne National Park; State Waters	2.7m[a]	700,000[c]	4,918[a]	551[a]
	Muscle	8	29	3.6	8	1	3					
	Brain	8	98	12.3	16	6	12.5					
	Liver	8	109	13.6	25	9	11.5					
Upper Keys	Plasma	6	39	6.5	11	4	6.5	Everglades National Park; FKNMS	18,943[a]	1.7m[b]	111[a]	170[a]
	Muscle	6	19	3.2	8	1	2.5					
	Brain	6	62	10.3	18	5	9.5					
	Liver	6	103	17.2	23	11	17.5					
Lower Keys	Plasma	5	25	5	9	1	5	FKNMS	22,622[a]	1.5m[b]	161[a]	141[a]
	Muscle	5	13	2.6	7	1	2					
	Brain	5	71	14.2	18	11	14					
	Liver	5	69	13.8	16	11	14					
Key West	Plasma	6	22	3.7	6	2	3.5	FKNMS	33,555[a]	2.8m[b]	47[a]	714[a]
	Muscle	6	27	4.5	7	0	5.5					
	Brain	6	77	12.8	16	11	12.5					
	Liver	6	114	19	26	15	17					
All Regions	Plasma	25	141	5.6	12	1	5		2.8m	6.0m	5,237	535
	Muscle	25	88	3.5	8	0	3					
	Brain	25	308	12.3	18	5	13					
	Liver	25	395	15.8	26	9	16					

[a] https://censusreporter.org/
[b] Rockport Analytics, 2018
[c] Thomas et al., 2021
[d] http://ournationalparks.us

cryovials (Corning Scientific™), and stored in a -20°C freezer. Bonefish were euthanized with an overdose of MS-222, adhering to FIU IACUC-21-058 protocol, shielded from sunlight using aluminum foil, stored on ice, and transferred to a -20°C freezer within 6 hours of collection. Internal tissues (muscle, liver, and brain) were extracted within 1 month of sample collection. A minimum of 0.2 g of each internal tissue was extracted using sterile disposable scalpels (Stoelting™) and placed in 2 mL cryovials (Corning Scientific™). To eliminate risk of cross-contamination, scalpels were disposed and workstations were cleaned with 95% ethyl alcohol (Thermo Fisher Scientific) between extraction of each internal tissue. Samples were then stored in a -20°C freezer until processing at the Department of Chemistry, Umeå University, Umeå, Sweden within 6 months of sample extraction.

2.4. Target pharmaceuticals, standards and analytical methods

A total of 91 pharmaceuticals were included in the analysis (Table S1), and target analyte selection was based on predicted ability to bioaccumulate in fish and detectability (Fick et al., 2010). A summary of analytical procedures is provided here and further details on QA/QC, LOQ, and recovery percentages are described in Section 1 of the Supplementary Materials document and Table S2 and are also detailed in Grabic et al., (2012), Lindberg et al., (2014), and Sedvall et al., (2022).

Surrogate and internal standards were classified as analytical grade (>98%) and +20 internal/pseudo labeled standards were used (Grabic et al., 2012; Lindberg et al., 2014), LC-MS/MS grade methanol and acetonitrile (Lichrosolv – hypergrade) were used for the mobile phase (Merck,Darmstadt, Germany). Purified water was prepared in-house using a Mili-Q Advantage system, including a UV radiation source (Millipore, Billerica, USA). Formic acid (Sigma-Aldrich, Steinheim, Germany) was used to prepare the 0.1% mobile phases for liquid chromatography.

After thawing, muscle, liver, and brain tissue samples from each fish were weighed (0.1 ± 0.01g) in 2 mL polypropylene (PP) tubes. After adding 50 ng of internal standards mixture, samples were extracted twice, sequentially using 1.5 mL of acetonitrile. Samples were homogenized for 4 min at 42,000 oscillations per minute with zirconium beads (Mini Beadbeater, Biospec, Bartlesville, OK) and then centrifuged at 17,500g for 10 min (Beckman Coulter Microfuge 22R Centrifuge). This protocol was followed for both eluent mixtures individually, and the supernatants were combined, evaporated to dryness (<20 µL), and reconstituted in 150 µL of methanol. Final extracts were transferred into the glass autosampler vials with a 200 µL insert and kept frozen at -18°C (for a minimum of 24 h). Directly before analysis, the samples were centrifuged again to settle precipitated proteins and other solid particles in the sample. Plasma samples (20 µl) were pretreated by adding 50 ng of each internal standard, 50 µl methanol and 20 µl of water (with 0.1% formic acid). Samples were then frozen at -18°C overnight, thawed, and centrifuged at 17,500 g for 10 minutes.

All samples were analyzed using a triple-stage quadrupole mass spectrometer (Quantum Ultra EMR,Thermo Fisher Scientific, San Jose, CA), coupled with a liquid chromatographic pump (Accela, Thermo Fisher Scientific) and an autosampler (PAL HTC, CTC Analytics AG, Zwingen, Switzerland). Heated electrospray (HESI), krypton 10.6 eV, in positive ion mode was used for ionization of pharmaceutical compounds. Chromatography was done using a C18 phase Hypersil GOLD column (50 mm, 2.1 mm ID, 5 µm particles, Thermo Fisher Scientific, San Jose, CA, USA), and a guard column (2 mm, 2.1 mm, i.d. 5 µm particles). Two MS/MS transitions were used for positive identifications of analytes with a criterion that the ratio between the transitions may not deviate more than +/−30% from the ratio in the corresponding calibration standard. Retention times for all analytes were within +/−2.5% of the retention time in the corresponding calibration standard. Limit of quantification (LOQ) was determined from standard curves based on repeated measurements of low-level spiked samples, and the lowest point in the standard curve that had a signal/noise ratio of 10 was considered to be equal to the LOQ. A seven-point matrix adjusted calibration curve over the range of 0.05–100 ng/mL was used for linearity evaluation and quantification. Carry-over effects were evaluated by injecting standards at 100 ng/L followed by two mobile phase blanks. Several instrumental and procedural blanks were included in each analytical run. Additional details on the determination of pharmaceuticals including HESI ionizations, polarities, precursor/product ions, collision energies, tube lens values, and retention times are described elsewhere (Supplemental Materials Section 1; Grabic et al., 2012; Lindberg et al., 2014; Sedvall et al., 2022).

2.5. Statistical analyses

We used a combined univariate and multivariate approach to assess variation in pharmaceutical number, pharmaceutical identity, and pharmaceutical composition (both in concentration and presence/absence) across tissues. To assess variation in pharmaceutical number across tissues, we used Generalized Linear Mixed Models (GLMMs). This approach was used to determine if the number of pharmaceuticals detected in each sample varied as a function of tissue. To assess variation in the identity of pharmaceuticals across tissues, a Generalized Linear Model (GLM) was used to evaluate if the number of detections for each pharmaceutical varied as a function of tissue and each pharmaceuticals log K_{ow} and biotransformation half-life (HL; days) normalized for a 10 g fish at 15°C. Last, we assessed differences in pharmaceutical composition for both concentration and presence/absence using four multivariate analyses: 1) Permutational analysis of variance (PERMANOVA) was used to test for differences across tissue, region of collection, and fish identity (hereafter fish ID); 2) Similarity percentage analysis (SIMPER) was used to identify pharmaceuticals of importance in driving multivariate assemblages, which were then visualized with vector overlays on nMDS plots; 3) Homogeneity of group dispersion (i.e., beta diversity), as a function of tissue and region of collection, was used to analyze the variation of pharmaceutical composition within and between groups; and 4) Hierarchical cluster analysis (HCA) was used to analyze variation in pharmaceutical composition across tissues and regions of collection. Details for each analysis are provided in the following sections. All statistical analyses were performed using R v 4.3.1 (R Core Team, 2023).

2.5.1. Variation in the number of pharmaceuticals

The influence of tissue on the mean number of pharmaceuticals detected in each sample was assessed using GLMMs with a Poisson distribution and tissue as a four-level fixed factor (plasma, muscle, brain, and liver). Since there could be differences in pharmaceutical exposure between sampling regions and differences in bioaccumulation between individual bonefish, GLMMs included region as a random effect with fish ID nested within region (McCallum et al., 2017). Preliminary analysis revealed no influence of fish size in pharmaceutical burdens, thus this factor was omitted from all statistical models (Castillo et al., *in revision*). GLMMs were performed using the R package lme4 (Bates et al., 2015) and tests of model assumptions and performance were conducted using the R package performance (Lüdecke et al., 2021). Pairwise comparisons of significant model contrasts for tissue were analyzed using Tukey's HSD tests with a Holm-Bonferroni adjustment using the R package emmeans (Lenth, 2022).

2.5.2. Variation in the identity of pharmaceuticals

Generalized Linear Models (GLMs) with a negative binomial distribution were used to assess the influence of tissue, log K_{ow}, and HL on the number of detections for each pharmaceutical (i.e., the number of detections across all samples independent of fish ID for a given pharmaceutical), in each tissue. Each pharmaceutical's log K_{ow} and HL (biotransformation rate in a 10 g fish at 15°C) was calculated using the Estimation Programs Interface (EPI Suite™; United States EPA, 2012; Table S1). Models included HL as a term to expand beyond accounting for solely physio-chemical properties of each pharmaceutical (e.g., log

K_{ow}), and consider the relationship between physio-chemical properties, fish physiology, and pharmacodynamic aspects of each pharmaceutical (Wang et al., 2022). Negative binomial distributions were used to account for overdispersion of the distribution using the R package MASS (Venables and Ripley, 2022). Since the relative influence of a pharmaceutical's log K_{ow} and HL on the number of detected pharmaceuticals could be tissue specific, an interaction between log K_{ow} and tissue, and HL and tissue, were included in the full model. We compared the full model with models containing every possible combination of terms ($n =$ 31 models) using the corrected Akaike information criterion (AICc) with the R package MuMIn (Bartón, 2022). All models that were < 4 AICc of the model having the lowest AICc were selected as candidates for the top model (Akaike, 1987; Anderson, 2008; Burnham and Anderson, 2004). If multiple models fell within 4 AICc of the model with the lowest AICc parsimony was used to select the top model (Aho et al., 2014). Plots of GLM main effects were generated using the R package ggeffects (Lüdecke et al., 2021). Tests of model assumptions, model performance, and pairwise comparisons of significant model contrasts were assessed as described in section 2.5.1.

2.5.3. Compositional differences in pharmaceutical assemblages

The influence of tissue and region in multivariate space on the concentration and presence/absence assemblages of all 91 pharmaceuticals was examined using PERMANOVAs with 999 permutations on a Bray-Curtis distance matrix with square-root transformed data for concentration and a Jaccard distance matrix for presence/absence. Pairwise PERMANOVA tests followed significant main effects with 999 permutations and a Holm-Bonferroni adjustment. Similarity in the concentration and presence/absence assemblages were visually represented in multidimensional ordination space using non-metric multidimensional scaling (nMDS). PERMANOVAs and nMDS were performed using the R package vegan (Oksanen et al., 2022), and multilevel pairwise comparisons (pairwise PERMANOVA) were performed using the vegan wrapper function pairwiseAdonis (Martinez Arbizu, 2017).

To assess differences in homogeneity of group dispersion (i.e., beta diversity) across tissues and regions for both the concentration and presence/absence assemblages, multivariate homogeneity of group dispersion was calculated on a Bray-Curtis distance matrix for concentration and on a Jaccard distance matrix for presence/absence using the R package vegan (Oksanen et al., 2022). In other words, the average distance of each sample within a group to their respective group centroid (i.e., each tissue or region's within group dispersion) was used to assess uniformity of pharmaceuticals accumulating in each tissue. The average distances to group centroids were then compared between tissues and regions to assess variability in pharmaceutical composition. Pairwise comparisons of mean group dispersion were performed using Tukey's HSD tests with a Holm-Bonferroni adjustment and visualized with box plots.

HCA was used to assess variation in pharmaceutical composition across tissues for both the concentration and presence/absence of detected pharmaceuticals, based on the average distance of each group centroid to the overall centroid of all samples (i.e., global centroid) in multivariate space. When all samples are plotted in multivariate space, a group centroid is the average position of all samples within a group (e.g., each tissue and region), while the global centroid is the average position of all samples regardless of the sample's respective group. HCA was assessed using the average detected concentration for each pharmaceutical for both tissue and region on a Bray-Curtis distance matrix for pharmaceutical concentration and on a Jaccard distance matrix for presence/absence and were visualized with a dendrogram using the R package ggdendro (de Vries and Ripley, 2022).

2.5.4. Influence of pharmaceutical identity on multivariate assemblages

The influence of individual pharmaceuticals on the observed pharmaceutical concentration and presence/absence assemblages, explained by ordination scores, was calculated using 'envfit()' then fitted to each nMDS plot using the R package vegan (Oksanen et al., 2022). This allowed for determination of which of the 91 pharmaceuticals were most important to driving correlations, similarities, and dissimilarities in both assemblages. Ordination score values were squared by their correlation (square root of the r^2), and arrow vectors were used to represent the magnitude and direction of the correlation between the ordination scores and the corresponding pharmaceutical. Arrow vectors point in the direction of the most rapid change in the gradient and arrow length indicates the strength of the gradient. The arrows representing the pharmaceuticals were adjusted to the plot dimensions using a constant multiplier, retaining the r^2 correlations. The significance of the fitted pharmaceutical vectors was assessed with 999 permutations, and pharmaceuticals displayed in the nMDS plots are those that had a p-value ≤ 0.001. Last, the contribution of specific pharmaceuticals in driving dissimilarities in the concentration and presence/absence assemblages across tissues and regions was assessed using similarity percentage analysis (SIMPER) with the R package vegan (Oksanen et al., 2022).

3. Results

3.1. Differential accumulation in pharmaceutical number

Pharmaceuticals were present across all tissues with tissue specific accumulation in pharmaceutical number. The number of pharmaceuticals detected in each sample was significantly different across tissues ($p < 0.001$; Table 2). Tukey pairwise comparisons found differences across all tissue contrasts (Table S3, Fig. 2). Across all samples ($n = 100$), 62 unique pharmaceuticals were detected, for a total of 932 pharmaceutical detections (Table S1). Every sample had at least one pharmaceutical, except for one muscle sample with no detections. Across tissues, liver had the most unique pharmaceuticals detected with 53 different pharmaceuticals, followed by brain (43 pharmaceuticals), plasma (30 pharmaceuticals), and muscle (30 pharmaceuticals). The highest number of pharmaceuticals was detected in liver (395 detections) with an average of 15.8 pharmaceuticals per sample, maximum of 26, and minimum of 9 pharmaceuticals in an individual sample, followed by brain (308 detections, 12.3 pharmaceuticals/sample, maximum of 18 and minimum of 5), plasma (141 detections, 5.6 pharmaceuticals/sample, maximum of 12 and minimum of 1), and muscle (88 detections, 3.5 pharmaceuticals/sample, maximum of 8 and minimum of 0; Table 1).

3.2. Differential accumulation in pharmaceutical concentration

Across all pharmaceutical detections ($n = 932$), the concentrations of detected pharmaceuticals ranged from 0.05 ng/g for diphenhydramine in muscle, to 289.3 ng/g for ketoconazole in liver, with an average concentration across all pharmaceuticals and samples of 8.86 (ng/g for muscle, plasma, and brain and ng/mL for plasma; Table 3, Table S1). Across all samples and pharmaceutical detections, the highest average concentration was in plasma (10.55 ng/mL), followed by liver (9.92 ng/g), brain (7.27 ng/g), and muscle (7.07 ng/g; Table S1). The highest concentration detected in plasma was for paracetamol (270 ng/mL), and the lowest was for risperidone (0.11 ng/mL; Table 3, Table S1). Further, paracetamol constituted the 7 highest plasma concentrations (ranging from 52 ng/mL – 270 ng/mL; Table S1). In liver, the top 5 highest concentrations were for ketoconazole (289.3 ng/g), ciprofloxacin (213.6 ng/g), tetracycline (124.10 ng/g), clonazepam (122 ng/g), and ciprofloxacin (114.8 ng/g), and the lowest concentration was 0.1 ng/g (20 liver detections were at this concentration; Table 3). In brain, ciprofloxacin was at high concentrations compared to the other 42 detected pharmaceuticals (ranging from 26.5 ng/g – 204.1 ng/g, 13 ciprofloxacin detections; Table S1). Further, in brain 4 of the top 5 highest detected concentrations were for ciprofloxacin (the second highest concentration was for trimethoprim at 109.4 ng/g), and 13 of the 23 highest detected concentrations were for ciprofloxacin (Table 3). Demonstrating

Table 2
Summary of the GLMM model for the number of pharmaceuticals per sample by tissue, with tissue as a fixed effect, region as a random effect, and fish ID (FID) nested within region.

Variable	Predictor	*p*	χ^2	R^2 (conditional)	R^2 (marginal)	AICc
Pharmaceutical Number	Tissue	<2.2E-16***	233.2	0.79	0.75	523.3
Random Effects						
Variable	**Groups**	**Variance**	**Standard Deviation**			
Pharmaceutical Number	FID:Region	1.90E-02	0.14			
	Region	4.10E-09	6.40E-05			
Fixed Effects						
Variable	**Groups**	**Estimate**	**Standard Error**	**Z value**	***p***	
Pharmaceutical Number	Intercept	2.5	0.06	39.5	<2.0E-16***	
	Muscle	-1.3	0.12	-10.4	<2.0E-16***	
	Plasma	-0.8	0.1	-7.7	1.43E-14***	
	Liver	0.2	0.08	3.3	0.001***	

p-value < 0.001 ***, p-value < 0.01 **, p-value < 0.05 *

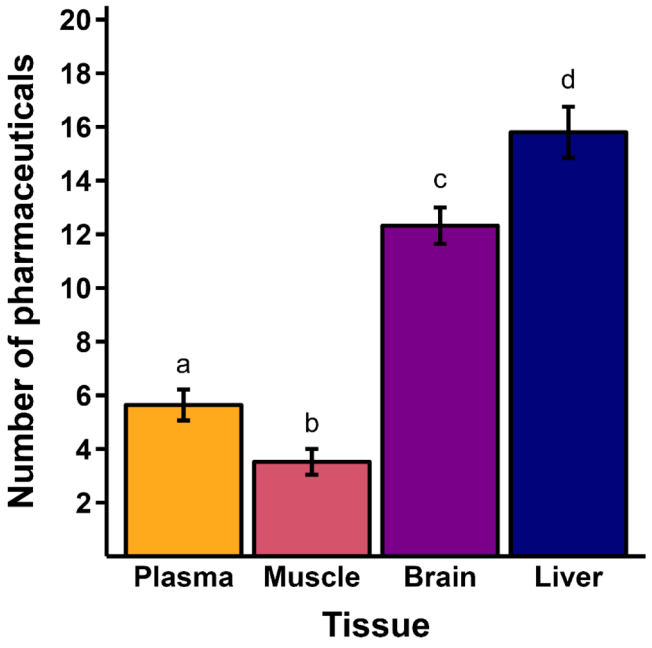

Fig. 2. Mean number of pharmaceuticals detected in each sample by tissue, per GLMM analysis. Letters indicate significant tissue contrasts per Tukey pairwise tests and error bars show standard errors.

similarity between brain and liver, ciprofloxacin was also frequently detected in liver at high concentrations (12 detections, 30.8 ng/g – 213.6 ng/g), with a higher average concentration (80.0 ng/g) in liver compared to brain (63.8 ng/g; Table 3, Table S1). In muscle, the highest concentration was for sulfamethoxazole (129 ng/g), followed by ciprofloxacin (63 ng/g), miconazole (53.5 ng/g), ciprofloxacin (31.6 ng/g), and venlafaxine (27.83 ng/g), and the lowest concentration was for diphenhydramine (0.05 ng/g; Table 3). Similar to brain and liver, ciprofloxacin was among the highest pharmaceutical concentrations detected in muscle, with the second highest average concentration (highest was for sulfamethoxazole at 67.44 ng/g; Table 3).

3.3. Variation in pharmaceutical identity

Differential accumulation of specific pharmaceuticals was also present across tissues. The GLM model that included tissue and log K_{ow} was selected as the top model. Both tissue ($p = 0.001$; Fig. 3a) and a pharmaceutical's log K_{ow} ($p < 0.001$; Fig. 3b) were found to influence the number of detections for each pharmaceutical (Table 4). Tukey pairwise comparisons revealed differences in the number of detections across pharmaceuticals and tissues (Table S4). Muscle was significantly different to both brain and liver (both $p < 0.001$; Table S4, Fig. 3a). The only other significant contrast was between plasma and brain ($p = 0.01$), while the plasma vs. muscle and brain vs. liver contrasts were not significant (Table S4, Fig. 3a). An inverse relationship between the number of detections for each pharmaceutical and the pharmaceutical's log K_{ow} was found, such that the predicted number of detections for each pharmaceutical decreased as a pharmaceutical's log K_{ow} increased (Fig. 3b).

Across all samples independent of tissue, the six most commonly detected pharmaceuticals were as follows: trimethoprim (62 detections), diphenhydramine (53 detections), bisoprolol (46 detections), alfuzosin (45 detections), and atracurium and hydroxyzine (both with 40 detections; Table 5). The most commonly detected pharmaceuticals differed across tissues. Trimethoprim, an antifolate antibiotic used to treat various infections, was the most common pharmaceutical in both brain (23 detections, 92% of samples) and liver (22 detections, 88% of samples; Table 5). Venlafaxine, a selective serotonin and norepinephrine reuptake inhibitor (SNRI), was the most common pharmaceutical in plasma (21 detections, 84% of samples) and the fourth most common pharmaceutical in muscle (9 detections, 36% of samples), while it was not detected in any brain or liver tissue samples (Table 5, Table S1). Although fluconazole, an antifungal used to treat various fungal infections and candidiasis, was the most common pharmaceutical in muscle (9 detections, 36% of samples), it was more frequently detected in liver samples (11 detections, 44% of samples), and also detected in plasma (6 detections, 24% of samples) and brain (4 detections, 16% of samples; Table 5, Table S1).

3.4. Variability in pharmaceutical composition – concentration

In multivariate ordination space, tissue ($p = 0.001$) and region of collection ($p = 0.02$) were significant drivers of the pharmaceutical concentration assemblage, while fish ID had no effect (Table 6, Fig. 4a). All tissue assemblages were distinct (all *adj.* $p = 0.006$), but all region pairwise comparisons were not significant (Table S5), indicating that tissue is a stronger driver in the concentration of detected pharmaceuticals. Further, this demonstrates that pharmaceuticals differentially accumulate in concentration across tissues.

Nine pharmaceuticals influenced the pharmaceutical concentration assemblage ($p \leq 0.001$; Fig. 4a). These included, in order of influence based on r^2: venlafaxine, atenolol, ciprofloxacin, cilazapril, miconazole, fluconazole, ketoconazole, mianserin, and clindamycin (Fig. 4a). Venlafaxine, ketoconazole, cilazapril, and ciprofloxacin were the most influential pharmaceuticals in driving dissimilarities of pharmaceutical concentrations across tissues based on the SIMPER analysis (Table S5).

Table 3
Summary of the top 5 highest detected average concentrations in plasma (ng/mL), muscle (ng/g), brain (ng/g), and liver (ng/g) with total detections, percent of samples, mean concentration, median concentration, and concentration range for each pharmaceutical.

Pharmaceutical	Tissue	Detections (%)	Mean Concentration	Median Concentration	Concentration Range
Paracetamol	Plasma	7 (28%)	134.14286	110.00	52 - 270
Atenolol	Plasma	15 (60%)	18.413333	9.30	6 - 49
Ranitidine	Plasma	4 (16%)	13.125	15.00	5.5 - 17
Hydroxyzine	Plasma	4 (16%)	8.5225	1.68	0.73 - 30
Metoprolol	Plasma	2 (8%)	5.755	5.76	5.32 - 6.19
Sulfamethoxazole	Muscle	2 (8%)	67.44	67.44	5.88 - 129
Ciprofloxacin	Muscle	4 (16%)	31.48	31.48	11 - 63
Clotrimazole	Muscle	2 (8%)	13.25	13.25	4.6 - 21.9
Atenolol	Muscle	3 (12%)	8.87	8.87	5 - 14.6
Naloxone	Muscle	4 (16%)	8.52	8.52	1.01 - 22.36
Ciprofloxacin	Brain	13 (52%)	63.79	48.30	26.5 - 204.1
Atenolol	Brain	9 (36%)	27.84	25.90	13.7 - 59
Ranitidine	Brain	3 (12%)	20.23	15.00	5.1 - 40.6
Ketoconazole	Brain	5 (20%)	14.82	15.20	10.7 - 19.2
Metoprolol	Brain	15 (60%)	13.52	8.90	5.1 - 66.2
Tetracycline	Liver	3 (12%)	94.97	92.60	68.2 - 124.1
Ciprofloxacin	Liver	12 (48%)	79.99	68.20	30.8 - 213.6
Ketoconazole	Liver	14 (56%)	52.56	25.85	10.5 - 289.3
Atenolol	Liver	9 (36%)	31.68	28.80	7.7 - 63.1
Clonazepam	Liver	7 (28%)	25.06	9.10	6 - 122

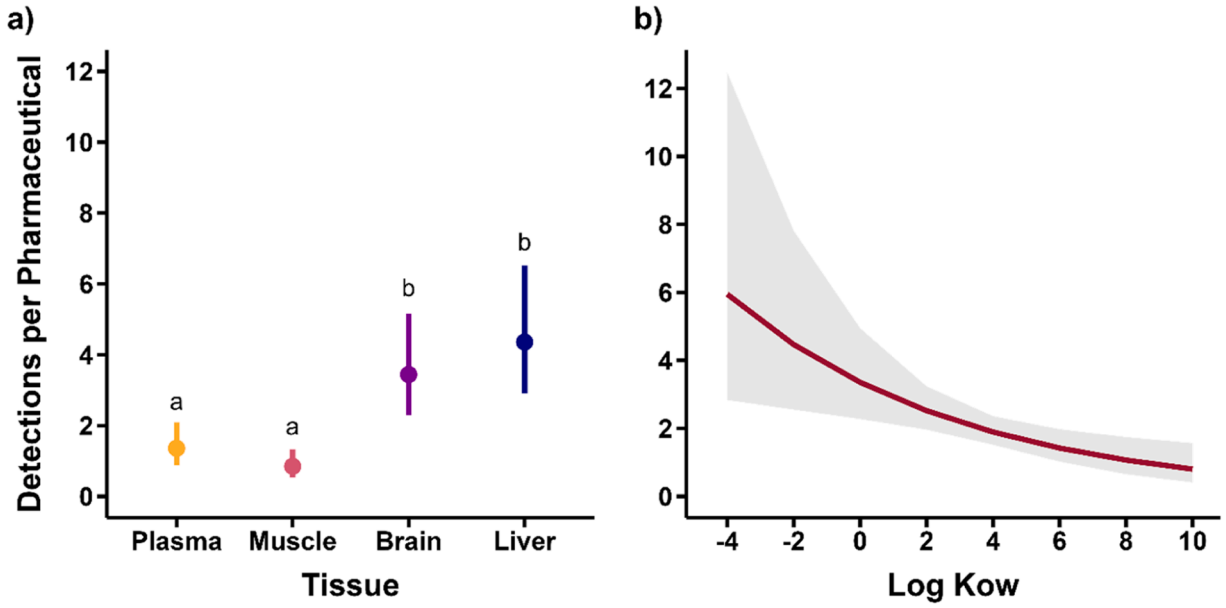

Fig. 3. Summary of GLM results showing a) the number of detections of each pharmaceutical by tissue. Dots denote the predicted mean number of detections per pharmaceutical for each tissue and bars show confidence intervals. And b) the predicted number of detections per pharmaceutical based on the pharmaceutical's log K_{ow}.

Table 4
Summary of the final GLM model for the number of detections per pharmaceutical by tissue and Log K_{ow}.

Variable	Predictor	*p*	χ^2	Null Deviance	Residual Deviance	AICc	D^2
Detections per Pharmaceutical	Tissue	1.1E-07***	35.2	346.3	305.6	1310.9	0.11
	Log K_{ow}	0.001***	10.7				

p-value < 0.001 ***, *p*-value < 0.01 **, *p*-value < 0.05 *

Venlafaxine was detected at higher concentrations in both brain and plasma than in muscle, and ketoconazole was detected at higher concentrations in liver than in plasma and brain. Cilazapril was detected at higher concentrations in liver than in muscle, and ciprofloxacin was detected at higher concentrations in the brain than in plasma (Table S5, Fig. 4a).

Results indicated variability in pharmaceutical concentration across samples as a function of tissue. Significant differences of within tissue variability of detected concentrations (i.e., beta diversity), or the mean distance of all samples in a tissue group to the respective tissue's group centroid in multivariate space, were found ($p = 0.001$; Table 7, Fig. 5a). Permutational tests of multivariate dispersion indicated that region was not a significant driver of dispersion (Table 7). Tukey's pairwise comparisons of dispersion revealed significant differences between muscle and all other tissues, driven by the greater average distance to group centroid in muscle (Table S6, Fig. 5a). This was likely driven by muscle

Table 5
Summary of the top 6 most commonly detected pharmaceuticals in plasma (ng/mL), muscle (ng/g), brain (ng/g), and liver (ng/g) with total detections, percent of samples, and the mean, median, and range of detected concentrations.

Pharmaceutical	Tissue	Detections (%)	Mean Concentration	Median Concentration	Concentration Range
Venlafaxine	Plasma	21 (84%)	3.30	2.09	0.76 - 10
Atenolol	Plasma	15 (60%)	18.41	9.30	6 - 49
Alfuzosin	Plasma	12 (48%)	0.33	0.36	0.12 - 0.66
Trimethoprim	Plasma	9 (36%)	1.15	0.22	0.13 - 5
Bisoprolol	Plasma	8 (32%)	0.46	0.26	0.17 - 1.2
Naloxone	Plasma	8 (32%)	2.89	2.70	1.3 - 5.08
Fluconazole	Muscle	9 (36%)	7.79	7.63	0.77 - 15.17
Diphenhydramine	Muscle	8 (32%)	0.97	0.30	0.051 - 5.82
Trimethoprim	Muscle	8 (32%)	0.59	0.20	0.1 - 2
Venlafaxine	Muscle	8 (32%)	7.95	1.98	0.92 - 27.83
Bisoprolol	Muscle	5 (20%)	0.54	0.20	0.1 - 1.73
Memantine	Muscle	5 (20%)	1.46	1.20	0.89 - 2.5
Trimethoprim	Brain	23 (92%)	5.13	0.30	0.1 - 109.4
Diphenhydramine	Brain	20 (80%)	0.52	0.30	0.1 - 3.5
Atracurium	Brain	19 (76%)	1.89	1.10	0.5 - 7.6
Hydroxyzine	Brain	19 (76%)	4.68	3.10	1.3 - 29.1
Bisoprolol	Brain	15 (60%)	0.70	0.70	0.1 - 1.8
Clotrimazole	Brain	15 (60%)	10.74	4.40	1.2 - 53.8
Metoprolol	Brain	15 (60%)	13.52	8.90	5.1 - 66.2
Trimethoprim	Liver	22 (88%)	0.51	0.30	0.1 - 3.7
Orphenadrine	Liver	20 (80%)	1.51	0.45	0.1 - 9
Diphenhydramine	Liver	19 (76%)	0.66	0.30	0.1 - 3.7
Bisoprolol	Liver	18 (72%)	0.96	0.90	0.1 - 2.8
Clindamycin	Liver	16 (64%)	3.99	2.90	1 - 12.1
Hydroxyzine	Liver	16 (64%)	4.37	3.00	0.7 - 9.8

Table 6
Summary of the PERMANOVA main effects for the pharmaceutical concentration and presence/absence assemblages by tissue, region, and fish ID (FID).

Model	Terms	df	Sum of sq	R^2	F Model	*p*
Concentration	Tissue	3	7.8	0.22	9.06	0.001***
	Region	3	1.3	0.04	1.57	0.021*
	FID	21	6.7	0.18	1.11	0.123
	Residual	71	20.4	0.56		
	Total	98	36.2	1.00		
Presence/ Absence	Tissue	3	6.2	0.15	5.80	0.001***
	Region	3	1.5	0.04	1.36	0.016*
	FID	21	8.0	0.19	1.07	0.173
	Residual	71	25.2	0.62		
	Total	98	40.8	1.00		

p-value < 0.001 ***, *p*-value < 0.01 **, *p*-value < 0.05 *

having the fewest detections (88 total), and high median concentration (1.4 ng/g) compared to other tissues with more detections (Table 1, Table S1). In other words, detected concentrations in muscle were more variable and unevenly distributed across samples, thus the variability in multivariate dispersion (i.e., average distance to centroid) was greatest in muscle.

Compositional differences in pharmaceutical concentration assemblage were further assessed by determining the distance of tissue and region group centroids to the global centroid with HCA and was visualized using a dendrogram. For tissue, results indicated that the greatest similarity was between brain and liver, brain and liver were more similar to plasma than to muscle, plasma assemblages were distinct to each other and to brain and liver, and muscle was the most distinct tissue (Fig. S1). The observed separation across region group centroids showed the greatest similarity between Key West and Lower Keys, while Key West and Lower Keys were more similar to Upper Keys than Biscayne Bay, and Biscayne Bay was the most distinct region (Fig. S2).

3.5. Variability in pharmaceutical composition – presence/absence

In multivariate ordination space, tissue ($p = 0.001$) and region of collection ($p = 0.02$) were significant drivers of pharmaceutical presence/absence assemblage across samples, while no effect was found for fish ID (Table 6, Fig. 4b). Significant differences were found for every tissue contrast (all *adj.* $p = 0.006$), while regional contrasts were not significant (Table S5). This indicates that tissue is a stronger driver than region of collection in the presence/absence of pharmaceuticals across samples.

Thirteen pharmaceuticals influenced the pharmaceutical presence/absence assemblage ($p \leq 0.001$; Fig. 4b). These included, in order of influence based on r^2: fluconazole, venlafaxine, ciprofloxacin, ketoconazole, orphenadrine, cilazapril, clomipramine, clindamycin, miconazole, fexofenadine, hydroxyzine, metoprolol, and naloxone (Fig. 4b). Ketoconazole, orphenadrine, ciprofloxacin, metoprolol, and venlafaxine were the most influential pharmaceuticals in driving dissimilarities across tissues based on the SIMPER analysis (Table S5). Ketoconazole was the most important pharmaceutical driving tissue dissimilarity, contributing to two significant contrasts, primarily due to its higher detections in the liver, driving liver dissimilarity to brain and plasma. Orphenadrine, ciprofloxacin, metoprolol, and venlafaxine each contributed to one significant contrast. Orphenadrine was more common in liver compared to muscle. Ciprofloxacin and metoprolol were more common in brain, driving dissimilarity to muscle and plasma, respectively. Venlafaxine, the most common pharmaceutical in plasma, was the strongest driver in its dissimilarity to muscle. Further, venlafaxine was absent in brain and liver, resulting in separation between the two tissues and plasma (Table S5, Fig. 4b).

Results indicated variability in pharmaceutical presence/absence across samples as a function of tissue. Permutational analyses of multivariate dispersion (i.e., beta diversity), revealed significant differences ($p = 0.001$) in pharmaceutical presence/absence across tissues, while there was no effect for region of collection (Fig. 5b, Table 7). Tukey's pairwise comparisons of dispersion revealed significant differences in 3 tissue contrasts, driven by the greater distance to group centroid in muscle, and between muscle and all other tissues (Table S6, Fig. 5b). As such, a greater diversity of pharmaceuticals accumulated in muscle compared to all other tissues, indicating a greater degree of uniformity in the presence/absence of pharmaceuticals in plasma, brain, and liver.

Compositional differences in pharmaceutical presence/absence were further assessed with HCA and visualized using a dendrogram. The observed separation across tissue group centroids in the presence/

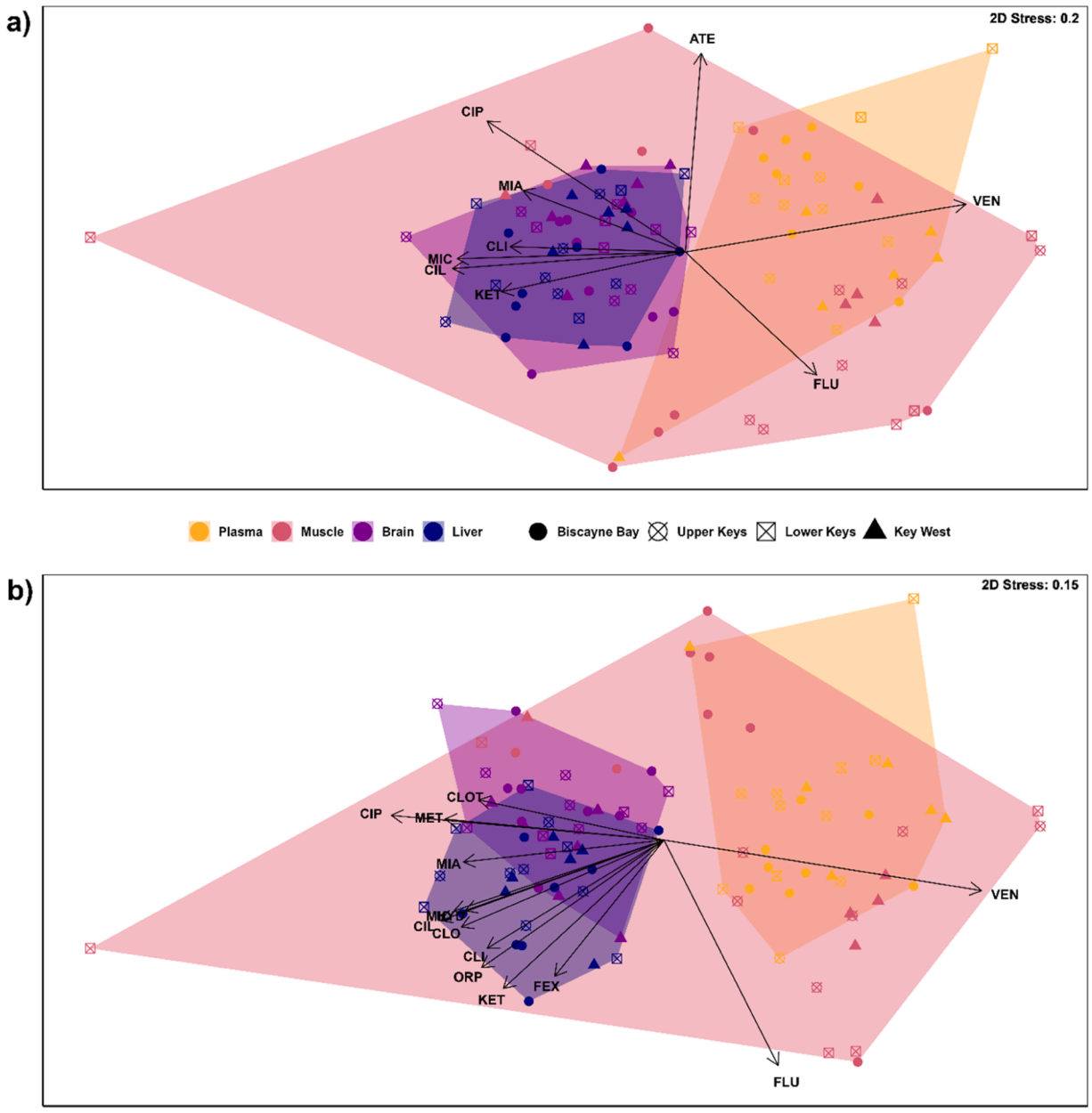

Fig. 4. nMDS plots showing the pharmaceutical assemblages in multidimensional ordination space color coded by tissue for a) the concentration of detected pharmaceuticals, and b) pharmaceutical presence/absence. Polygons denote the assemblage boundaries of each tissue. Shapes denote region of collection. Vector arrows show the relative direction and magnitude of pharmaceutical influence ($p \geq 0.001$). Abbreviations are as follows; ATE = atenolol, VEN = venlafaxine, FLU = fluconazole, FEX = fexofenadine, KET = ketoconazole, ORP = orphenadrine, CLI = clindamycin, CLO = clomipramine, CIL = cilazapril, MIC = miconazole, MIA = mianserin, MET = metoprolol, CIP = ciprofloxacin, CLOT = clotrimazole.

Table 7
Summary of beta diversity permutational tests of multivariate dispersions for the concentration and presence/absence assemblages by tissue and region.

Model	Groups	df	Sum of sq	Mean of Sq	F Model	*p*
Concentration	Tissue	3	0.373	0.124	15.1	0.001***
	Residual	95	0.784	0.008		
	Region	3	0.012	0.004	0.7	0.564
	Residual	95	0.519	0.005		
Presence/ Absence	Tissue	3	0.140	0.047	11.1	0.001***
	Residual	95	0.340	0.004		
	Region	3	0.006	0.002	0.8	0.499
	Residual	95	0.227	0.002		

p-value < 0.001 ***, *p*-value < 0.01 **, *p*-value < 0.05 *

absence assemblage was similar to the separation observed in the pharmaceutical concentration assemblage (Fig. S1, Fig. S3), such that the greatest similarity was between brain and liver, brain and liver were more similar to plasma than to muscle, and muscle was the most distinct tissue. However, a greater degree of similarity between brain and liver was observed in the concentration assemblage compared to the presence/absence assemblage (Fig. S1, Fig. S3). In other words, brain and liver were more similar in detected concentrations than in they were in presence/absence of pharmaceuticals. Last, the observed separation across region group centroids was similar to the separation observed in the pharmaceutical concentration assemblage (Fig. S2, Fig. S4), such that the greatest similarity was between Key West and Lower Keys, Key West and Lower Keys were more similar to Upper Keys than Biscayne Bay, and Biscayne Bay was the most distinct region (Fig. S4).

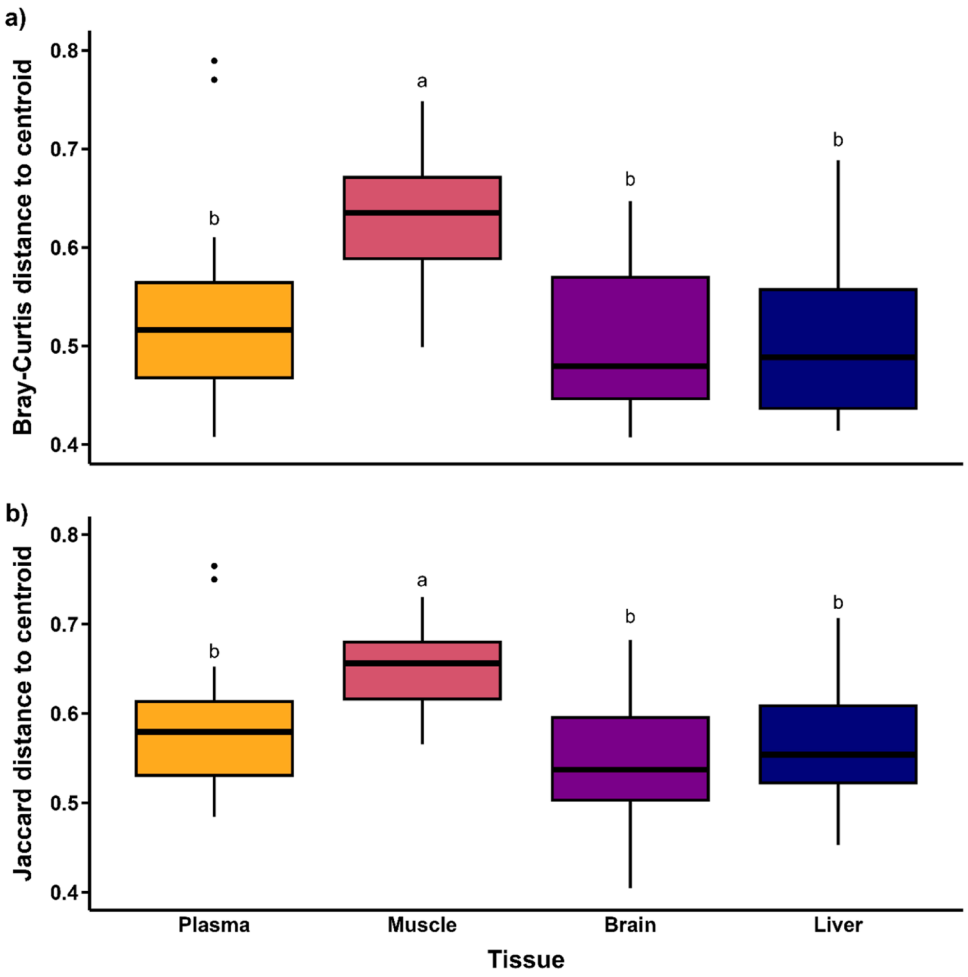

Fig. 5. Summary of beta diversity permutational analysis of multivariate dispersions showing the mean distance of each sample to their respective tissue group's centroid for a) pharmaceutical concentration, and b) pharmaceutical presence/absence. Letters indicate significant differences per Tukey pairwise tests. For the box and whisker plot, the shaded area represents the interquartile range, the solid horizontal black line represents the median value, the whiskers are 1.5 ± the interquartile range, and the black dots are any points that fall outside 1.5 ± the interquartile range.

4. Discussion

Our examination of pharmaceutical accumulation and distribution revealed tissue specific differences in number of pharmaceuticals, detections of specific pharmaceuticals, and in pharmaceutical composition across tissues. Differential accumulation in pharmaceutical number and concentration was present across all tissues, and pharmaceuticals accumulated the most in liver > brain > plasma > muscle, while the highest concentrations were found in plasma > liver > brain > muscle. Differences in the number of pharmaceuticals and the concentrations to which they accumulate underscores the need to consider the goals of pharmaceutical surveys when selecting target tissues. The identity of the most frequently detected pharmaceuticals varied across tissues and an inverse relationship was present between a pharmaceutical's log K_{ow} and the predicted number of pharmaceuticals in each sample. Muscle was most dissimilar to brain and liver in the identity of accumulated pharmaceuticals. Since the observed correlation between detections of each pharmaceutical and their respective log K_{ow} was contrary to what would be predicted, results emphasize that even when assessing differential accumulation in terms of pharmaceutical specific accumulation, homogeneity of variance, and pharmaceutical composition, other physio-chemical properties have an influence in bioconcentration/bioaccumulation potential. It is likely that differences in lipid content between tissues are not consistent enough to result in a consistent or uniform influence of log K_{ow} in accumulation. Region of sample collection influenced the concentration and presence/absence assemblages across samples but was not an influential driver of within group composition (i.e., beta diversity) for both assemblages, indicating that accumulation of pharmaceuticals in each sample was uniform across regions, but varied across tissues. With the same bonefish included in this study, Castillo et al., (2024b) assessed the influence of environmental compartments (water, sediment, and bonefish prey) on pharmaceutical accumulation in bonefish plasma in Biscayne Bay, Upper Keys, and Lower Keys, concluding that environmental pharmaceutical burdens across regions did not influence pharmaceutical accumulation in bonefish plasma. Tissues varied to each other, and variability was also present within tissue groups (i.e., dispersion of tissue samples from their respective group centroids), demonstrating differences in uniformity of accumulated pharmaceuticals across tissues. Muscle was found to be the most variable in composition of both pharmaceutical concentration and presence/absence. Within group variability was also more pronounced in concentration than in the presence/absence of pharmaceuticals, indicating more uniformity in the identity of pharmaceuticals that accumulate in each tissue and greater variability in the concentration to which they accumulate.

4.1. Differential accumulation in pharmaceutical number and concentration

The number of pharmaceuticals detected in each sample varied

between tissues. Liver had the most, with 53 unique pharmaceuticals and 15.8 pharmaceuticals/sample, followed by brain with 43 pharmaceuticals (12.3 pharmaceuticals/sample), plasma (30 pharmaceuticals, 5.6 pharmaceuticals/sample), and muscle (30 pharmaceuticals, 3.5 pharmaceuticals/sample). Both laboratory studies (Huerta et al., 2016; McCallum et al., 2017), and field studies (Liu et al., 2015), have demonstrated higher concentrations and number of pharmaceuticals in liver and brain compared to muscle. Few studies have included all four tissues considered in this study (e.g., Heynen et al., 2016; McCallum et al., 2017), frequently testing liver, brain, and muscle, and less frequently including plasma in analyses (Gómez-Regalado et al., 2023). Studies often examine uptake of only a few pharmaceuticals in controlled laboratory settings (Heynen et al., 2016), and when examining uptake of multiple pharmaceuticals, usually focus on concentrations in the context of bioconcentration factors and omit interpretation of differential accumulation pertaining to the number of pharmaceuticals (Grabicova et al., 2014; Liu et al., 2018). In a laboratory setting accumulation and behavioral study, McCallum et al., (2017) examined uptake of a similar suite of pharmaceuticals (93 pharmaceuticals total) in perch exposed to wastewater, with results partially in line with ours, but with important differences. Far fewer pharmaceuticals were detected in the laboratory exposure study (11 of 93 pharmaceuticals) compared to this study detecting 62 of 91 pharmaceuticals. Further, McCallum et al., (2017) found the most pharmaceuticals in plasma > gonads > brain > liver > muscle, while in our study the most pharmaceuticals were found in liver > brain > plasma > muscle. This suggests that *in situ* exposure leads to differential accumulation, and accumulation of more pharmaceuticals, when compared to laboratory studies (Duarte et al., 2023), since prolonged exposure allows for achievement and maintenance of steady state concentrations of many pharmaceuticals considering pharmaceutical specific differences in metabolism and excretion (Gómez-Regalado et al., 2023; Mackay et al., 2018). Consequently, laboratory studies could misrepresent risk estimates of exposure in wild fish (Gómez-Regalado et al., 2023).

Concentrations of accumulated pharmaceuticals also proved to be tissue specific. The highest average concentration was detected in plasma > liver > brain > muscle, which is different to the number of pharmaceuticals across tissues (liver > brain > plasma > muscle). In laboratory settings, previous literature has found higher concentrations in plasma compared to liver, brain, and muscle, concluding that plasma could be an indicator of the highest bodily pharmaceutical concentrations (Heynen et al., 2016; McCallum et al., 2017). Our results support this observation; however, they suggest that liver or brain could be a better indicator of overall chronic exposure in pharmaceutical number, and plasma for concentration. Further, Garcia et al., (2012) examined differential tissue accumulation of carbamazepine across plasma, muscle, brain and liver tissues in both a field setting and laboratory exposure experiment, finding results similar to ours. At one sampling point in the field, the highest concentrations were found in plasma > liver > muscle (brain was not sampled in the field). Notably, during the 14-day flow-through exposure component, the highest concentrations were found in plasma from day 1 – day 3, after which brain, liver, and muscle were higher for the remainder of the study. This supports the notion that in the field, plasma could be indicative of recent or acute exposure (highest pharmaceutical concentrations), and liver could be indicative of more long-term or chronic exposure (Burkina et al., 2015).

4.2. Differential accumulation in pharmaceutical identity

The identity of the most frequently detected pharmaceuticals was different across tissues, demonstrating differential accumulation of specific pharmaceuticals. We accounted for pharmaceuticals' physiochemical, ADME, and pharmacodynamic characteristics (log K_{ow} and HL), in addition to tissue, in the accumulation model of specific pharmaceuticals. Although log K_{ow} had a significant influence on tissue uptake of specific pharmaceuticals, the observed negative trend is contrary to what would be expected based on the theoretical influence of log K_{ow} on accumulation of compounds (Armitage et al., 2017; Gómez-Regalado et al., 2023), but has been observed in studies examining pharmaceuticals (Duarte et al., 2022).

As predominantly ionizable compounds (Armitage et al., 2017), and therefore frequently present in a charged polar form, log K_{ow} is often not sufficient in predicting accumulation of pharmaceuticals (Carter et al., 2022; Duarte et al., 2022; Hermens et al., 2013). Thus, based on ionization estimates, the majority of pharmaceuticals would be expected to have low rates of bioconcentration/bioaccumulation. However, data has demonstrated that some highly ionized compounds (some up to >90% ionized) can accumulate in aquatic organisms (Burkhard, 2021). Further, some pharmaceuticals with no predicted bioconcentration/bioaccumulation potential (e.g., log K_{ow} < 3; Organization for Economic Co-operation Development Guidline, 2005) have been found to extensively accumulate in fish (Duarte et al., 2022). Importantly, log K_{ow} does not properly account for the influence of abiotic factors present in field settings (e.g., pH, temperature, dissolved organic matter), and compounds with low log K_{ow} can be highly influenced by these parameters (Arnot and Gobas, 2006; Gómez-Regalado et al., 2023). Clearly, a more nuanced set of factors dictate the extent of pharmaceutical accumulation of distribution of pharmaceuticals in exposed biota. For example, Lu et al., (2018) examined the effects of dissolved organic matter (DOM) and water flow (i.e., hydrodynamics) on the bioconcentration of diclofenac in crucian carp (*Carassius auratus*), finding an inverse relationship between bioconcentration and increasing DOM and water flows. Accordingly, additional field studies investigating the accuracy of log K_{ow} as a predictor of bioconcentration/bioaccumulation potential across multiple tissues in fish is necessary, and consideration of alternative predictors for pharmaceutical accumulation in fish and aquatic biota.

Specific pharmaceuticals were found to differentially accumulate in each tissue. SIMPER analysis revealed that more pharmaceuticals preferentially accumulated in brain and liver, and others in plasma, driving dissimilarity between all tissues. In muscle, pharmaceuticals accumulated with less specificity. As a result, when specific pharmaceuticals were found to preferentially accumulate in a certain tissue, it was most frequently in brain and liver followed by plasma, while fluconazole was the only pharmaceutical influencing dissimilarities between tissues by preferentially accumulating in muscle. This could be an artifact of the significantly higher number of pharmaceuticals accumulating in liver, brain, and plasma. Antifungal agents have be demonstrated to elicit inhibitory effects in pharmaceutical metabolism in humans (Venkatakrishnan et al., 2000), as such the potential influence of interactive effects in pharmaceutical metabolism and any resulting effects in pharmaceutical accumulation across different tissues merits further investigation.

4.3. Differential accumulation in pharmaceutical composition

Composition varied in distribution across tissues, within each tissue, and between tissues for both pharmaceutical concentration and presence/absence. Within tissue indices of differential accumulation (i.e., beta diversity) revealed that for both pharmaceutical concentration and presence/absence, plasma, brain, and liver were similarly uniform in pharmaceutical composition, while the greatest variability of detected pharmaceuticals was present in muscle. Thus, a similarly high degree of specificity in pharmaceutical accumulation was present in brain and liver while a greater diversity of pharmaceuticals accumulated in muscle. The multivariate approach used in this study for assessment of composition across tissues appears to be unique in the literature, as such we do not have comparable studies available for comparison. Nevertheless, inferences based on the pharmacodynamic processes of ADME can be made to evaluate potential drivers of the observed variability in composition across tissues (Armitage et al., 2017; Carter et al., 2022; Gómez-Regalado et al., 2023; Matthee et al., 2023).

In general, pharmaceutical metabolism involves transformation processes aimed at reducing lipophilicity and increase hydrophilicity to facilitate elimination via urine and/or bile, which are catalyzed by phase I and phase II enzymes (Baron et al., 2017; Burkina et al., 2015; Schlenk et al., 2008). The differential distribution of these enzymes across various internal tissues greatly influences the extent to which pharmaceuticals accumulate throughout the body (Matthee et al., 2023; Rizk et al., 2017). The most significant enzymes involved in pharmaceutical metabolism in humans are cytochrome P450 (CYPs) enzymes (Matthee et al., 2023), which have been identified in fish, but the extent they are involved is not fully understood (Li et al., 2023; Schlenk et al., 2008). Regardless, CYPs are extensively present in liver tissue (Gomez et al., 2010), which could explain liver having the highest number of pharmaceuticals. In order to be present in brain, pharmaceuticals must have the capability of crossing the blood-brain barrier (Pardridge, 2012), which requires pharmaceuticals to be molecularly small and lipophilic for accumulation (Lin et al., 2022; Matthee et al., 2023). Naturally, this means that only a distinctive subset of pharmaceuticals is capable of accumulation in brain tissue, the characteristics of which are similar to those that influence accumulation in the liver, which could explain the observed similarly high incidences of accumulation in brain and liver. In muscle, the factors that influence accumulation are primarily those dealing with lipid content (Liu et al., 2015; Zhao et al., 2015), which is variable across different muscle tissues (Escher et al., 2011). For example, (Zhang et al., 2010) sampled two different muscle tissues, finding differential accumulation likely due to differences in lipid content. Even though muscle samples were collected from the same location in every fish, variability in lipid composition across samples could have resulted in the greater diversity of accumulated pharmaceuticals. It is also important to note that differences in blood perfusion across tissues was not experimentally nor mathematically accounted for in our analyses, and although the analytical approach used accounts for multiple variables influencing differential accumulation across tissues, variability in blood perfusion could have influenced the results. Collectively, our assessment of pharmaceutical assemblages across tissues revealed consistent patterns in tissue specific composition and underscores the influence of tissue selection in variability of detected pharmaceuticals.

5. Conclusion

This study documents differential uptake of pharmaceuticals across multiple tissues with tissue specific accumulation in pharmaceutical number, concentration, identity, and composition in a wild subtropical marine mesoconsumer fish. Pharmaceuticals were detected in all but one sample (muscle tissue), with number highest in liver > brain > plasma > muscle, while concentrations were highest in plasma > liver > brain > muscle. Composition of accumulated pharmaceuticals was different between tissues, with variability highest in muscle, moderately variable in plasma, and most uniform in brain and liver. This demonstrates a higher degree of specificity in pharmaceutical accumulation in brain and liver, followed by plasma and muscle. Our results highlight the utility of plasma and liver in providing comprehensive estimates of exposure for wild fish populations, both in pharmaceutical number and concentration. This underscores the importance of tissue selection when examining pharmaceuticals in aquatic systems. Further, the higher number in liver and higher concentration in plasma suggests that liver could be an indicator of chronic exposure while plasma more indicative of acute and recent exposure, particularly when considering differences in metabolism and distribution rates between pharmaceuticals. As such, field studies could implore a combination of plasma and liver sampling to capture both the pharmaceuticals accumulating to the highest number and highest concentration, thus diversifying available indices of exposure and the ability to accurately quantify risk when surveying for a large suite of pharmaceuticals. Using the same bonefish analyzed in this study, Castillo et al., 2024a and Castillo et al., (*in revision*) related detected pharmaceutical concentrations to each pharmaceutical's respective human therapeutic plasma concentration (H_TPC) and evaluated the potential for pharmacological effects from pharmaceutical exposure in wild fish. The researchers found that 39% of bonefish sampled across the Caribbean Basin had at least one pharmaceutical at a concentration exceeding 1/3 of the H_TPC, with 23 of 49 detected pharmaceutical exceeding the 1/3 H_TPC threshold in at least one fish, and a maximum of 11 pharmaceuticals exceeding the 1/3 H_TPC threshold in a single bonefish (Castillo et al., 2024a). As such, future research should consider using the metric proposed by Castillo et al., (2024a) to add an assessment of risk of pharmacological effect when evaluating the extent of pharmaceutical exposure in wild fish. Last, to better understand differential uptake of pharmaceuticals in wild fish species, future research needs to prioritize sampling of multiple tissues for more comprehensive assessments of pharmaceutical exposure in both number and concentration, allowing for a more accurate evaluation of the potential for physiological and behavioral alterations in exposed biota.

CRediT authorship contribution statement

N.A. Castillo: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **R.O. Santos:** Writing – review & editing, Validation, Supervision, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **W.R. James:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **R. Rezek:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **D. Cerveny:** Writing – review & editing, Validation, Formal analysis. **R.E. Boucek:** Writing – review & editing, Validation. **A.J. Adams:** Writing – review & editing, Conceptualization. **J. Fick:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **T. Brodin:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **J.S. Rehage:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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