Chemosphere 349 (2024) 140949

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Understanding pharmaceutical exposure and the potential for effects in marine biota: A survey of bonefish (*Albula vulpes*) across the Caribbean Basin

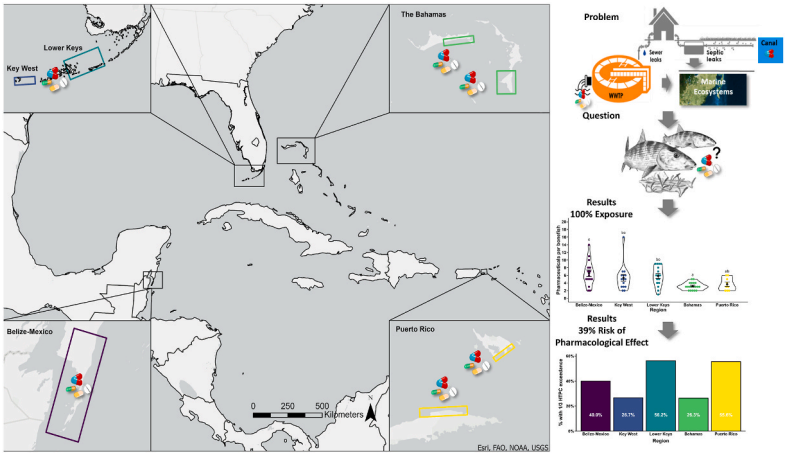
N.A. Castillo [a,*], W.R. James [a,b], R.O. Santos [b], R. Rezek [c], D. Cerveny [d,e], R.E. Boucek [f], A.J. Adams [f,g], T. Goldberg [h], L. Campbell [h], A.U. Perez [f], J.J. Schmitter-Soto [i], J.P. Lewis [f], J. Fick [j], T. Brodin [d], J.S. Rehage [a]

[a] *Earth and Environment Department, Institute of Environment, Florida International University, Miami, FL, USA*
[b] *Department of Biology, Institute of Environment, 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 Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA*
[i] *Departmento de Sistemática y Ecología Acuática, El Colegio de la Frontera Sur, Chetumal, Mexico*
[j] *Department of Chemistry, Umeå University, Umeå, Sweden*

HIGHLIGHTS

- Pharmaceuticals were detected in all fish from Caribbean coastal ecosystems.
- 49 of 102 pharmaceuticals detected and 23 exceeded the 1/3 H_TPC.
- 39% of fish had pharmaceuticals at concentrations capable of pharmacological effects.
- Number of detections and pharmaceutical concentrations varied across regions.
- Exposure to pharmacologically active concentrations was unrelated to detections.

GRAPHICAL ABSTRACT



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

https://doi.org/10.1016/j.chemosphere.2023.140949
Received 22 September 2023; Received in revised form 8 December 2023; Accepted 11 December 2023
Available online 12 December 2023

ARTICLE INFO

Handling Editor: Magali Houde

Keywords:
Pharmaceutical
Aquatic contamination
Therapeutic concentrations
Marine environments
Coastal environments
Bonefish

ABSTRACT

Most research on pharmaceutical presence in the environment to date has focused on smaller scale assessments of freshwater and riverine systems, relying mainly on assays of water samples, while studies in marine ecosystems and of exposed biota are sparse. This study investigated the pharmaceutical burden in bonefish (*Albula vulpes*), an important recreational and artisanal fishery, to quantify pharmaceutical exposure throughout the Caribbean Basin. We sampled 74 bonefish from five regions, and analyzed them for 102 pharmaceuticals. We assessed the influence of sampling region on the number of pharmaceuticals, pharmaceutical assemblage, and risk of pharmacological effects. To evaluate the risk of pharmacological effects at the scale of the individual, we proposed a metric based on the human therapeutic plasma concentration (H_TPC), comparing measured concentrations to a threshold of 1/3 the H_TPC for each pharmaceutical. Every bonefish had at least one pharmaceutical, with an average of 4.9 and a maximum of 16 pharmaceuticals in one individual. At least one pharmaceutical was detected in exceedance of the 1/3 H_TPC threshold in 39% of bonefish, with an average of 0.6 and a maximum of 11 pharmaceuticals exceeding in a Key West individual. The number of pharmaceuticals (49 detected in total) differed across regions, but the risk of pharmacological effects did not (23 pharmaceuticals exceeded the 1/3 H_TPC threshold). The most common pharmaceuticals were venlafaxine (43 bonefish), atenolol (36), naloxone (27), codeine (27), and trimethoprim (24). Findings suggest that pharmaceutical detections and concentration may be independent, emphasizing the need to monitor risk to biota regardless of exposure diversity, and to focus on risk quantified at the individual level. This study supports the widespread presence of pharmaceuticals in marine systems and shows the utility of applying the H_TPC to assess the potential for pharmacological effects, and thus quantify impact of exposure at large spatial scales.

1. Introduction

Pharmaceutical contaminants have become a concern worldwide as production and consumption increases at an exponential rate, far outpacing the ability to conduct risk assessments and monitoring (Persson et al., 2022). Globally in 2020, 4.5 trillion 30-day prescriptions were dispensed (IQVIA Institute, 2022). This represents a 24% increase relative to 2015 (IQVIA Institute, 2021), and a 2–6% annual growth rate is expected by 2026 (IQVIA Institute, 2022). There is now substantial evidence for the presence of pharmaceuticals in the terrestrial and aquatic environments, and the potential for deleterious effects in humans and exposed fauna (Branchet et al., 2021; Chaturvedi et al., 2021; Mezzelani and Regoli, 2021; Świacka et al., 2022), yet the extent of their presence and ecological effects is not fully understood. Although generally present at low concentrations without an immediate risk (Fabbri and Franzellitti, 2016), pharmaceuticals bioaccumulate in fish and marine biota at sublethal concentrations capable of eliciting behavioral and physiological alterations with ecosystem-wide implications (Bertram et al., 2022; Kidd et al., 2023; Lagesson et al., 2016; Prichard and Granek, 2016; Saaristo et al., 2018).

Pharmaceuticals reach the aquatic environment mainly via industrial (pharmaceutical manufacturing, agricultural operations, and landfill leachate) and domestic sources (human and animal excretion of unmetabolized pharmaceuticals, wastewater treatment plants, and septic system discharge), among others (Bavumiragira et al., 2022; Gaw et al., 2014; Kumar et al., 2023). With conventional treatment methodologies, wastewater treatment plants (WWTPs) are ineffective in complete removal of pharmaceuticals, sometimes even increasing their concentrations (Kumar et al., 2022), and consistent discharge combined with potentially long half-lives results in pseudo-persistence and prolonged environmental exposure (Fahlman et al., 2018; Gros et al., 2010; Mezzelani et al., 2018). As a result, recent surveys show widespread presence of pharmaceuticals in aquatic environments, including in remote and seemingly pristine ecosystems (Castillo et al., 2023; Husk et al., 2019; Jiang et al., 2020; Kallenborn et al., 2018). For example, Duarte et al. (2021) detected 70 different emerging contaminants in Antarctic phytoplankton, 40 of which were pharmaceuticals, concluding that exposure to organisms at the bottom of the marine food web threatens the entire ecosystem's trophic structure and function.

The majority of studies examining the presence of pharmaceutical contaminants in aquatic environments have focused on surveys of freshwater and riverine systems on smaller spatial scales, primarily utilizing water samples as means of detection (Świacka et al., 2022). National surveys of riverine water and biota have been conducted, such as a United States survey of 139 streams with detections in 80% of locations (Kolpin et al., 2002), numerous studies in China with detections in over 40 different riverine and freshwater locations (Liu and Wong, 2013; Zhu et al., 2019), and an Australian survey of 73 rivers with detections in 92% of samples, with 13 pharmaceuticals found at concentrations capable of adverse effects (Scott et al., 2014). Having shown large scale contamination across riverine and freshwater systems, the next step is to expand investigation of pharmaceuticals' presence and effects in marine and coastal systems, which are often the final recipient of riverine pollution (Kötke et al., 2019; Mezzelani and Regoli, 2021; Zandaryaa and Frank-Kamenetsky, 2021).

Further, across marine and coastal pharmaceutical assessments, certain geographic areas remain understudied, such as the Caribbean Basin. For example, a review of pharmaceutical research in Latin America through 2019 identified 24 studies conducted throughout Mexico (Valdez-Carrillo et al., 2020), but only one study examined marine environments, finding seven pharmaceuticals at four coastal locations in the Yucatan Peninsula (Metcalfe et al., 2011). To our knowledge, surveys of Bahamian and Belizean aquatic systems have not been conducted. Puerto Rico, in contrast, has a long history of pharmaceutical production and documented contamination (Ramcharran, 2011). Despite extensive pharmaceutical manufacturing and its importance to Puerto Rico's economy, only four environmental studies were conducted through 2019 (Valdez-Carrillo et al., 2020), with one in coastal marine systems detecting carbamazepine at 16 of 17 sampling sites (Wade et al., 2015). Most recently, Bradley et al. (2021) detected pharmaceuticals in 9 of 14 drinking water sampling locations. In subtropical South Florida, the extent of pharmaceutical presence and ecological effects are also limited. Freshwater surveys detected pharmaceuticals in water samples (Wang, 2012; Wang and Gardinali, 2013a), and mosquito fish (*Gambusia affinis*; Wang and Gardinali, 2013b; Wang and Gardinali, 2012), from systems directly affected by reclaimed water and irrigation. Additional studies have detected pharmaceuticals and indicators of wastewater contamination in marine systems (Cejas, 2010; Gardinali and Zhao, 2002; Henderson et al., 2020). Ng et al. (2021) recently detected hormones, pharmaceuticals, and endocrine disrupting compounds at 28 of 29 sites throughout South Florida, with hormones at concentrations high enough to elicit endocrine disruption. Clearly, the presence of pharmaceuticals in freshwater and marine systems throughout the Caribbean Basin is likely, the extent of their environmental exposure is not fully understood, and additional sampling is needed.

In this study, we examined the pharmaceutical burden and evaluated the risk of pharmacological effects in a tropical and subtropical coastal marine fish across the Caribbean Basin. Our focal species was bonefish (*Albula vulpes*) due to their reliance on coastal and nearshore habitats, potential high incidence of exposure, and substantial socio-economic value in support of recreational and artisanal fisheries (Adams et al., 2023; Rennert, 2017; Rennert et al., 2019). Additionally, as a mobile higher order consumer, bonefish can provide information on spatio-temporal trends in pharmaceutical contamination beyond that possible from abiotic samples (Treu et al., 2022). Our study addressed two questions: 1) To what extent were Caribbean bonefish exposed to pharmaceutical contaminants? and 2) Were Caribbean bonefish exposed to concentrations high enough to pose a risk of pharmacological effects? To address these questions, we sampled bonefish blood plasma across five distinct coastal regions in the Caribbean. We hypothesized that: 1) Exposure would be highest in more urbanized and populous regions compared to less populated and developed regions; and 2) Pharmaceuticals would be detected at concentrations capable of eliciting pharmacological effects, with the highest risk of these effects in regions with the highest degree of pharmaceutical exposure.

2. Materials and methods

2.1. Study species

We selected bonefish, an economically and culturally-important species that supports a recreational catch-and-release fishery throughout the Caribbean Sea and western North Atlantic Ocean (Adams et al., 2014), and an artisanal fishery in some locations (e.g., Cuba; Rennert et al., 2019), because their ecology makes them particularly susceptible to exposure of pharmaceutical contaminants. Bonefish rely on shallow nearshore habitats consisting of seagrass beds, intertidal sand flats, mangroves, and hardbottom, which can be in close proximity to urbanized coastal areas and associated pollution sources (Brownscombe et al., 2017, 2019; Larkin, 2011). In addition to exposure via respiration (i.e., dissolved exposure), bonefish primarily feed on benthic vertebrates and invertebrates (Ault, 2008; Campbell et al., 2022; Colton and Alevizon, 1983; Crabtree et al., 1998), which places them at an elevated risk of pharmaceutical exposure since benthic invertebrates can have higher levels of pharmaceuticals (Du et al., 2014a; Lagesson et al., 2016). Bonefish exhibit high site fidelity with an average home range of 10 km (Boucek et al., 2019; Murchie et al., 2013; Pina-Amargós et al., 2023), but undergo long-distance spawning migrations ranging from 20 km to 80 km (Adams et al., 2019, 2021; Boucek et al., 2019; Perez et al., 2019b; Larkin et al., 2023), and recent South Florida tracking data show longer-distance movements during the spawning season (>100 km, October through April; Boucek et al. unp. data). This adds another source of risk of pharmaceutical exposure since mobile mesoconsumers can accumulate contaminants from numerous sources across a large spatial area (Treu et al., 2022).

2.2. Sampling regions

The study expanded across five regions (Fig. 1, Table 1) of importance to the Caribbean bonefish fisheries (Boucek et al., 2022; Perez et al., 2021; Sherman et al., 2018). The five regions were: the transboundary region of Belize and Mexico (hereafter referred to as Belize-Mexico), locations west of Key West (hereafter referred to as Key

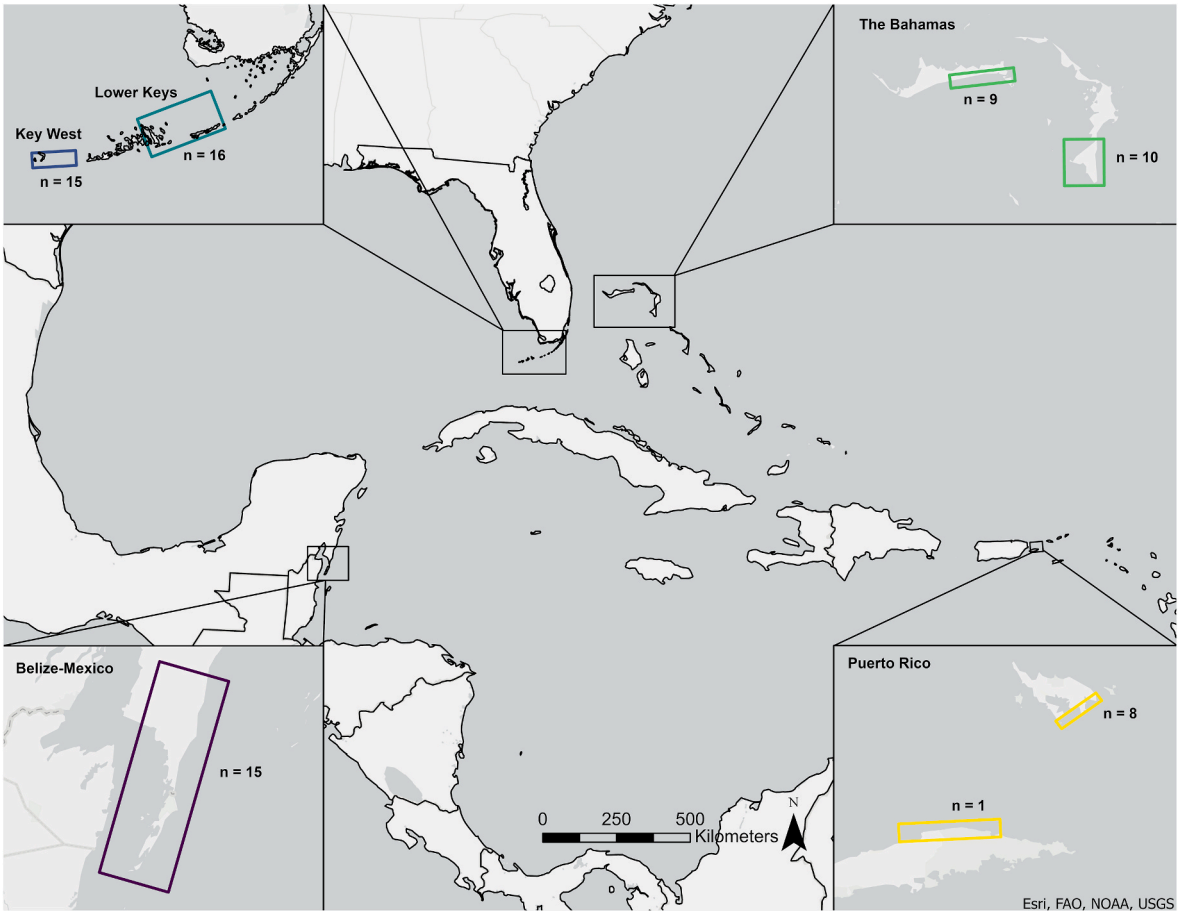


Fig. 1. Map of the five Caribbean sampling regions. Exact bonefish sampling locations are omitted due to their status as a prohibited and protected species and the sensitive nature of the fishing locations and instead polygons including all sampling locations are shown.

Table 1
Sampling effort, summary of pharmaceutical findings, and characteristics of the five regions sampled. Shown are the number of bonefish sampled per region, pharmaceutical detections (total and mean), total and mean1/3 H_TPC (human therapeutic plasma concentration) exceedances, total number with at least one 1/3 H_TPC exceedance, and % of bonefish with at least one 1/3 H_TPC exceedance. Total marine area and protected area pertains to the entire region (e.g., the entirety of The Bahamas national waters). Annual visitation statistics for the Belize-Mexico and Puerto Rico regions were not available.

Summary	Belize-Mexico	Key West	Lower Keys	Bahamas	Puerto Rico	Total
Total Bonefish	15	15	16	19	9	74
Total Detections	98	80	93	60	33	364
Mean Detections	6.5	5.3	5.8	3.2	3.7	4.9
Total 1/3 H_TPC	8	14	12	5	5	44
Mean 1/3 H_TPC	0.53	0.93	0.75	0.26	0.55	0.59
Total with ≥1 1/3 H_TPC	6	4	9	5	5	29
% with ≥1 1/3 H_TPC	40.0%	26.7%	56.3%	26.3%	55.6%	39.2%
Total Marine Area (sq/km)	61,488[a]	11,200[a]	11,200[a]	619,785[a]	172,958[a]	865,431
Protected Area (% of Total)	424 (<1%)[a]	488 (4.4%)[a]	488 (4.4%)[a]	710 (<1%)[a]	2144 (1.2%)[a]	<1%
Human Population	17,685[c,d,e]	33,555[b]	22,622[b]	64,062[f]	9,812[g]	147,776
Annual Visitation	NA	2.8 m[h]	1.5 m[h]	59,000[i]	NA	4.4 m
Land Area (sq/km)	62	47[b]	161[b]	1331	73[g]	684
People per sq/km	243[c,d]	706[b]	141[b]	48[f]	61[g]	83

[a] https://mpatlas.org/.
[b] https://censusreporter.org/.
[c] https://worldpopulationreview.com/.
[d] https://en.mexico.pueblosamerica.com/i/xcalak/.
[e] https://sib.org.bz/statistics/population/.
[f] **Bahamas Census Office, 2011.**
[g] **https://census.gov.**
[h] **Rockport Analytics, 2019.**
[i] **Fedler, 2018.**

West), Lower Florida Keys (hereafter referred to as Lower Keys), two islands in The Bahamas, Abaco and Grand Bahama (hereafter collectively referred to as The Bahamas), and two islands east of Puerto Rico, Culebra and Vieques (hereafter collectively referred to as Puerto Rico; Fig. 1). Each region has varying degrees of urbanization, population levels, and anthropogenic influence, allowing us to examine a wide range of pharmaceutical pollution risk across a large geographic scale and suite of environmental characteristics (Table 1).

The Lower Keys experiences a lower degree of anthropogenic influence and development compared to Key West, which has the highest population density of all regions. Belize-Mexico and Puerto Rico are less urbanized and have the lowest human population sizes. The Bahamas is the most populated of all regions, yet has the lowest population density and annual visitation rates. Overall, metrics of anthropogenic influence (e.g., total population, population density, and annual visitation) are variable across sampling regions (Table 1).

2.3. Sample collection

Bonefish were sampled using hook and line angling in Florida, Puerto Rico, and The Bahamas, and using seine nets in Belize-Mexico and for a subset of Bahamian bonefish. Key West (n = 15) and Lower Keys (n = 16) samples were collected between August 2019 and October 2020, Belize-Mexico samples were collected in June 2019 (n = 15), Bahamas samples were collected in June 2018 (n = 9) and November 2018 (n = 10), and Puerto Rico samples were collected in February 2019 (n = 1) and December 2019 (n = 8). All fish were captured from shallow, nearshore habitats (<10 m to 15 km from a shoreline with human presence; Table 1, Fig. 1). Due to the difficulty of capture, bonefish were opportunistically sampled within each region, but a concerted effort was made for a broad spatial distribution within regions (e.g., 10 km between collection sites within a region). Upon capture, bonefish total length, fork length, and girth measurements were taken, and GPS coordinates of sampling location were recorded. 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), adhering to FIU IACUC-21-058 protocol. 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 h of collection, samples were centrifuged for 15 min at 3500 rpm (LW Scientific USA E8 Portable Centrifuge) to separate plasma. Plasma was then aliquoted using sterile polyethylene transfer pipets (Corning Scientific™), placed in 2 mL cryovials (Corning Scientific™), and stored in a −20 °C freezer until processing at the Department of Chemistry, Umeå University, Umeå, Sweden within 6 months of sampling.

2.4. Target pharmaceuticals, standards and analytical methods

A total of 102 pharmaceuticals were included in the analysis (Table S1), and target analyte selection was based on detectability and predicted ability to bioaccumulate in fish (Fick et al., 2010). A summary of analytical procedures is provided here, and additional details are provided in Grabic et al. (2012), Lindberg et al. (2014), and Sedvall et al. (2022).

All 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.

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 min. 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 plasma 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 (Grabic et al., 2012; Lindberg et al., 2014; Sedvall et al., 2022).

2.5. Human therapeutic plasma concentration (H_TPC)

To evaluate the potential for pharmacological effects from pharmaceutical plasma concentrations, we drew upon the Biological Read-Across Hypothesis, which asserts that pharmacological effects can occur in non-target organisms as a result of conservation of mammalian pharmaceutical target sites (Huggett et al., 2003). Behavioral and/or physiological alterations in fish can occur at internal plasma concentrations within the human therapeutic plasma concentration (H_TPC), or the concentration required for a pharmaceutical to elicit a therapeutic effect in humans (Sumpter and Margiotta-Casaluci, 2022; Valenti et al., 2012). Application of this hypothesis in hazard and risk estimates in fish has been substantiated since up to 86% of human drug targets are conserved (Brown et al., 2014; Gunnarsson et al., 2008; Rand-Weaver et al., 2013; Schreiber et al., 2011). For our study, we selected a threshold of 1/3 the H_TPC as a conservative estimate of potential pharmacological effect, informed by Huerta et al. (2016), who found behavioral effects in fathead minnows (*Pimephales promelas*) with plasma concentrations at 1/3 the H_TPC for oxazepam. Even though the utility of comparing measured pharmaceutical concentrations to their respective H_TPCs as an assessment of risk for pharmacological effects has been shown to be applicable (Fabbri, 2015; Fabbri and Franzellitti, 2016; Sumpter and Margiotta-Casaluci, 2022), it assumes the presence of the pharmaceutical's drug target in fish, which is consistent with the read across hypothesis, yet this information is absent for many pharmaceuticals. Thus, in our study, we apply a 1/3 H_TPC threshold and interpret pharmaceutical concentrations at or above 1/3 the H_TPC as those showing a moderate to high potential for pharmacological effects. Use of the 1/3 H_TPC as a threshold allows us to compare the potential of pharmacological effects for the large number of pharmaceuticals in our study, since for many of those lowest observable effect concentrations are not available. The H_TPC values used for comparison were those reported by Fick et al. (2010), and Schulz et al. (2020).

2.6. Statistical analyses

We used a combined univariate and multivariate analytical approach to examine variation in the number of pharmaceuticals and the number of 1/3 H_TPC exceedances (i.e., concentrations above the 1/3 H_TPC threshold). All univariate and multivariate models quantified variation as a function of sampling region, and all statistical analyses were performed using R v 4.3.1 (R Core Team, 2023).

2.6.1. Variation in the number of pharmaceutical detections

The influence of region on the number of pharmaceuticals detected per bonefish was assessed using Generalized Linear Models (GLMs) with a Poisson distribution. GLMs were performed using the base R package stats (R Core Team, 2023), tests of model assumptions were performed using R package performance (Lüdecke et al., 2021), and model performance was assessed using R packages MuMInN (Bartón, 2023) and car (Fox and Weisberg, 2008). Pairwise comparisons of model contrasts for region were analyzed using Tukey's HSD tests in the R package emmeans (Lenth, 2023), and *p*-values with Holm-Bonferroni adjustments were derived using R package multcomp (Hothorn et al., 2008).

The influence of region in multivariate space using the presence and absence of pharmaceuticals was examined using Permutational Analysis of Variance (PERMANOVA) with 999 permutations on a Jaccard distance matrix. Pairwise PERMANOVA tests followed a significant region effect using Holm-Bonferroni *p*-value adjustments. Similarity in the presence/absence of pharmaceuticals 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).

2.6.2. Variation in pharmaceutical risk

GLMs with a negative binomial distribution were used to assess the influence of region on the number of 1/3 H_TPC exceedances per bonefish. Negative binomial distributions were used to account for overdispersion of the distribution using the R package MASS (Venables and Ripley, 2002). Model performance was assessed as described in section 2.6.1, and pairwise comparisons of model contrasts for region were analyzed using Tukey's HSD tests.

To assess the potential risk of pharmacological effects posed by pharmaceutical exposure in multivariate space using pharmaceutical concentrations, we calculated a proportion of the measured pharmaceutical concentration to a 1/3 H_TPC threshold for each pharmaceutical detected. In other words, for each pharmaceutical detected, we divided the observed concentration by 1/3 of the pharmaceutical's H_TPC to obtain a proportion. This proportion, rather than the raw concentration values, was used to scale the risk of each detected pharmaceutical concentration to its respective threshold of effect, accounting for differences in concentrations necessary to elicit an effect unique to each pharmaceutical. The influence of region on this 1/3 H_TPC pharmaceutical assemblage was assessed using PERMANOVA (with 999 permutations) based on a Bray-Curtis distance matrix, with square-root transformed data of 1/3 H_TPC exceedances. Pairwise PERMANOVAs with a Holm-Bonferroni adjusted *p*-value were used to compare individual regions. Variation in the 1/3 H_TPC proportions across all pharmaceuticals and samples was visualized using nMDS.

2.6.3. Influence of pharmaceutical identity on multivariate assemblages

The influence of individual pharmaceuticals on the observed pharmaceutical presence and absence and on the 1/3 H_TPC assemblages, explained by ordination scores, was calculated with 999 permutations. This allowed us to examine which of the 102 pharmaceuticals were most important to driving correlations, similarities, and dissimilarities in both assemblages. Scores were then fitted to each nMDS plot using the R package vegan (Oksanen et al., 2022). 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. This is equivalent to fitting a linear trend surface (plane in 2 dimensions), with the arrows showing its gradient (direction of steepest increase). The arrows representing the pharmaceuticals were adjusted to the plot dimensions using a constant multiplier, retaining the scaled 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 $\leq$0.01. Last, the contribution of specific pharmaceuticals to driving dissimilarities among regions (regional contrasts) was assessed using similarity percentage analysis (SIMPER) with the R package vegan (Oksanen et al., 2022).

3. Results

3.1. Variation in pharmaceutical detections across bonefish samples

Pharmaceuticals were detected in all 74 plasma samples tested, indicating widespread exposure throughout the Caribbean (Fig. 1, Table 1). All samples had at least one pharmaceutical, with a maximum of 16 pharmaceuticals in an individual bonefish from Key West. On average, we detected 4.9 pharmaceuticals per bonefish across the 5 regions (Table 1). Across all samples, 49 unique pharmaceuticals were detected, for a total of 364 pharmaceutical detections (Table S1). Of the 364 detections, the 10 most frequently detected pharmaceuticals accounted for 232 (63.7%) of all detections (Table S1).

Venlafaxine, atenolol, naloxone, codeine, and trimethoprim were the top 5 most frequently detected pharmaceuticals across all samples (accounting for 43.1% of detections; Table S1). Venlafaxine, a selective serotonin and norepinephrine reuptake inhibitor (SNRI) frequently prescribed for the treatment of major depressive disorder, generalized anxiety, social anxiety disorder, and panic disorder, was detected in 43 bonefish (58.1% of samples, 0.61–22.28 ng/mL). Atenolol, a beta-1 selective blocker used in the management of hypertension and chronic angina, was detected in 36 bonefish (48.6% of samples, 5.1–49 ng/mL). Both codeine (0.57–5.52 ng/mL), an opioid analgesic used to treat moderate to severe pain, and naloxone (1.23–9.66 ng/mL), an opioid receptor antagonist used to rapidly reverse an opioid overdose, were detected in 27 bonefish (36.5% of samples). Trimethoprim, an antifolate antibiotic used to treat various infections, was detected in 24 bonefish (32.4% of samples, 0.11–58 ng/mL; Table S1).

3.2. Regional differences in pharmaceutical exposure

Across regions, Belize-Mexico had the most detections (98 detections), followed by the Lower Keys (93 detections), Key West (80 detections), The Bahamas (60 detections), and Puerto Rico (33 detections; Table 1, Table S1). Region was significant ($p < 0.001$) in driving variation in the number of pharmaceuticals detected per bonefish (Table 2, Fig. 2a).

Tukey pairwise comparisons indicated significant differences between The Bahamas and three other regions; Belize-Mexico ($p < 0.001$), Lower Keys ($p = 0.002$), and Key West ($p < 0.05$; Table S2). The number of pharmaceuticals detected in The Bahamas (3.2 pharmaceuticals/bonefish) was lower than Belize-Mexico (6.5 pharmaceuticals/bonefish), Lower Keys (5.8 pharmaceuticals/bonefish), and Key West (5.3 pharmaceuticals/bonefish; Table 1). And the number detected in Belize-Mexico was higher than those detected in Puerto Rico (3.7 pharmaceuticals/bonefish, $p < 0.05$; Fig. 2a).

In multivariate ordination space, region significantly influenced the presence/absence pharmaceutical assemblage ($p = 0.001$; Fig. 3a, Table 3). In particular, Lower Keys differed significantly from the other 4 regions sampled, suggesting a distinct assemblage of pharmaceuticals (Table S3). Two other regions showed significant differences from at least 2 regions, and those included Key West and The Bahamas. Seven pharmaceuticals influenced the pharmaceutical assemblage ($p \leq 0.01$). These included, in order of influence; atenolol, paracetamol, naloxone, risperidone, ranitidine, trimethoprim, and azelastine. Atenolol, paracetamol, and naloxone were found to be the most influential, all with a p-value = 0.001 (Fig. 3a). In SIMPER analysis, paracetamol, trimethoprim, and azelastine were the most influential pharmaceuticals driving regional dissimilarities (Table S3). Paracetamol was the most important pharmaceutical driving this dissimilarity, contributing to four of the seven significant regional contrasts, and because of its higher detections in the Lower Keys, separating that region from the other 4 regions. Trimethoprim was more commonly detected in The Bahamas, driving dissimilarity from Key West and Belize-Mexico. Azelastine was most commonly detected in Puerto Rico, driving dissimilarity from Key West (Table S3, Fig. 3a).

3.3. Variation in 1/3 H_TPC exceedances across bonefish samples

Pharmaceutical exposure exceeding the 1/3 H_TPC threshold was present in all sampling regions. Across all regions 23 pharmaceuticals of the 49 detected (46.9%) were observed at least once at a concentration exceeding 1/3 of the H_TPC. Of the 364 total detections, 44 were at concentrations in exceedance (12.1%; Fig. 4). Notably, almost 40% of the 74 bonefish sampled (29 bonefish, 39.2%) had at least one pharmaceutical at a concentration exceeding the 1/3 H_TPC threshold, with a maximum of 11 pharmaceuticals in exceedance of the 16 total pharmaceuticals detected in a Key West bonefish (Table 1, Table S1). The percentage of bonefish with at least 1 1/3 H_TPC was highest in the Lower Keys and Puerto Rico (>55%), intermediate in Belize-Mexico (40%), and lowest in Key West and The Bahamas (<27%; Fig. 4).

Naloxone, azelastine, metoprolol, biperiden, and clemastine were the top 5 pharmaceuticals detected above 1/3 H_TPC (Table 1, Table S1). Naloxone was most frequently detected in exceedance – in 9 bonefish (12.2% of samples). Azelastine, a histamine H1-receptor antagonist used intranasally to treat allergic and vasomotor rhinitis, and in an ophthalmic solution to treat allergic conjunctivitis, was detected above the 1/3 H_TPC threshold in 4 bonefish (5.4% of samples). Metoprolol, biperiden, and clemastine were all detected at concentrations exceeding the 1/3 H_TPC threshold in 3 bonefish (4.1% of samples; Table S1). Metoprolol is a beta-blocker used in the treatment of hypertension and angina, and used to reduce mortality due to myocardial infarction. Biperiden is a muscarinic receptor antagonist used to treat parkinsonism and control extrapyramidal side effects of neuroleptic drugs. Clemastine is an antihistamine with sedative and anticholinergic effects used to treat the symptoms of allergic rhinitis. Importantly, of these 5 pharmaceuticals detected above 1/3 H_TPC, only naloxone had a high incidence of detections – third most detected and in 27 bonefish (Table S1). This suggests that the number of detections does not necessarily correlate with the potential for pharmacological effects.

3.4. Regional differences in 1/3 H_TPC exceedances

In contrast to the number of pharmaceutical detections, there was no significant regional variation for 1/3 H_TPC exceedances ($p = 0.3$; Fig. 2b, Table 2). In multivariate ordination space, however, region ($p = 0.001$) drove dissimilarity in the 1/3 H_TPC pharmaceutical assemblage

Table 2
Summary of the GLM model for the number of pharmaceuticals per bonefish and the number of 1/3 H_TPC exceedances per bonefish by sampling region.

Variable	Predictor	p	Null Deviance	Residual Deviance	AICc	χ^2	D^2
Pharmaceutical Number	Region	1.9E-05***	106.2	79.1	336.3	27.0	0.24
1/3 H_TPC Exceedances	Region	0.30	65.4	60.6	164.2	4.87	0.01

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

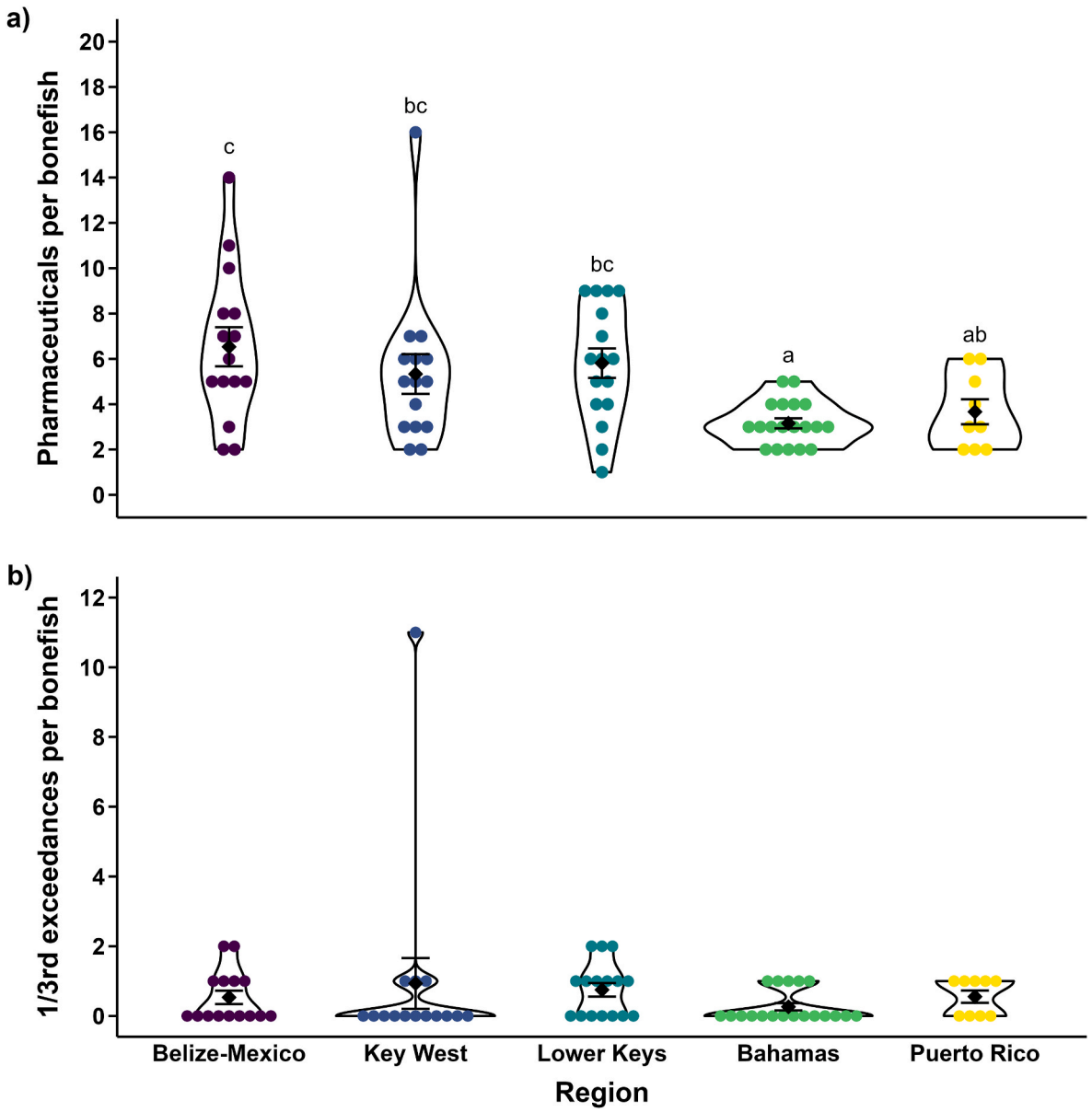


Fig. 2. Violin plots with: a) the number of pharmaceuticals detected per bonefish, and b) the number of 1/3 H_TPC exceedances, across the five regions sampled. In the violin plot, colored points are samples values, width shows the distribution of those values, black diamonds denote regional means, and black bars show standard errors. Letters indicate significant regional differences per Tukey pairwise tests (only detected for number of pharmaceuticals).

(Fig. 3b, Table 3). In particular, the Lower Keys and Key West had assemblages that despite their geographical proximity were distinct from each other, and from all other regions (Table S3). Five pharmaceuticals were found to influence the observed pharmaceutical risk assemblage: trimethoprim, codeine, naloxone, desloratadine, and azelastine (Fig. 3b). Of these, codeine, naloxone, and azelastine were found to be the most influential in regional dissimilarities (Table S3). Of the eight significant regional contrasts, azelastine and codeine were the most influential, both contributing to three significant contrasts, while naloxone contributed to two significant regional contrasts. Codeine drove the dissimilarities between the Lower Keys and The Bahamas (more frequently exceeding 1/3 of the H_TPC in The Bahamas), and between Key West and The Bahamas, and Key West and Belize-Mexico (more frequently exceeding 1/3 H_TPC in Key West for both contrasts). Azelastine was highest in concentration (i.e., more frequently in exceedance of the 1/3 H_TPC) in Puerto Rico, driving the dissimilarity between Puerto Rico and the Lower Keys, Key West, and The Bahamas. Last, naloxone was most influential in the dissimilarities between the Lower Keys and Key West (more frequently exceeding 1/3 of the H_TPC in Key West), and Lower Keys and Belize-Mexico (more frequently exceeding 1/3 of the H_TPC in Belize-Mexico; Table S3).

4. Discussion

Our examination of bonefish exposure shows widespread presence of pharmaceuticals throughout coastal marine ecosystems of the Caribbean Basin. Pharmaceuticals were detected in every fish sampled across all five regions, yet showed significant regional differences. Results indicated that pharmaceutical exposure is high in both urban populous regions and those less developed and less populous. Further, our results demonstrate the potential for pharmaceuticals to bioaccumulate in marine biota at concentrations capable of eliciting pharmacological effects. Pharmaceuticals in exceedance of the 1/3 H_TPC threshold were present in 39.2% of all bonefish, indicating a potential risk posed by pharmaceutical exposure throughout the Caribbean Basin. The incidence of pharmaceuticals detected at concentrations capable of

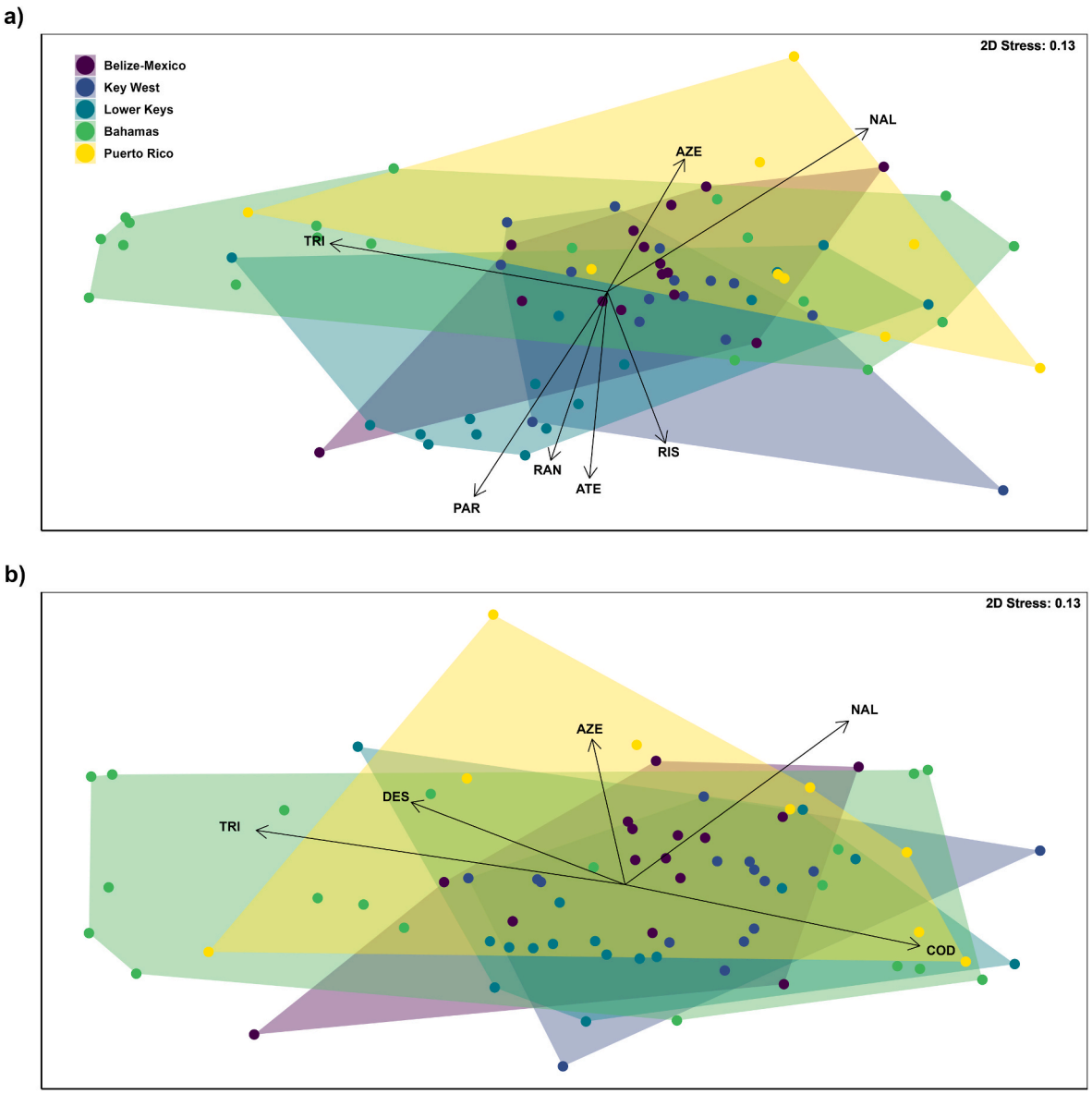


Fig. 3. nMDS plots showing: a) the presence/absence pharmaceutical assemblage, and b) the 1/3 H_TPC proportion assemblage, in multidimensional ordination space color coded by sampling region across all 74 samples. Vector arrows show the relative direction and magnitude of pharmaceutical influence ($p \geq 0.01$). Abbreviations are as follows: TRI = trimethoprim, AZE = azelastine, NAL = naloxone, RIS = risperidone, ATE = atenolol, RAN = ranitidine, PAR = paracetamol, DES = desloratadine, COD = codeine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Summary of PERMANOVA main effects for the presence/absence of pharmaceuticals and for the 1/3 H_TPC exceedances (calculated as a proportion of each pharmaceutical's human therapeutic concentration, please see text for details).

Model	Terms	df	Sum of sq	R^2	F Model	*p*
Presence/Absence	Region	4	4.4	0.14	2.9	0.001***
	Residual	69	26.6	0.86		
	Total	73	31.1	1.00		
1/3 H_TPC Exceedances	Region	4	3.7	0.14	2.9	0.001***
	Residual	69	22.2	0.86		
	Total	73	25.8	1.00		

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

pharmacological effects did not vary across regions, nor did the number of 1/3 H_TPC exceedances correspond to elevated levels of exposure. A total of 49 pharmaceuticals were detected, and 23 of these were detected at concentrations above the1/3 H_TPC.

4.1. Patterns in number of pharmaceuticals detected across regions

We hypothesized that pharmaceutical exposure would positively correlate with population density; however, exposure was prevalent across both densely and sparsely populated regions. The number of

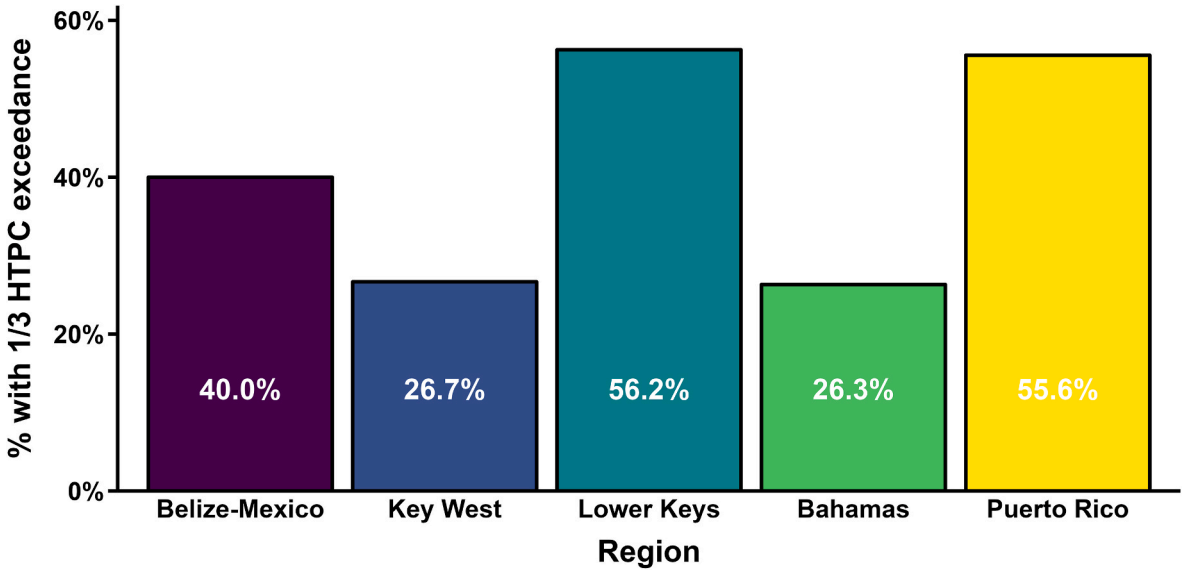


Fig. 4. Percentage of bonefish in each region with at least 1 detected pharmaceutical exceeding 1/3 of the H_TPC.

pharmaceuticals detected in the two most populated regions, Lower Keys and Key West, and the second least populated region, Belize-Mexico, were similarly high. The Bahamas had the lowest number of pharmaceutical detections across all regions, followed by Puerto Rico, the least populated region. Further, the number of detections in The Bahamas was significantly different to all other regions except Puerto Rico. Although pharmaceutical exposure is often highest near urban and populous areas, which are influenced by consistent wastewater effluent discharge from WWT and septic systems, and industrial livestock operations (Osorio et al., 2016; Tang et al., 2021), other factors can result in pharmaceutical contamination of less developed areas (Kallenborn et al., 2018; Mandaric et al., 2017; Wilkinson et al., 2022). A survey of 258 rivers in 104 countries found that exposure was not exclusively related to population and degree of urbanization; rather, some of the least developed areas had elevated pharmaceutical detections, likely as a result of ineffective or entirely absent wastewater treatment (Bouzas-Monroy et al., 2022; Wilkinson et al., 2022).

Many of the potential sources of pharmaceutical contamination associated with populous areas are present in the Lower Keys and Key West, and although likely present in the other 3 Caribbean regions, specific information is lacking. Even though Belize-Mexico is less populated compared to the other regions, inadequate wastewater infrastructure and a high density of people per sq/km could result in elevated pharmaceutical exposure (Jameel et al., 2020; Osorio et al., 2016). Such instances of inadequate infrastructure and a relatively high population density resulting in pharmaceutical exposure in less populous regions have been documented. Mandaric et al. (2017) found extensive pharmaceutical contamination in remote Alpine rivers and that the extent of contamination was influenced by visitation, increasing during the tourist season. Further, pharmaceutical contamination sources such as distant coastal wastewater discharge (Lara-Martín et al., 2020), proximity to shipping lanes (Alygizakis et al., 2016), and ocean current transport (Alygizakis et al., 2021; Brumovský et al., 2017) may have influenced the observed high number of detections in the less populated regions. With variable degrees of pharmaceutical exposure in populous and less populated regions, our results support the necessity for further examination of pharmaceutical exposure at large spatial scales (Branchet et al., 2021; Bu et al., 2016), and provide additional evidence for widespread pharmaceutical contamination independent of anthropogenic influence. A Key West bonefish had comparatively high pharmaceutical detections (16 total pharmaceuticals) and 1/3 H_TPC exceedances (11 total exceedances), the causes of which could be diverse and are difficult to determine. It is possible that individual variability, such as increased feeding of contaminated prey or a higher residence time in an area with increased pharmaceutical contamination led to higher pharmaceutical number and concentrations. Additional variables outside of those tested in our study would be necessary to determine the potential causes of this increased exposure.

Temporal variability in pharmaceutical exposure to aquatic systems may have also influenced our observed regional differences. Bonefish sample collections across all regions were cross-sectional and not temporally matched, limiting our ability to examine the influence of time of collection on the observed number of pharmaceutical detections. Seasonal variation of pharmaceutical detections occurs in freshwater tributaries (Burns et al., 2018; Ebele et al., 2020; Im et al., 2020), and in estuarine and marine ecosystems (Branchet et al., 2021; Lu et al., 2020; Tanabe and Ramu, 2012), the causes of which can be diverse. Seasonal patterns in pharmaceutical use can also impact the number and identity of pharmaceuticals in aquatic systems (Kot-Wasik et al., 2016; Narita et al., 2021; Sui et al., 2011). The effects of seasonal tourism on pharmaceutical exposure can also be substantial in coastal systems adjacent to populated areas (Maasz et al., 2019), and in less populated areas (Mandaric et al., 2017), particularly when visitation is spatially concentrated. Last, seasonal variation in hydrological conditions, physio-chemical water properties, and seasonal fluctuations in climate are additional factors driving pharmaceutical presence in the environment (Bayen et al., 2013; Branchet et al., 2021).

Variation in bonefish movement associated with spawning could have influenced the observed regional differences. Castillo et al., (2023) documented differences in pharmaceutical detections and concentrations in South Florida bonefish associated with their spawning season, finding that a lower degree of variability in pharmaceutical assemblage and concentrations during the spawning season. Bonefish have localized spatial preferences, with an average home range of 10 km (Boucek et al., 2019; Murchie et al., 2013; Pina-Amargós et al., 2023), but undertake seasonal long-distance spawning migrations ranging from 20 km to 80 km (Adams et al., 2019, Boucek et al., 2019; Perez et al., 2019; Adams et al., 2021). This variability in bonefish movement patterns could contribute to the observed variation in pharmaceutical assemblage. Additional temporally explicit sampling is needed to identify the specific mechanisms (e.g., seasonal bonefish movement patterns vs. seasonal pharmaceutical use) on patterns of pharmaceutical exposure and pharmaceutical assemblage.

4.2. Patterns in the potential for pharmacological effects

We hypothesized that the number of detected pharmaceutical concentrations exceeding the 1/3 H_TPC threshold would be present in regions with the highest degree of pharmaceutical exposure, but our results did not show a positive correlation between exposure and risk of pharmacological effects. In contrast to the number of pharmaceuticals detected, region did not affect the incidence of exposure to concentrations capable of pharmacological effects. The greatest risk of pharmacological effects was present in the Lower Keys (56.3% of samples), which had the second highest number of pharmaceutical detections (5.8 pharmaceuticals/bonefish). Puerto Rico had a comparably high incidence of detected pharmaceutical concentrations exceeding 1/3 the H_TPC (55.6% of samples), yet the region had the second lowest number of pharmaceuticals detected (3.7 pharmaceuticals/bonefish), showing that despite a lower degree of pharmaceutical exposure, there can still be a higher risk of pharmacological effects. Collectively, our results highlight the variability in the relationship between elevated risk of pharmacological effect and high pharmaceutical exposure and population density (Hong et al., 2018; Letsinger et al., 2019; Nödler et al., 2014), suggesting that other factors (e.g., wastewater treatment infrastructure, tourism, and currents) may contribute to risk distribution (Dehm et al., 2021; Fonseca et al., 2020; Wilkinson et al., 2022). Consequently, regions with more pharmaceuticals in exposed biota are not necessarily those at highest risk of exposure to pharmacologically active concentrations.

Although similar mechanisms that influence regional variation in pharmaceutical diversity and exposure can also influence pharmaceutical concentrations (Branchet et al., 2021; Im et al., 2020; Lu et al., 2020; Tanabe and Ramu, 2012), this was not the case in our bonefish samples. Regional variation in environmental conditions (e.g., hydrological dynamics, ocean currents, water physio-chemical properties, climate), could influence the ability for pharmaceuticals to remain present in water at high concentrations and subsequent bioaccumulation to higher concentrations in bonefish (Branchet et al., 2021; Cerveny et al., 2021; Chen et al., 2021; Dehm et al., 2021). Anthropogenic factors (e.g., population density, tourism, WWT processing), could also reduce the ability to identify a clear regional trend in 1/3 H_TPC exceedances (Osorio et al., 2016; Wilkinson et al., 2017, 2018). Thus, we suggest pharmaceutical identity and exposure can be more regionally dependent, yet factors influencing concentration may be more nuanced and variable. In order to determine the most influential drivers of risk posed by bioaccumulation of pharmaceuticals to concentrations capable of pharmacological effect, additional investigation accounting for a greater set of variables is required (e.g., pharmaceutical physio-chemical properties, production and consumption rates, WWTP removal efficiency, seasonal variability, population density, climate, and ecological composition).

4.3. Strengths and limitations

Our study allows for comparison of pharmaceutical exposure on a large spatial scale across multiple regions. By comparing measured concentrations to 1/3 of each pharmaceutical's H_TPC, we were able to combine analysis of pharmaceutical exposure with an assessment of potential risk of pharmacological effect posed by exposure. This method can be implored in future studies to determine the potential for risks to exposed biota while assessing the prevalence of pharmaceuticals in aquatic systems and can serve as a preliminary assessment of risk. The lack of available data on effect concentrations in relationship to each pharmaceutical's H_TPC is a limitation of this study in that the presence of effects cannot be extrapolated, rather we can only report the potential risk of pharmacological effects. Future studies should expand the information on sublethal effect concentrations to a wide range of pharmaceuticals frequently detected in natural systems. Further, the individual variability, mismatch in timing of sampling, and unequal distribution of bonefish size could also have influenced relative exposure and is a limiting factor in our study. Continued research into patterns of pharmaceutical exposure and the potential for pharmacological effects in biota should expand the variables tested by imploring a balanced temporal design and also reduce morphological variability across individuals.

5. Conclusion

Our study establishes the widespread presence of pharmaceuticals in subtropical coastal marine environments, bioaccumulating to concerning concentrations in exposed fish. Regional numbers in pharmaceutical detections did not directly correlate with exposure to pharmacologically active concentrations, highlighting the need to go beyond documenting the presence of pharmaceuticals to evaluating detected concentrations in relation to their potential for pharmacological effects at the scale of individual organisms. For this, we propose the use of thresholds relative to pharmaceutical effect concentrations in humans, such as the 1/3 H_TPC, used in this study. The use of these thresholds can provide additional information on the levels of exposure, serve as preliminary risk assessments, and be used as starting points for quantifying the consequences of exposure. In our study, the presence of multiple pharmaceuticals (average of 4.9 pharmaceuticals per bonefish) in every bonefish and the high frequency of concentrations exceeding a threshold of pharmacological effect (39.2%) poses concerns for the regions, due to the uncertain and often considerable effects posed by pharmaceutical mixtures (Backhaus, 2014; Kidd et al., 2023). Future research needs to expand beyond pharmaceutical detection and relate concentrations to the potential for pharmacological effects at the scale of the individual and investigate possible behavioral and physiological alterations to better understand the effects of exposure.

CRediT authorship contribution statement

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

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jennifer S. Rehage reports financial support and equipment, drugs, or supplies were provided by Bonefish & Tarpon Trust. Jennifer S. Rehage reports financial support was provided by United States Environmental Protection Agency.

Data availability

Data will be made available on request.

Acknowledgments

Authors thank the instrumental assistance from Capts. Brett Greco, Mark Johnson, Eric Lund, the Florida Keys Fishing Guides Association, the Lower Keys Fishing Guides Association, and all other fishing guides who assisted in sample collection. All bonefish handling and processing techniques followed the approved Florida International University protocol IACUC-21-058-CR01 and permit numbers: SAL-22-2100-SRP, BISC-2023-SCI-0031, EVER-2022-SCI-0028, DRTO-2022-SCI-0008, 036-19 from the Belize Fisheries Department, IBANQROO/DANP/DANPZS/0018/V-2019 from the Instituto de Biodiversidad y Áreas Naturelles Protegidas del Estado de Quintana Roo in Mexico, PPF/DGOPA-053/15 from the Comisión Nacional de Pesca in Mexico, 2018-IC-083 from the Departmento de Recursos Naturales y Ambiantes in Puerto Rico, and permitted by the Department of Marine Resources, Ministry of Agriculture and Marine Resources, for The Bahamas sample collection. This material was developed in collaboration with the Florida Coastal Everglades Long-Term Ecological Research program under the National Science Foundation Grant No. DEB-1832229 and DEB-2025954. This material was funded by the Bonefish and Tarpon Trust under Grant No. 800010342 and EPA Grant No. 800015295. This material is based upon work supported by the National Science Foundation under Grant No. HRD-1547798 and Grant No. HRD-2111661. These NSF Grants were awarded to Florida International University as part of the Centers for Research Excellence in Science and Technology (CREST) Program. This is contribution number 1668 from the Institute of Environment, a Preeminent Program at Florida International University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.140949.

References

Adams, A., Danylchuk, A.J., Cooke, S.J., 2023. Conservation connections: incorporating connectivity into management and conservation of flats fishes and their habitats in a multi-stressor world. Environ. Biol. Fish. 106, 117–130. https://doi.org/10.1007/s10641-023-01391-4.

Adams, A.J., Horodysky, A.Z., McBride, R.S., Guindon, K., Shenker, J., MacDonald, T.C., Harwell, H.D., Ward, R., Carpenter, K., 2014. Global conservation status and research needs for tarpons (*Megalopidae*), ladyfishes (*Elopidae*) and bonefishes (*Albulidae*). Fish Fish. 15, 280–311. https://doi.org/10.1111/faf.12017.

Adams, A.J., Lewis, J.P., Kroetz, A.M., Grubbs, R.D., 2021. Bonefish (*Albula vulpes*) home range to spawning site linkages support a marine protected area designation. Aquat. Conserv. Mar. Freshw. Ecosyst. 31, 1346–1353. https://doi.org/10.1002/aqc.3534.

Adams, A.J., Shenker, J.M., Jud, Z.R., Lewis, J.P., Carey, E., Danylchuk, A.J., 2019. Identifying pre-spawning aggregation sites for bonefish (*Albula vulpes*) in the Bahamas to inform habitat protection and species conservation. Environ. Biol. Fish. 102, 159–173. https://doi.org/10.1007/s10641-018-0802-7.

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci. Total Environ. 541, 1097–1105. https://doi.org/10.1016/j.scitotenv.2015.09.145.

Alygizakis, N.A., Slobodnik, J., Thomaidis, N.S., 2021. Sources and occurrence of pharmaceutical residues in offshore seawater. In: Pharmaceuticals in Marine and Coastal Environments. Elsevier Ltd. https://doi.org/10.1016/b978-0-08-102971-8.00011-1.

Ault, J.S., 2008. Biology and Management of the World Tarpon and Bonefish Fisheries. CRC Press.

Backhaus, T., 2014. Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. Phil. Trans. Biol. Sci. 369 https://doi.org/10.1098/rstb.2013.0585.

Bahamas Census Office. The official website of the government of The Bahamas. https://www.bahamas.gov.bs/wps/portal/public/Key%20Statistics/Social%20Statistics/Population%20and%20Census/!ut/p/b1/vZDJkqJAElafpR_ApliE8kghIsqOsl0IBATZN4Gup5-e5TCXHi8TnXnKiC_jy_yJgPCIoInmRxZNj7aJqp9zwIYmAqat7Gge7kUG8CzDc2hr0RLDEi7hOT6z7hl5OciX0M4T6ToKgmeSEqJU4PvKoTIipN33EZDTU7hjpSJtnvMw56kac0V66XAylk8Mn8OuzXnnbFNRppiN1UCpz3VHqdEUmXI-y2yHSidMtZpubvRGoNm1b4ago1i9gfrotaVQgoH31QiasJlRj3gDFUNW3Uxytkkdc XL8OJpboWNlWu7cqjqX8KPvvXbMsRxmo8HGIUw_jHp5e_t83P98HHxRPHiVy-_9LwBwoF7nGvxCaCCpPM9AVbo4AMgk4C2bNIFOU3-Af534Lwk4kK8AivA_Ae7LMyFLXAgPMKFdfHQyLrFVYEtTr0_SFlsMSoC1snG1a3VWE4lUFXcBynWZMFLGgl_VwlLV5GgkjnVF_L4uVS16JeS-Wagz3y0kv1v43ZEy_z_SExE8bvX7Etfv4J2BDENBkgY7iuO2EBJO4bM7IZNXUbbu26C_0n1EieCqHEz3Fgn61tNNwb74ZYo3m9mf3HwNXBWP3B7B9nkMxMiuy1JXGbJnTlMsZltkQUU818WZMgc70DBlHzRUnKxWuVFgEe2xKCJvut9pOT-6T72aaOnUgS4-DHnLDUv2mMu9HD5jHempcad3RcN5Cc9w7t2SHGPexJbPJrkbrZOC8RGumIu6_qIHbWDUYsWRA7fiQkj2mfO0HZ2ODQm1ur-0yjYzDUrkLzB2HjBSR-NBaMe2Tomuns8Ka4l37e9m3n4Am6UKZw!!/dl4/d5/L2dBISEvZ0FBIS9nQSEh/.

Bartón, K.. MuMIn: Multi-model inference. R package version 1.47.5. https://CRAN.R-project.org/package=MuMIn.

Bavumiragira, J.P., Ge, J., Yin, H., 2022. Fate and transport of pharmaceuticals in water systems: a processes review. Sci. Total Environ. 823, 153635 https://doi.org/10.1016/j.scitotenv.2022.153635.

Bayen, S., Zhang, H., Desai, M.M., Ooi, S.K., Kelly, B.C., 2013. Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: influence of hydrodynamics and physical–chemical properties. Environ. Pollut. 182, 1–8. https://doi.org/10.1016/j.envpol.2013.06.028.

Bertram, M.G., Martin, J.M., McCallum, E.S., Alton, L.A., Brand, J.A., Brooks, B.W., Cerveny, D., Fick, J., Ford, A.T., Hellström, G., Michelangeli, M., Nakagawa, S., Polverino, G., Saaristo, M., Sih, A., Tan, H., Tyler, C.R., Wong, B.B.M., Brodin, T., 2022. Frontiers in quantifying wildlife behavioural responses to chemical pollution. Biol. Rev. 46, 1346–1364. https://doi.org/10.1111/brv.12844.

Boucek, R.E., Lewis, J.P., Stewart, B.D., Jud, Z.R., Carey, E., Adams, A.J., 2019. Measuring site fidelity and homesite-to-pre-spawning site connectivity of bonefish (*Albula vulpes*): using mark-recapture to inform habitat conservation. Environ. Biol. Fish. 102, 185–195. https://doi.org/10.1007/s10641-018-0827-y.

Boucek, R.E., Rehage, J.S., Castillo, N.A., Dwoskin, E., Lombardo, S.M., Santos, R., Navarre, C., Larkin, M., Adams, A.J., 2022. Using recreational tournament records to construct a 53 - year time series of the Florida Keys recreational Bonefish fishery. Environ. Biol. Fish. https://doi.org/10.1007/s10641-022-01299-5.

Bradley, P.M., Padilla, I.Y., Romanok, K.M., Smalling, K.L., Focazio, M.J., Breitmeyer, S. E., Cardon, M.C., Conley, J.M., Evans, N., Givens, C.E., Gray, J.L., Gray, L.E., Hartig, P.C., Higgins, C.P., Hladik, M.L., Iwanowicz, L.R., Lane, R.F., Loftin, K.A., McCleskey, R.B., McDonough, C.A., Medlock-Kakaley, E., Meppelink, S., Weis, C.P., Wilson, V.S., 2021. Pilot-scale expanded assessment of inorganic and organic tapwater exposures and predicted effects in Puerto Rico, USA. Sci. Total Environ. 788, 147721 https://doi.org/10.1016/j.scitotenv.2021.147721.

Branchet, P., Arpin-Pont, L., Piram, A., Boissery, P., Wong-Wah-Chung, P., Doumenq, P., 2021. Pharmaceuticals in the marine environment: what are the present challenges in their monitoring? Sci. Total Environ. 766, 142644 https://doi.org/10.1016/j.scitotenv.2020.142644.

Brown, A.R., Gunnarsson, L., Kristiansson, E., Tyler, C.R., 2014. Assessing variation in the potential susceptibility of fish to pharmaceuticals, considering evolutionary differences in their physiology and ecology. Phil. Trans. Biol. Sci. 369, 20130576 https://doi.org/10.1098/rstb.2013.0576.

Brownscombe, J.W., Cooke, S.J., Danylchuk, A.J., 2017. Spatiotemporal drivers of energy expenditure in a coastal marine fish. Oecologia 183, 689–699. https://doi.org/10.1007/s00442-016-3800-5.

Brownscombe, J.W., Griffin, L.P., Gagne, T.O., Haak, C.R., Cooke, S.J., Finn, J.T., Danylchuk, A.J., 2019. Environmental drivers of habitat use by a marine fish on a heterogeneous and dynamic reef flat. Mar. Biol. 166, 1–13. https://doi.org/10.1007/s00227-018-3464-2.

Brumovský, M., Bečanová, J., Kohoutek, J., Borghini, M., Nizzetto, L., 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean. Environ. Pollut. 229, 976–983. https://doi.org/10.1016/j.envpol.2017.07.082.

Bouzas-Monroy, A., Wilkinson, J.L. Melling, M., Boxall, A.B.A., 2022. Assessment of the potential ecotoxicological effects of pharmaceuticals in the world's rivers. Environmental Toxicology and Chemistry. 41(8), 2008-2020. doi: 10.1002/etc.5355.

Bu, Q., Shi, X., Yu, G., Huang, J., Wang, B., 2016. Assessing the persistence of pharmaceuticals in the aquatic environment: challenges and needs. Emerging Contam. 2, 145–147. https://doi.org/10.1016/j.emcon.2016.05.003.

Burns, E.E., Carter, L.J., Kolpin, D.W., Thomas-Oates, J., Boxall, A.B.A., 2018. Temporal and spatial variation in pharmaceutical concentrations in an urban river system. Water Res. 137, 72–85. https://doi.org/10.1016/j.watres.2018.02.066.

Campbell, L.J., Castillo, N.A., Shenker, J., Owens, L.A., Santos, R.O., Adams, A.J., Rehage, J.S., Denton, K.E., Goldberg, T.L., 2022. Bone appétit: DNA metabarcoding as a non-lethal alternative to morphological dietary assessment in Atlantic bonefish (*Albula vulpes*). Environ. Biol. Fish. https://doi.org/10.1007/s10641-022-01328-3.

Castillo, N.A., Santos, R.O., James, W.R., Rezek, R., Cerveny, D., Boucek, R.E., Adams, A. J., Fick, J., Brodin, T., Rehage, J.S., [Preprint]. March 26, 2023. Widespread pharmaceutical exposure at concentrations of concern for a subtropical coastal fishery: Bonefish (*Albula vulpes*). https://doi.org/10.2139/ssrn.4394268.

Cejas, M.J., 2010. Sucralose : a Unique Tracer for Wastewater Intrusion (Master of Science Chemistry). Florida International University. https://doi.org/10.25148/etd.FI14060126.

Cerveny, D., Fick, J., Klaminder, J., McCallum, E.S., Bertram, M.G., Castillo, N.A., Brodin, T., 2021. Water temperature affects the biotransformation and accumulation of a psychoactive pharmaceutical and its metabolite in aquatic organisms. Environ. Int. 155, 106705 https://doi.org/10.1016/j.envint.2021.106705.

Chaturvedi, P., Shukla, P., Giri, B.S., Chowdhary, P., Chandra, R., Gupta, P., Pandey, A., 2021. Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: a review on emerging contaminants. Environ. Res. 194, 110664 https://doi.org/10.1016/j.envres.2020.110664.

Chen, L., Guo, C., Sun, Z., Xu, J., 2021. Occurrence, bioaccumulation and toxicological effect of drugs of abuse in aquatic ecosystem: a review. Environ. Res. 200, 111362 https://doi.org/10.1016/j.envres.2021.111362.

Colton, D.E., Alevizon, W.S., 1983. Feeding ecology of bonefish in Bahamian waters. Trans. Am. Fish. Soc. 112, 178–184. https://doi.org/10.1577/1548-8659(1983)112<178:feobib>2.0.co;2.

Crabtree, R.E., Stevens, C., Snodgrass, D., Stengard, F.J., 1998. Feeding habits of bonefish, *Albula vulpes*, from the waters of the Florida Keys. Fish. Bull. 96, 754–766.

Dehm, J., Singh, S., Ferreira, M., Piovano, S., Fick, J., 2021. Screening of pharmaceuticals in coastal waters of the southern coast of Viti Levu in Fiji, South Pacific. Chemosphere 276, 130161. https://doi.org/10.1016/j.chemosphere.2021.130161.

Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A., Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., Brooks, B.W., 2014a. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. Phil. Trans. Biol. Sci. 369 https://doi.org/10.1098/rstb.2014.0058.

Duarte, B., Gameiro, C., Matos, A.R., Figueiredo, A., Silva, M.S., Cordeiro, C., Caçador, I., Reis-Santos, P., Fonseca, V., Cabrita, M.T., 2021. First screening of biocides, persistent organic pollutants, pharmaceutical and personal care products in Antarctic phytoplankton from Deception Island by FT-ICR-MS. Chemosphere 274, 129860. https://doi.org/10.1016/j.chemosphere.2021.129860.

Ebele, A.J., Oluseyi, T., Drage, D.S., Harrad, S., Abou-Elwafa Abdallah, M., 2020. Occurrence, seasonal variation and human exposure to pharmaceuticals and personal care products in surface water, groundwater and drinking water in Lagos State, Nigeria. Emerging Contam. 6, 124–132. https://doi.org/10.1016/j.emcon.2020.02.004.

Fabbri, E., 2015. Pharmaceuticals in the environment: expected and unexpected effects on aquatic fauna: adverse effects of environmental pharmaceuticals. Ann. N. Y. Acad. Sci. 1340, 20–28. https://doi.org/10.1111/nyas.12605.

Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: focus on exposure and biological effects in animal species. Environ. Toxicol. Chem. 35, 799–812. https://doi.org/10.1002/etc.3131.

Fahlman, J., Fick, J., Karlsson, J., Jonsson, M., Brodin, T., Klaminder, J., 2018. Using laboratory incubations to predict the fate of pharmaceuticals in aquatic ecosystems. Environ. Chem. 15, 463–471. https://doi.org/10.1071/EN18154.

Fick, J., Lindberg, R.H., Tysklind, M., Larsson, D.G.J., 2010. Predicted critical environmental concentrations for 500 pharmaceuticals. Regul. Toxicol. Pharmacol. 58, 516–523. https://doi.org/10.1016/J.YRTPH.2010.08.025.

Fonseca, V.F., Reis-Santos, P., Duarte, B., Cabral, H.N., Caçador, M.I., Vaz, N., Dias, J.M., Pais, M.P., 2020. Roving pharmacies: modelling the dispersion of pharmaceutical contamination in estuaries. Ecol. Indicat. 115, 106437 https://doi.org/10.1016/j.ecolind.2020.106437.

Fox, J., Weisberg, S., 2008. An R companion to applied regression, Third Edition. Sage, Thousand Oaks, CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Gardinali, P.R., Zhao, X., 2002. Trace determination of caffeine in surface water samples by liquid chromatography - atmospheric pressure chemical ionization - mass spectrometry (LC-APCI-MS). Environ. Int. 28, 521–528. https://doi.org/10.1016/S0160-4120(02)00080-6.

Gaw, S., Thomas, K.V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Phil. Trans. Biol. Sci. 369, 20130572 https://doi.org/10.1098/rstb.2013.0572.

Grabic, R., Fick, J., Lindberg, R.H., Fedorova, G., Tysklind, M., 2012. Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry. Talanta 100, 183–195. https://doi.org/10.1016/j.talanta.2012.08.032.

Gros, M., Petrović, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36, 15–26. https://doi.org/10.1016/j.envint.2009.09.002.

Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Larsson, D.G.J., 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ. Sci. Technol. 42, 5807–5813. https://doi.org/10.1021/es8005173.

Henderson, A., Ng, B., Landeweer, S., Quinete, N., Gardinali, P., 2020. Assessment of sucralose, caffeine and acetaminophen as anthropogenic tracers in aquatic systems across Florida. Bull. Environ. Contam. Toxicol. 105, 351–357. https://doi.org/10.1007/s00128-020-02942-6.

Hong, B., Lin, Q., Yu, S., Chen, Yongshan, Chen, Yuemin, Chiang, P., 2018. Urbanization gradient of selected pharmaceuticals in surface water at a watershed scale. Sci. Total Environ. 634, 448–458. https://doi.org/10.1016/j.scitotenv.2018.03.392.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical Journal 50 (3), 346–363.

Huerta, B., Margiotta-Casaluci, L., Rodríguez-Mozaz, S., Scholze, M., Winter, M.J., Barceló, D., Sumpter, J.P., 2016. Anti-anxiety drugs and fish behavior: establishing the link between internal concentrations of oxazepam and behavioral effects. Environ. Toxicol. Chem. 35, 2782–2790. https://doi.org/10.1002/etc.3448.

Huggett, D.B., Cook, J.C., Ericson, J.F., Williams, R.T., 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. Hum. Ecol. Risk Assess. 9, 1789–1799. https://doi.org/10.1080/714044797.

Husk, B., Sanchez, J.S., Leduc, R., Takser, L., Savary, O., Cabana, H., 2019. Pharmaceuticals and pesticides in rural community drinking waters of Quebec, Canada – a regional study on the susceptibility to source contamination. Water Qual. Res. J. Can. 54, 88–103. https://doi.org/10.2166/wqrj.2019.038.

Im, J.K., Kim, S.H., Noh, H.R., Yu, S.J., 2020. Temporal-spatial variation and environmental risk assessment of pharmaceuticals in tributaries of the Han River watershed, South Korea. Sci. Total Environ. 741, 140486 https://doi.org/10.1016/j.scitotenv.2020.140486.

IQVIA Institute, 2022. The Global Use of Medicines 2022 Outlook to 2026.

IQVIA Institute, 2021. The Use of Medicines in the U.S. Spending and Using Trends and Outlook to 2025.

Jameel, Y., Valle, D., Kay, P., 2020. Spatial variation in the detection rates of frequently studied pharmaceuticals in Asian, European and North American rivers. Sci. Total Environ. 724, 137947 https://doi.org/10.1016/j.scitotenv.2020.137947.

Jiang, X., Zhu, Y., Liu, L., Fan, X., Bao, Y., Deng, S., Cui, Y., Cagnetta, G., Huang, J., Yu, G., 2020. Occurrence and variations of pharmaceuticals and personal-care products in rural water bodies: a case study of the Taige Canal (2018–2019). Science of the Total Environment 143138. https://doi.org/10.1016/j.scitotenv.2020.143138.

Kallenborn, R., Brorström-Lundén, E., Reiersen, L.O., Wilson, S., 2018. Pharmaceuticals and personal care products (PPCPs) in Arctic environments: indicator contaminants for assessing local and remote anthropogenic sources in a pristine ecosystem in change. Environ. Sci. Pollut. Control Ser. 25, 33001–33013. https://doi.org/10.1007/s11356-017-9726-6.

Kidd, K.A., Backhaus, T., Brodin, T., Inostroza, P.A., McCallum, E.S., 2023. Environmental risks of pharmaceutical mixtures in aquatic ecosystems: reflections on a decade of research. Enviro Toxic and Chemistry etc 5726. https://doi.org/10.1002/etc.5726.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999−2000: a national reconnaissance. Environ. Sci. Technol. 36, 1202–1211. https://doi.org/10.1021/es011055j.

Kötke, D., Gandrass, J., Xie, Z., Ebinghaus, R., 2019. Prioritised pharmaceuticals in German estuaries and coastal waters: occurrence and environmental risk assessment. Environ. Pollut. 255, 113161 https://doi.org/10.1016/j.envpol.2019.113161.

Kot-Wasik, A., Jakimska, A., Śliwka-Kaszyńska, M., 2016. Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. Environ. Monit. Assess. 188, 661. https://doi.org/10.1007/s10661-016-5637-0.

Kumar, M., Ngasepam, J., Dhangar, K., Mahlknecht, J., Manna, S., 2022. Critical review on negative emerging contaminant removal efficiency of wastewater treatment systems: concept, consistency and consequences. Bioresour. Technol. 352, 127054 https://doi.org/10.1016/j.biortech.2022.127054.

Kumar, M., Sridharan, S., Sawarkar, A.D., Shakeel, A., Anerao, P., Mannina, G., Sharma, P., Pandey, A., 2023. Current research trends on emerging contaminants pharmaceutical and personal care products (PPCPs): a comprehensive review. Sci. Total Environ. 859, 160031 https://doi.org/10.1016/j.scitotenv.2022.160031.

Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., Klaminder, J., 2016. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web - insights from a field experiment. Sci. Total Environ. 568, 208–215. https://doi.org/10.1016/j.scitotenv.2016.05.206.

Lara-Martín, P.A., Chiaia-Hernández, A.C., Biel-Maeso, M., Baena-Nogueras, R.M., Hollender, J., 2020. Tracing urban wastewater contaminants into the Atlantic Ocean by nontarget screening. Environ. Sci. Technol. 54, 3996–4005. https://doi.org/10.1021/acs.est.9b06114.

Larkin, M.F., 2011. Assessment of South Florida's Bonefish Stock, vol. 214. Dissertation. https://scholarship.miami.edu/discovery/fulldisplay/alma991031447484702976/01UOML_INST:ResearchRepository.

Larkin, M.F., Kroetz, A.M., Boucek, R.E., 2023. Bonefish do not respect international boerders: the Florida-Bahamas connection. Marine Biology 170, 149. https://doi.org/10.1007/s00227-023-04292-3.

Lenth, R.. emmeans: estimated marginal means, aka least-squares means. R package version 1.8.7. https://CRAN.R-project.org/package=emmeans.

Letsinger, S., Kay, P., Rodríguez-Mozaz, S., Villagrassa, M., Barceló, D., Rotchell, J.M., 2019. Spatial and temporal occurrence of pharmaceuticals in UK estuaries. Sci. Total Environ. 678, 74–84. https://doi.org/10.1016/j.scitotenv.2019.04.182.

Lindberg, R.H., Östman, M., Olofsson, U., Grabic, R., Fick, J., 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. 58, 221–229. https://doi.org/10.1016/j.watres.2014.03.076.

Liu, J.L., Wong, M.H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ. Int. 59, 208–224. https://doi.org/10.1016/j.envint.2013.06.012.

Lu, S., Lin, C., Lei, K., Wang, B., Xin, M., Gu, X., Cao, Y., Liu, X., Ouyang, W., He, M., 2020. Occurrence, spatiotemporal variation, and ecological risk of antibiotics in the water of the semi-enclosed urbanized Jiaozhou Bay in eastern China. Water Res. 184, 116187 https://doi.org/10.1016/j.watres.2020.116187.

Lüdecke, et al., 2021. performance: An R package for assessment, comparison and testing of statistical models. J. Open Source Software 6 (60), 2021, 3139. https://doi.org/10.21105/joss.03139.

Maasz, G., Mayer, M., Zrinyi, Z., Molnar, E., Kuzma, M., Fodor, I., Pirger, Z., Takács, P., 2019. Spatiotemporal variations of pharmacologically active compounds in surface waters of a summer holiday destination. Sci. Total Environ. 677, 545–555. https://doi.org/10.1016/j.scitotenv.2019.04.286.

Mandaric, L., Diamantini, E., Stella, E., Cano-Paoli, K., Valle-Sistac, J., Molins-Delgado, D., Bellin, A., Chiogna, G., Majone, B., Diaz-Cruz, M.S., Sabater, S., Barcelo, D., Petrovic, M., 2017. Contamination sources and distribution patterns of pharmaceuticals and personal care products in Alpine rivers strongly affected by tourism. Sci. Total Environ. 590, 484–494. https://doi.org/10.1016/j.scitotenv.2017.02.185. –591.

Martinez Arbizu, P., 2017. pairwiseAdonis: Pairwise multilevel comparison using Adonis. R package version 0.4.1.

Metcalfe, C.D., Beddows, P.A., Bouchot, G.G., Metcalfe, T.L., Li, H., Van Lavieren, H., 2011. Contaminants in the coastal karst aquifer system along the Caribbean coast of the Yucatan Peninsula, Mexico. Environ. Pollut. 159, 991–997. https://doi.org/10.1016/j.envpol.2010.11.031.

Mezzelani, M., Gorbi, S., Regoli, F., 2018. Pharmaceuticals in the aquatic environments: evidence of emerged threat and future challenges for marine organisms. Mar. Environ. Res. 140, 41–60. https://doi.org/10.1016/j.marenvres.2018.05.001.

Mezzelani, M., Regoli, F., 2021. The biological effects of pharmaceuticals in the marine environment. Ann. Rev. Mar. Sci 14, 1–24. https://doi.org/10.1146/annurev-marine-040821-075606.

Murchie, K.J., Cooke, S.J., Danylchuk, A.J., Danylchuk, S.E., Goldberg, T.L., Suski, C.D., Philipp, D.P., 2013. Movement patterns of bonefish (*Albula vulpes*) in tidal creeks and coastal waters of Eleuthera, the Bahamas. Fish. Res. 147, 404–412. https://doi.org/10.1016/j.fishres.2013.03.019.

Narita, K., Hoshide, S., Kario, K., 2021. Seasonal variation in blood pressure: current evidence and recommendations for hypertension management. Hypertens. Res. 44, 1363–1372. https://doi.org/10.1038/s41440-021-00732-z.

Ng, B., Quinete, N., Maldonado, S., Lugo, K., Purrinos, J., Briceño, H., Gardinali, P., 2021. Understanding the occurrence and distribution of emerging pollutants and endocrine disruptors in sensitive coastal South Florida Ecosystems. Sci. Total Environ. 757, 143720 https://doi.org/10.1016/j.scitotenv.2020.143720.

Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85, 50–59. https://doi.org/10.1016/j.marpolbul.2014.06.024.

Osorio, V., Larrañaga, A., Aceña, J., Pérez, S., Barceló, D., 2016. Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers. Sci. Total Environ. 540, 267–277. https://doi.org/10.1016/j.scitotenv.2015.06.143.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J., 2022. vegan: Community Ecology Package. R package version 2.6-4, https://CRAN.R-project.org/package=vegan.

Perez, A.U., Schmitter-Soto, J.J., Adams, A.J., 2021. The first estimate of interjurisdictional population dynamics for bonefish, *Albula vulpes*, a shared resource in the western Caribbean. Environ. Biol. Fish. 104, 341–356. https://doi.org/10.1007/s10641-021-01081-z.

Perez, A.U., Schmitter-Soto, J.J., Adams, A.J., Heyman, W.D., 2019. Connectivity mediated by seasonal bonefish (*Albula vulpes*) migration between the Caribbean Sea and a tropical estuary of Belize and Mexico. Environ. Biol. Fish. 102, 197–207. https://doi.org/10.1007/s10641-018-0834-z.

Persson, L., Carney Almroth, B.M., Collins, C.D., Cornell, S., De Wit, C.A., Diamond, M.L., Fantke, P., Hassellöv, M., MacLeod, M., Ryberg, M.W., Søgaard Jørgensen, P., Villarrubia-Gómez, P., Wang, Z., Hauschild, M.Z., 2022. Outside the safe operating space of the planetary boundary for novel entities. Environ. Sci. Technol. 56, 1510–1521. https://doi.org/10.1021/acs.est.1c04158.

Pina-Amargós, F., Figueredo-Martin, T., Pérez, A., Olivera-Espinosa, Y., Adams, A.J., 2023. The first examination of the movements of flats fishes to evaluate the effectiveness of marine protected areas in Cuba. Environ. Biol. Fish. 106, 147–160. https://doi.org/10.1007/s10641-022-01343-4.

Prichard, E., Granek, E.F., 2016. Effects of pharmaceuticals and personal care products on marine organisms: from single-species studies to an ecosystem-based approach. Environ. Sci. Pollut. Control Ser. 23, 22365–22384. https://doi.org/10.1007/s11356-016-7282-0.

Ramcharran, H., 2011. The pharmaceutical industry of Puerto Rico: ramifications of global competition. J. Pol. Model. 33, 395–406. https://doi.org/10.1016/j.jpolmod.2010.11.003.

Rand-Weaver, M., Margiotta-Casaluci, L., Patel, A., Panter, G.H., Owen, S.F., Sumpter, J. P., 2013. The read-across hypothesis and environmental risk assessment of pharmaceuticals. Environ. Sci. Technol. 47, 11384–11395. https://doi.org/10.1021/es402065a.

Rennert, J.J., 2017. Age and Growth of Bonefish, *Albula Species* Among Cuban Habitats. University of North Carolina Willmington.

Rennert, J.J., Shenker, J.M., Angulo-Valdés, J.A., Adams, A.J., 2019. Age, growth, and age at maturity of bonefish (*Albula species*) among Cuban habitats. Environ. Biol. Fish. 102, 253–265. https://doi.org/10.1007/s10641-018-0836-x.

Rockport Analytics, 2019. 2018 Tourism in the Florida Keys & Key West: Stable Growth Despite Challenging Times. Rockport Analytics.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., Arnold, K.E., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. Proc. Biol. sci. 285 https://doi.org/10.1098/rspb.2018.1297.

Schreiber, R., Gündel, U., Franz, S., Küster, A., Rechenberg, B., Altenburger, R., 2011. Using the fish plasma model for comparative hazard identification for pharmaceuticals in the environment by extrapolation from human therapeutic data. Regul. Toxicol. Pharmacol. 61, 261–275. https://doi.org/10.1016/J.YRTPH.2011.08.006.

Schulz, M., Schmoldt, A., Andresen-Streichert, H., Iwersen-Bergmann, S., 2020. Revisited: therapeutic and toxic blood concentrations of more than 1100 drugs and other xenobiotics. Crit. Care 24, 1–4. https://doi.org/10.1186/s13054-020-02915-5.

Scott, P.D., Bartkow, M., Blockwell, S.J., Coleman, H.M., Khan, S.J., Lim, R., McDonald, J.A., Nice, H., Nugegoda, D., Pettigrove, V., Tremblay, L.A., Warne, M. StJ., Leusch, F.D.L., 2014. A national survey of trace organic contaminants in Australian rivers. J. Environ. Qual. 43, 1702–1712. https://doi.org/10.2134/jeq2014.01.0012.

Sedvall, E., Fick, J., Pettersson, C., Hedeland, M., 2022. Pharmaceuticals are identified in insects in River Fyris – a study with both tandem quadrupole and quadrupole-time-of-flight mass spectrometry. Environ. Adv. 8, 100194 https://doi.org/10.1016/j.envadv.2022.100194.

Sherman, K.D., Shultz, A.D., Dahlgren, C.P., Thomas, C., Brooks, E., Brooks, A., Brumbaugh, D.R., Gittens, L., Murchie, K.J., 2018. Contemporary and emerging fisheries in the Bahamas—conservation and management challenges, achievements and future directions. Fish. Manag. Ecol. 25, 319–331. https://doi.org/10.1111/fme.12299.

Sui, Q., Huang, J., Deng, S., Chen, W., Yu, G., 2011. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ. Sci. Technol. 45, 3341–3348. https://doi.org/10.1021/es200248d.

Sumpter, J.P., Margiotta-Casaluci, L., 2022. Environmental occurrence and predicted pharmacological risk to freshwater fish of over 200 neuroactive pharmaceuticals in widespread use. Toxics 10, 233. https://doi.org/10.3390/toxics10050233.

Świacka, K., Maculewicz, J., Kowalska, D., Caban, M., Smolarz, K., Świeżak, J., 2022. Presence of pharmaceuticals and their metabolites in wild-living aquatic organisms – current state of knowledge. J. Hazard Mater. 424 https://doi.org/10.1016/j.jhazmat.2021.127350.

Tanabe, S., Ramu, K., 2012. Monitoring temporal and spatial trends of legacy and emerging contaminants in marine environment: results from the environmental specimen bank (es-BANK) of Ehime University, Japan. Mar. Pollut. Bull. 64, 1459–1474. https://doi.org/10.1016/j.marpolbul.2012.05.013.

Tang, J., Sun, J., Wang, W., Yang, L., Xu, Y., 2021. Pharmaceuticals in two watersheds in Eastern China and their ecological risks. Environ. Pollut. 277, 116773 https://doi.org/10.1016/j.envpol.2021.116773.

Treu, G., Slobodnik, J., Alygizakis, N., Badry, A., Bunke, D., Cincinelli, A., Claßen, D., Dekker, R.W.R.J., Göckener, B., Gkotsis, G., Hanke, G., Duke, G., Jartun, M., Movalli, P., Nika, M.-C., Rüdel, H., Tarazona, J.V., Thomaidis, N.S., Tornero, V., Dulio, V., 2022. Using environmental monitoring data from apex predators for chemicals management: Towards better use of monitoring data from apex predators in support of prioritisation and risk assessment of chemicals in Europe. Environmental Sciences Europe 34 (1), 82. https://doi.org/10.1186/s12302-022-00665-5.

Valdez-Carrillo, M., Abrell, L., Ramírez-Hernández, J., Reyes-López, J.A., Carreón-Diazconti, C., 2020. Pharmaceuticals as emerging contaminants in the aquatic environment of Latin America: a review. Environ. Sci. Pollut. Res. 27, 44863–44891. https://doi.org/10.1007/s11356-020-10842-9.

Valenti, T.W., Gould, G.G., Berninger, J.P., Connors, K.A., Keele, N.B., Prosser, K.N., Brooks, B.W., 2012. Human therapeutic plasma levels of the selective serotonin reuptake inhibitor (SSRI) sertraline decrease serotonin reuptake transporter binding and shelter-seeking behavior in adult male fathead minnows. Environ. Sci. Technol. 46, 2427–2435. https://doi.org/10.1021/es204164b.

Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with S, Fourth Edition. Springer, New York, ISBN 0-387-95457-0.

Wade, C., Otero, E., Poon-Kwong, B., Rozier, R., Bachoon, D., 2015. Detection of human-derived fecal contamination in Puerto Rico using carbamazepine, HF183 Bacteroides, and fecal indicator bacteria. Mar. Pollut. Bull. 101, 872–877. https://doi.org/10.1016/j.marpolbul.2015.11.016.

Wang, C., 2012. Assessment of the Occurrence and Potential Effects of Pharmaceuticals and Personal Care Products in South Florida Waters and Sediments.

Wang, C., Gardinali, P.R., 2013. Detection and occurrence of microconstituents in reclaimed water used for irrigation - a potentially overlooked source. Anal. Bioanal. Chem. 405, 5925–5935. https://doi.org/10.1007/s00216-013-6799-z.

Wang, J., Gardinali, P.R., 2013. Uptake and depuration of pharmaceuticals in reclaimed water by mosquito fish (*Gambusia holbrooki*): a worst-case, multiple-exposure scenario. Environ. Toxicol. Chem. 32, 1752–1758. https://doi.org/10.1002/etc.2238.

Wang, J., Gardinali, P.R., 2012. Analysis of selected pharmaceuticals in fish and the fresh water bodies directly affected by reclaimed water using liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 404, 2711–2720. https://doi.org/10.1007/s00216-012-6139-8.

Wilkinson, J., Hooda, P.S., Barker, J., Barton, S., Swinden, J., 2017. Occurrence, fate and transformation of emerging contaminants in water: an overarching review of the

field. Environ. Pollut. 231, 954–970. https://doi.org/10.1016/j.envpol.2017.08.032.

Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M., Luarte, T., Petrosyan, V., Perikhanyan, Y., Mahon, C.S., McGurk, C.J., Hofmann, T., Kormoker, T., Iniguez, V., Guzman-Otazo, J., Tavares, J. L., Gildasio De Figueiredo, F., Razzolini, M.T.P., Dougnon, V., Gbaguidi, G., Traoré, O., Blais, J.M., Kimpe, L.E., Wong, M., Wong, D., Ntchantcho, R., Pizarro, J., Ying, G.-G., Chen, C.-E., Páez, M., Martínez-Lara, J., Otamonga, J.-P., Poté, J., Ifo, S. A., Wilson, P., Echeverría-Sáenz, S., Udikovic-Kolic, N., Milakovic, M., Fatta-Kassinos, D., Ioannou-Ttofa, L., Belušová, V., Vymazal, J., Cárdenas-Bustamante, M., Kassa, B.A., Garric, J., Chaumot, A., Gibba, P., Kunchulia, I., Seidensticker, S., Lyberatos, G., Halldórsson, H.P., Melling, M., Shashidhar, T., Lamba, M., Nastiti, A., Supriatin, A., Pourang, N., Abedini, A., Abdullah, O., Gharbia, S.S., Pilla, F., Chefetz, B., Topaz, T., Yao, K.M., Aubakirova, B., Beisenova, R., Olaka, L., Mulu, J. K., Chatanga, P., Ntuli, V., Blama, N.T., Sherif, S., Aris, A.Z., Looi, L.J., Niang, M., Traore, S.T., Oldenkamp, R., Ogunbanwo, O., Ashfaq, M., Iqbal, M., Abdeen, Z., O'Dea, A., Morales-Saldaña, J.M., Custodio, M., de la Cruz, H., Navarrete, I., Carvalho, F., Gogra, A.B., Koroma, B.M., Cerkvenik-Flajs, V., Gombač, M., Thwala, M., Choi, K., Kang, H., Ladu, J.L.C., Rico, A., Amerasinghe, P., Sobek, A., Horlitz, G., Zenker, A.K., King, A.C., Jiang, J.-J., Kariuki, R., Tumbo, M., Tezel, U., Onay, T.T., Lejju, J.B., Vystavna, Y., Vergeles, Y., Heinzen, H., Pérez-Parada, A., Sims, D.B., Figy, M., Good, D., Teta, C., 2022. Pharmaceutical pollution of the world's rivers. Proc. Natl. Acad. Sci. USA 119, e2113947119. https://doi.org/10.1073/pnas.2113947119.

Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio) accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864–875. https://doi.org/10.1016/j.envpol.2017.11.090.

Zandaryaa, S., Frank-Kamenetsky, D., 2021. A source-to-sea approach to emerging pollutants in freshwater and oceans: pharmaceuticals in the Baltic Sea region. Water Int. 46, 195–210. https://doi.org/10.1080/02508060.2021.1889867.

Zhu, Y., Snape, J., Jones, K., Sweetman, A., 2019. Spatially explicit large-scale environmental risk assessment of pharmaceuticals in surface water in China. Environ. Sci. Technol. 53, 2559–2569. https://doi.org/10.1021/acs.est.8b07054.