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Widespread pharmaceutical exposure at concentrations of concern for a subtropical coastal fishery: Bonefish (*Albula vulpes*)

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ABSTRACT

Pharmaceuticals have been acknowledged as an important contaminant of emerging concern with the potential to cause adverse effects in exposed fauna. Most research has focused on temperate freshwater systems; therefore, there is a pressing need to quantify pharmaceutical exposure in subtropical coastal marine systems. This study investigated the prevalence of pharmaceutical exposure to bonefish (*Albula vulpes*) in subtropical South Florida, USA, and evaluated the relative risk of detected concentrations to elicit pharmacological effects. The influence of sampling region, season (within or outside spawning season), and bonefish length on pharmaceutical assemblage, detection frequency, and risk was assessed. Both spatial (multiple regions) and temporal (spawning season) components were considered in order to incorporate bonefish biology biological in our exploration of pharmaceutical exposure and potential risk of effect. To quantify risk of pharmacological effects, concentrations were compared to a 1/3 threshold of the human therapeutic plasma concentration (H_TPC). In total, 53 different pharmaceuticals were detected with an average of 7.1 pharmaceuticals per bonefish and 52.3 % had at least one pharmaceutical exceeding the 1/3 H_TPC threshold. The presence of pharmaceutical cocktails at concentrations capable of eliciting pharmacological effects is of particular concern considering the potential for unknown interactions. For exposure and risk of pharmacological effect, region and season were significant, while bonefish length was not. Pharmaceutical exposure and risk were highest in the most remote sampling region. Results establish pharmaceuticals' widespread prevalence in subtropical coastal marine ecosystems, exposure and risk to biota, and the necessity to examine marine systems.

1. Introduction

Over recent years, pharmaceuticals have become an important class of emerging contaminants worldwide. There is now substantial evidence for the presence of pharmaceuticals in the aquatic environment, and for the potential of harmful effects on exposed fauna (Arnold et al., 2014; Branchet et al., 2021; Brodin et al., 2013; Hellström et al., 2016; López-Pacheco et al., 2019; Madikizela et al., 2020; Mezzelani et al., 2018; Świacka et al., 2022). A recent survey of 258 rivers in 104 countries found that pharmaceuticals pose a global threat to aquatic systems, with detections in all but two rivers, and with 25 % of samples having at least one pharmaceutical at a concentration exceeding safe levels for aquatic organisms (Bouzas-Monroy et al., 2022; Wilkinson et al., 2022).

The overwhelming majority of pharmaceutical pollution studies in aquatic environments focus on freshwater riverine systems (Świacka et al., 2022). In contrast, few studies address pharmaceutical pollution in marine and coastal systems (Branchet et al., 2021; Mezzelani et al., 2018; Miller et al., 2018; Ojemaye and Petrik, 2019), and most marine

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and coastal studies have been conducted in temperate regions (Maranho et al., 2021). Yet, connectivity between freshwater and coastal marine systems directly places receiving marine waters at risk of contamination (Bonnefille et al., 2018; Zandaryaa and Frank-Kamenetsky, 2021). In particular, since over 40 % of the world's population lives within 100 km of a coastline, and tropical regions have experienced the most rapid urbanization, tropical marine systems are at a heightened risk of pharmaceutical contamination (Day et al., 2021; Finkl and Makowski, 2019). Furthermore, their presence in offshore seawater (>50 km from shore) suggests a potential ubiquitous presence of pharmaceuticals in open marine environments (Alygizakis et al., 2016; Biel-Maeso et al., 2018; Nödler et al., 2014). Remote coastal systems are also not free from pharmaceutical exposure. A recent study of coastal Antarctic phytoplankton identified over 40 different pharmaceutical compounds at concentrations capable of ecosystem-wide trophic structure alteration (Duarte et al., 2021). Thus, there is a need for additional comprehensive monitoring of pharmaceuticals in open coastal and marine environments, including those located in tropical and subtropical regions.

There is also a need to go beyond documenting the presence of pharmaceuticals to assessing the potential for deleterious effects on exposed marine fauna (Branchet et al., 2021; Miller et al., 2018). Although concentrations of pharmaceuticals in water and sediment are generally in the low ng/L range, without an immediate lethal risk (Fabbri and Franzellitti, 2016), pharmaceuticals can bioaccumulate in fish and other marine biota to sublethal concentrations capable of eliciting effects such as behavioral and physiological alterations, with ecosystem-wide repercussions (Brodin et al., 2013; Miller et al., 2021; Moreno-González et al., 2016; Saaristo et al., 2018). For instance, marine bivalves, a key food source supporting marine food webs, bioconcentrate anti-inflammatory pharmaceuticals to concentrations causing sublethal effects (Almeida et al., 2020), thus posing a threat to the entire marine ecosystem through trophic-transfer and biomagnification. Upper trophic levels in coastal food webs are also at risk. A pharmaceutical survey in Delaware Bay, USA, detected pharmaceutical concentrations in fish plasma that were orders of magnitude higher than concentrations in water, with several pharmaceuticals in plasma at concentrations exceeding a recommended safety factor for pharmaceuticals in humans (based on plasma concentrations; Bean et al., 2018).

In subtropical South Florida, USA, we are beginning to investigate the presence and risk of pharmaceuticals in coastal waters, but the extent of the problem is still poorly understood. Freshwater assessments detected pharmaceuticals in reclaimed water used in irrigation (Wang, 2012; Wang and Gardinali, 2013), and in mosquito fish (*Gambusia affinis*) collected from waterbodies directly affected by reclaimed water irrigation (Wang and Gardinali, 2012). Pharmaceuticals, hormones and steroids have been detected in water and sediment samples from the Miami River, a coastal urban river that runs through the city of Miami (Yang et al., 2015), and in two coastal Florida Keys locations (Singh et al., 2010). Additional water sampling has identified the presence of pharmaceuticals in the Florida Keys and Miami via proxies of wastewater contamination such as sucralose, caffeine, and paracetamol (Cejas, 2010; Gardinali and Zhao, 2002; Henderson et al., 2020). In the most comprehensive assessment of coastal South Florida, Ng et al. (2021) collected water samples at 29 locations along a gradient of heavily-urbanized to remote coastal areas and analyzed them for the presence of 6 hormones and endocrine disrupting compounds, and 4 pharmaceuticals. Using a hazard quotient assessment, Ng et al. (2021) found that all but one location had compounds high enough to elicit endocrine disruption effects on aquatic organisms. These studies establish the presence of pharmaceuticals in South Florida coastal waters, likely posing a risk for marine biota; however, more investigation is needed to understand overall exposure and risk.

In this study, our goal was to examine the extent to which a coastal South Florida fish is exposed to pharmaceutical contaminants. We selected bonefish (*Albula vulpes*) due to their reliance on coastal and nearshore habitats, potential high risk of exposure, and high socioeconomic value as a recreational fishery. Further, as a mobile higher order consumer, bonefish can provide spatiotemporal information on pharmaceutical contamination beyond that possible from water and sediment samples alone (Treu et al., 2022). Our study addressed two questions: 1) To what extent are bonefish exposed to pharmaceutical contaminants across South Florida and what factors drive the observed patterns in exposure?; and 2) Are bonefish exposed to concentrations high enough to pose a risk of pharmacological effect, and what factors drive that risk? To address these questions, we sampled bonefish blood plasma across five coastal regions, expanding 330 km of the South Florida coastline. To assess the relative risk of pharmacological effects posed by the detected pharmaceutical concentrations, we compared the measured plasma concentrations to a 1/3 threshold of the human therapeutic plasma concentration (H_TPC; Castillo et al., 2024a). We hypothesized that: 1) Bonefish exposure would be high in highly urbanized areas and decrease in areas less densely populated and remote sampling regions; 2) Pharmaceuticals would be detected at concentrations capable of eliciting pharmacological effects, with the highest risk in densely urbanized areas; and 3) Exposure and risk would also be affected by timing of sampling and fish body size (bonefish total length).

2. Material and methods

2.1. Study species

We selected bonefish (*Albula vulpes*), as their ecology makes them particularly susceptible to exposure of pharmaceutical contaminants. Bonefish are 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). Aside from their planktonic larval phase, bonefish rely upon 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 (Brownscombe et al., 2017, 2019; Larkin, 2011). In the Florida Keys region of South Florida, the estimated economic impact of the catch-and-release flats fishery, which includes bonefish, tarpon (*Megalops atlanticus*), and permit (*Trachinotus falcatus*), is $473 million USD annually (Fedler, 2013; Smith et al., 2023). Anglers and researchers have observed a decline of unknown cause in the South Florida bonefish population beginning in the mid-1990s, while populations elsewhere in the Caribbean Sea have remained relatively stable (Boucek et al., 2022; Rehage et al., 2019; Santos et al., 2017, 2019). Mark recapture studies in Belize, Bahamas, and Cuba show that bonefish appear to exhibit a high degree of site fidelity, with an average home range of 10 km (Boucek et al., 2019; Murchie et al., 2013; Perez et al., 2019; Pina-Amargós et al., 2023), but recent South Florida tracking data shows larger movements above 100 km during the spawning season (October through April; Boucek et al., 2022). Prior to spawning, bonefish throughout South Florida migrate to a common location to form prespawning aggregations, after which they travel offshore and undergo a series of deep dives in order to disperse their larvae into the gulf stream (Lombardo et al., 2020). During a spawning season, bonefish have exhibited long-range migrations transiting multiple regions considered in this study, such that bonefish originally tagged in the Lower Keys have been detected in Biscayne Bay during spawning season and then returned to the Lower Keys outside of spawning season (Fig. 1; Boucek et al., 2022). These long-range migrations and increased mobility during the spawning season could increase pharmaceutical exposure, yet decrease variation in exposure across individuals, as individuals are more likely to be exposed to the same sources of contaminants. In addition to exposure via respiration, bonefish primarily feed on seagrass 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 and invertebrate organisms tend to have higher levels of pharmaceuticals (Du et al., 2014a; Lagesson et al., 2016; Miller et al., 2021; Zhang et al., 2020).

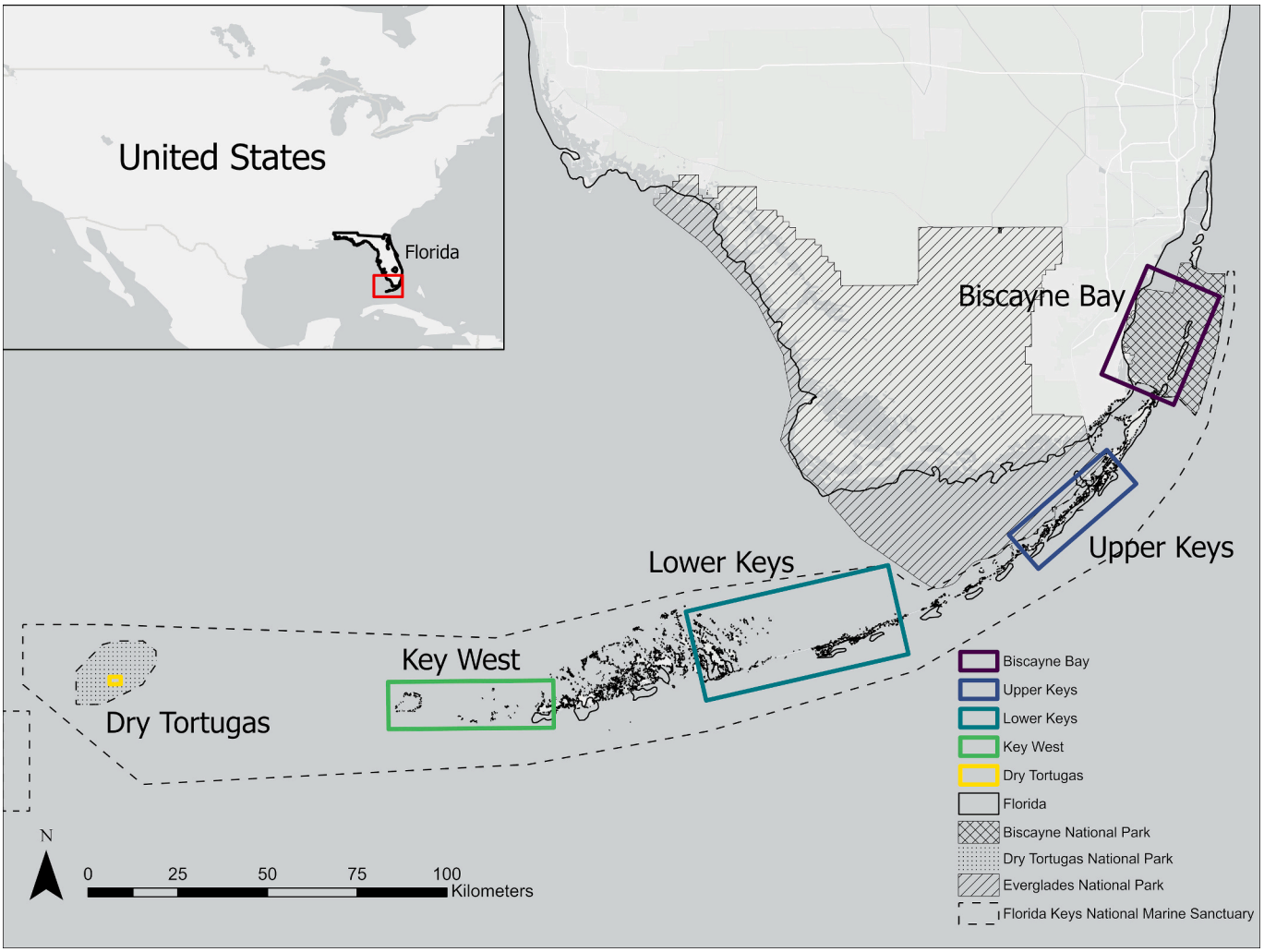

Fig. 1. Map of the five South Florida sampling regions. Exact bonefish sampling locations are omitted due to their status as a prohibited and protected species and the sensitive nature of the fishing locations.

2.2. Sampling regions

Bonefish plasma samples were collected over a 330 km expanse of coastal South Florida, from Biscayne Bay in the northeast to the Dry Tortugas in the southwest. The study area consisted of five sampling regions (Fig. 1) 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

Table 1
Sampling effort, summary of pharmaceutical findings, and characteristics of the five regions sampled. Shown are number of bonefish sampled per region, pharmaceutical detections (total and mean), 1/3 H_TPC exceedances (total, mean per bonefish), total number, and % with at least 1 1/3 H_TPC exceedance. Jurisdiction indicates the management entity of each region. FKNMS = Florida Keys National Marine Sanctuary.

Summary	Biscayne Bay	Upper Keys	Lower Keys	Key West	Dry Tortugas	Total
Total bonefish	24	23	16	15	15	93
Total detections	191	138	93	80	158	660
Mean detections	8.0	6.0	5.8	5.3	10.5	7.0
Total 1/3 H_TPC	24	12	12	14	33	95
Mean 1/3 H_TPC	1.00	0.52	0.75	0.93	2.20	1.02
Total with ≥1 1/3 H_TPC	13	9	9	4	14	49
% with ≥1 1/3 H_TPC	54.2 %	39.1 %	56.3 %	26.7 %	93.3 %	52.7 %
Jurisdiction	Biscayne National Park; State Waters	Everglades National Park; FKNMS	FKNMS	FKNMS	Dry Tortugas National Park	
Human population	2.7m[a]	18,943[a]	22,622[a]	33,555[a]	15[d]	2.8m
Annual visitation	700,000[c]	1.7m[b]	1.5m[b]	2.8m[b]	80,000[c]	6.1m
sq/mi	1899[a]	43[a]	62[a]	18[a]	0.06[c]	2022
People per sq/mi	1,424[a]	441[a]	364[a]	1,829[a]	15[d]	1372

[a] https://censusreporter.org/.
[b] Rockport Analytics (2019).
[c] Thomas et al. (2021).
[d] http://ournationalparks.us.

bonefish fishery (Boucek et al., 2022). The five regions included from north to south: Biscayne Bay, Upper Keys, Lower Keys, areas west of Key West (hereafter referred to as Key West), and the Dry Tortugas (Fig. 1, Table 1). Each region has varying degrees of urbanization and anthropogenic influence, allowing us to examine a wide range of pharmaceutical pollution exposure and risk across a large geographic scale. Biscayne Bay spans the length of Miami-Dade County, the most populous county in Florida (Browder et al., 2005; U. S. Census Bureau, 2021), bordered by the city of Miami, and contains Biscayne National Park (BNP; Browder et al., 2005). Of the five regions, Biscayne Bay is the most densely populated region, with a population of 2.7 million, and annual visitation of 700,000 people to BNP (Thomas et al., 2021). The bonefish fishery in Biscayne Bay remained the most stable during the historic bonefish decline (Rehage et al., 2019).

The Upper Keys, Lower Keys, and Key West (Monroe County, FL, USA) 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). The Upper Keys, which receives 1.7 million visitors annually, consists of a string of islands with Florida Bay to the northwest and the Atlantic Ocean to the southeast. The Upper Keys was historically the epicenter of the South Florida bonefish fishery but experienced the most severe decline of all regions in bonefish size, quantity, and fishing quality (Larkin, 2011; Larkin et al., 2010; Rehage et al., 2019; Santos et al., 2019). The Lower Keys experiences a lower degree of anthropogenic influence and development, and receives 1.4 million visitors annually (Rockport Analytics, 2019). At a population of 26,000, Key West has a comparatively small land mass to population ratio but experiences the largest number of annual visitors within the Keys region at 1.8 million (Rockport Analytics, 2019; U. S. Census Bureau, 2021). Finally, located 120 km west of Key West, the Dry Tortugas and Dry Tortugas National Park (DRTO) encompass 119 km^2 of protected no-take preserves (Schmidt et al., 2004). The Dry Tortugas is the most remote and least populous region with <10 permanent residents and 80,000 visitors annually (Table 1; Thomas et al., 2021).

2.3. Sample collection

Bonefish were collected throughout the five regions using hook and line angling between January 2019 and December 2020 (n = 86 bonefish), and July–September 2021 (n = 7 bonefish). All fish were captured from shallow, nearshore seagrass habitats (<10 m to 15 km from a shoreline with human presence and potential pharmaceutical exposure; 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. 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 >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), and then 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) until plasma was clear. 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 of 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 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 are provided here, and additional details are provided in Grabic et al. (2012), Lindberg et al. (2014), and Sedvall et al. (2022).

Reference standards 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 ultrapure water system (Millipore, Billerica, USA). Formic acid at 0.1 % (Sigma-Aldrich, Steinheim, Germany) was used to prepare the mobile chromatographic phases.

Plasma samples (20 μL) were pretreated by adding 50 ng of each internal surrogate standard, 50 μl methanol and 20 μl of water (with 0.1 % formic acid). Samples were then frozen at −18 °C for 1 h and 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). A gradient of methanol and acetonitrile in water (all solvents acidified with 0.1 % formic acid) was used for the elution of analytes, starting with 200 mL/min, 5 % methanol in water for 1 min, followed by a gradient change to 20/20/60 water/acetonitrile/methanol at a flow-rate of 250 mL/min over 8 min and a final gradient change to acetonitrile/methanol 40/60 at 300 mL/min 1 in 11 min. These parameters were held for 1 min and then changed back to starting conditions and held for 4 min. 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-1 was used for linearity evaluation and quantification. Carry-over effects were evaluated by injecting standards at 100 ng l^{-1} 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 relative risk posed to bonefish, defined by the potential for pharmacological effects from the detected pharmaceutical concentrations, we drew upon the Biological Read-Across Hypothesis, which asserts that therapeutic/pharmacological effects can occur in non-target organisms as a result of conservation of mammalian pharmaceutical target sites (Huggett et al., 2003). Application of this hypothesis to fish has been shown to be valid, since up to 86 % of human drug targets are conserved (Brown et al., 2014; Fabbri, 2015; Gould et al., 2021; Gunnarsson et al., 2008; Margiotta-Casaluci et al., 2014; Rand-Weaver et al., 2013). Further, significant behavioral and/or physiological alterations for fish can occur at internal plasma concentrations below the full human therapeutic plasma concentration (H_TPC), or the concentration required for a drug to elicit an effect in humans (Duarte et al., 2023; Gould et al., 2021; Huerta et al., 2016; Margiotta-

Casaluci et al., 2014; Sumpter and Margiotta-Casaluci, 2022). We selected a threshold of 1/3 the H_TPC as a conservative estimate of effect, following the methods outlined by Castillo et al. (2024a), and informed by Huerta et al. (2016), which found behavioral effects in fathead minnows (*Pimephales promelas*) that had plasma concentrations of oxazepam at 1/3 the H_TPC. Even though the utility of comparing measured pharmaceutical concentrations to their respective H_TPCs as an assessment of risk for pharmacological effect has been shown to be valid (Gould et al., 2021), it assumes the presence of the pharmaceutical's drug target, information that is absent for many pharmaceuticals. Due to this assumption, the incidence of a measured concentration exceeding the 1/3 H_TPC threshold does not definitively conclude that a pharmacological effect will occur, rather it indicates that the pharmaceutical has the capability of causing an effect, thus indicating potential risk posed by exposure. Given the large number of pharmaceuticals considered in this study, and the variation in effect concentrations of each pharmaceutical, utilization of the H_TPC as a comparison to measured concentrations and method to estimate risk of pharmacological effect allows for standardization of comparison across many different pharmaceuticals, despite the method's inherent assumption. 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 assess variation in the frequency of pharmaceuticals and 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 3 factors: region (for the five regions sampled), spawning season (i.e., whether sampling was conducted inside or outside the spawning season, October through April), and bonefish length (total length). All statistical analyses were performed using R v 4.3.1 (R Core Team, 2023).

2.6.1. Variation in the frequency of pharmaceutical detections

The influence of the region, spawning season, and bonefish length on the number of pharmaceuticals detected per bonefish was assessed with Generalized Linear Models (GLMs). Negative binomial distributions were used to account for overdispersion in the data. GLMs were performed using R package MASS (Venables and Ripley, 2002), and tests of model assumptions were performed using R package performance (Lüdecke et al., 2021). We compared the full model to each model with just region, season, or bonefish length using the corrected Akaike information criterion (AICc), using R package MuMIn (Bartón, 2022) and car (Fox and Weisberg, 2019). Models that fell within 4 AICc of the model with the lowest AICc were considered candidate top models. We then used parsimony to select the final model (Aho et al., 2014; Akaike, 1987; Anderson, 2008; Burnham and Anderson, 2004; Wong and Li, 1998). Pairwise comparisons of model contrasts for region were analyzed using Tukey's HSD tests in the R packages emmeans (Lenth, 2022), and multcomp (Hothorn et al., 2008).

The influence of region, spawning season, and bonefish length on the presence and absence of pharmaceuticals across all samples was further examined using Permutational Analysis of Variance (PERMANOVA) tests with 999 permutations and a Jaccard distance metric. Pairwise PERMANOVA tests were then conducted on significant main effects with 999 permutations and a Holm-Bonferroni *p*-value adjustment. Similarity in the presence/absence of pharmaceuticals were visually represented in multidimensional ordination space using non-metric multidimensional scaling (nMDS). PERMANOVAs 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 were also used to assess the influence of region, spawning season, and bonefish length on the number of 1/3 H_TPC exceedances per bonefish. Negative binomial distributions were used to account for overdispersion in the data. We compared the full model to each model with just region, spawning season, or bonefish length using the corrected Akaike information criterion (AICc). Models were compared as described above using AICc (Aho et al., 2014; Akaike, 1987; Anderson, 2008; Burnham and Anderson, 2004; Wong and Li, 1998). Pairwise comparisons of model contrasts for region were analyzed using Tukey's HSD tests.

To further assess the relative risk of pharmacological effects posed by pharmaceutical exposure in multivariate space using pharmaceutical concentrations, we calculated a proportion of the pharmaceutical concentration to the 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 their respective thresholds of effect, accounting for differences in concentrations necessary to elicit an effect unique to each pharmaceutical. PERMANOVA with a Bray-Curtis distance metric, 999 permutations, and a square-root transformation was then used to determine the influence of region, spawning season, and bonefish length. The dissimilarity among significant main effects was assessed using pairwise PERMANOVA with 999 permutations and a Holm-Bonferroni adjusted *p*-value. Variation in the 1/3 H_TPC proportions across all pharmaceuticals and all samples was visualized using a nMDS.

2.6.3. Influence of pharmaceutical identity on multivariate assemblages

The influence of individual pharmaceuticals in multivariate space on the observed pharmaceutical assemblage for both pharmaceutical presence and absence and the proportion of the 1/3 H_TPC analyses, explained by ordination scores, was calculated with 999 permutations, and 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 show the magnitude of the correlation between the ordination scores and the corresponding pharmaceutical. Arrow vectors point in the direction of 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 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 ≤ 0.01.

3. Results

3.1. Variation in pharmaceutical numbers across bonefish samples

Results indicate the widespread prevalence of pharmaceutical contamination throughout South Florida and for all bonefish. A total of 93 bonefish plasma samples were collected across the five South Florida regions (Fig. 1, Table 1). All samples had at least one pharmaceutical, with a maximum of 17 pharmaceuticals in an individual bonefish. On average, we detected 7.1 pharmaceuticals per bonefish (Table 1). Across all samples, 53 unique pharmaceuticals were detected, for a total of 660 pharmaceutical detections (Table S1). Of the 660 total detections, the 20 most frequently detected pharmaceuticals accounted for 551 (83.5 %) of all detections (Table 2).

Atenolol, venlafaxine, alfuzosin, trimethoprim and paracetamol were the top 5 most frequently detected pharmaceuticals across all samples (40.0 % of detections, Table 2), see Table S1 for a detailed summary of measured concentrations. Atenolol, a beta-1 selective blocker used in the management of hypertension and chronic angina, was detected in 67 bonefish (72.0 % of samples). Venlafaxine, a selective serotonin and norepinephrine reuptake inhibitor (SNRI) frequently

Table 2
Summary of the 20 most frequently detected pharmaceuticals across all bonefish samples (n = 93). There were 551 detections across the top 20, and 660 total detections across all bonefish. Percent reflects the percentage of bonefish with that pharmaceutical. Of the 20 most detected, 6 pharmaceuticals had the most 1/3 H_TPC exceedances, shown in bold, which accounted for 53 of the 95 total 1/3 H_TPC exceedances across all bonefish. Concentration range reflects the minimum and maximum detected concentration for each pharmaceutical.

Pharmaceutical	Total detections (%)	Total 1/3 H_TPC exceedances	Concentration range (ng/mL)
Atenolol	67 (72.0 %)	**14**	1.6–120
Venlafaxine	65 (69.9 %)	0	18–328.5
Alfuzosin	46 (49.5 %)	**7**	0.11–47.24
Trimethoprim	43 (46.2 %)	0	0.11–58
Paracetamol	40 (43.0 %)	0	1.8–690
Bisoprolol	34 (36.6 %)	2	0.11–10
Ranitidine	26 (28.0 %)	**10**	5.1–290
Risperidone	25 (26.9 %)	1	0.1–24
Diphenhydramine	24 (25.8 %)	0	0.06–4
Bupropion	22 (23.7 %)	3	0.18–39
Codeine	21 (22.6 %)	0	0.72–5.52
Fluconazole	20 (21.5 %)	0	0.52–25
Atracurium	19 (20.4 %)	0	0.59–8.2
Memantine	19 (20.4 %)	0	0.5–9.4
Naloxone	18 (19.4 %)	**6**	1.23–5.08
Hydroxyzine	13 (14.0 %)	**6**	0.32–120
Irbesartan	13 (14.0 %)	2	0.51–165.03
Clotrimazole	12 (12.9 %)	0	1.1–13
Flecainide	12 (12.9 %)	0	0.12–0.85
Haloperidol	12 (12.9 %)	**10**	0.16–3.5

prescribed for the treatment of major depressive disorder, generalized anxiety, social anxiety disorder and panic disorder, was detected in 65 bonefish (69.9 % of samples). The next three most frequently detected pharmaceuticals were alfuzosin, an alpha-1 adrenergic antagonist used in the symptomatic management of benign prostatic hypertrophy (BPH), detected in 46 bonefish (49.5 % of samples); trimethoprim, an antifolate antibiotic often used in combination with sulfamethoxazole to treat various infections, in 43 bonefish (46.2 % of samples); and paracetamol (acetaminophen), the most common analgesic worldwide, used in pain management and for its antipyretic effects (fever reducing), detected in 40 bonefish (43.0 % of samples).

3.2. Factors influencing pharmaceutical exposure

While pharmaceutical exposure was ubiquitous, regional differences were found. In terms of pharmaceutical detections, Biscayne Bay had the highest (191 detections), followed by the Dry Tortugas (158 detections), Upper Keys (138 detections), Lower Keys (93 detections), and Key West (80 detections); Table 1.

The model containing sampling region only was selected as the best model ($p < 0.001$, Table 3, Fig. 2), and Tukey pairwise comparisons indicated significant differences between the Dry Tortugas and three regions: Key West, Upper Keys, and Lower Keys (all $p < 0.001$, Table S2). These comparisons reveal a higher level of contamination in the Dry Tortugas (10.5 pharmaceuticals/bonefish), relative to the Florida Keys regions, where the average number of pharmaceuticals was below 6 for all regions combined (Fig. 2).

In multivariate ordination space, region ($p = 0.001$, Fig. 3a) and spawning season ($p = 0.02$, Fig. 3b) were significant drivers of the assemblage of pharmaceutical presence/absence (Table 4). Significant regional pairs, from highest to lowest r^2 value, were between Key West vs. Dry Tortugas, Biscayne Bay vs. Key West, Biscayne Bay vs. Upper Keys, Lower Keys vs. Key West, Upper Keys vs. Dry Tortugas, Upper Keys vs. Lower Keys and Upper Keys vs. Key West (all *adj.* $p = 0.01$, Table S3). A larger spread among points (bonefish) in multidimensional ordination space for samples collected outside the spawning season indicated a higher degree of variance in pharmaceutical assemblage relative to samples collected during spawning season (Fig. 3b). Six pharmaceuticals were found to influence the pharmaceutical assemblage with a *p*-value ≤ 0.01, and these were, in order of influence: atenolol, paracetamol, naloxone, codeine, ranitidine, and azithromycin (Fig. 3a, b). The separation between the Dry Tortugas and Key West and Upper Keys was driven primarily by the presence of paracetamol, while naloxone and codeine influenced the pharmaceutical assemblage along an opposite gradient, primarily driving the distinctiveness of Key West, Lower Keys, and Biscayne Bay (Fig. 3a, b).

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

The risk of pharmaceutical exposure, at concentrations exceeding the 1/3 H_TPC concentration, was present across all of South Florida. Across all regions, a total of 31 pharmaceuticals of the 53 detected (58.5 %), were observed at least once at a concentration exceeding the 1/3 H_TPC threshold. Of the 660 total detections, 95 were at concentrations in exceedance (14.5 %). Importantly, above half of the 93 bonefish sampled (49 bonefish, 52.7 %) had at least one pharmaceutical at a concentration above the 1/3 H_TPC threshold (Table 1, Table S1).

Atenolol, haloperidol, ranitidine, alfuzosin, hydroxyzine, naloxone, and metoprolol were the top 7 pharmaceuticals (in order of most to least) detected above the 1/3 H_TPC threshold (Table 2, Table S1). Atenolol was detected above a 1/3 H_TPC exceedance the most – in 14 bonefish (15.1 % of samples). Haloperidol, an antipsychotic agent used to treat schizophrenia and other psychoses, as well as symptoms of agitation, irritability and delirium, and ranitidine, a histamine H2 antagonist used to prevent and treat gastric-acid conditions (e.g., duodenal ulcers), both had 10 1/3 H_TPC exceedances (10.8 % of samples). Alfuzosin had 7 1/3 H_TPC exceedances (7.5 % of samples). Hydroxyzine, a histamine H_1-receptor antagonist used to treat anxiety and tension associated with psychoneuroses, as well as allergic conditions (e.g., urticaria); naloxone, an opioid receptor antagonist used to rapidly reverse an opioid overdose; and metoprolol, a selective beta-1 blocker used in the treatment of hypertension and angina, all had 6 exceedances (6.5 % of samples, Table S1).

3.4. Factors influencing exposure risk

Similar to the number of pharmaceuticals, 1/3 H_TPC exceedances varied spatially (Fig. 4). The model with region only was selected as the top model ($p = 0.007$, Table 3). Tukey pairwise comparisons indicated significant differences between Dry Tortugas and the Upper Keys ($p = 0.006$, Table S2). Dry Tortugas had an average of 2.2 1/3 H_TPC exceedances per bonefish, with 14 out of 15 bonefish having at least one pharmaceutical above the 1/3 H_TPC threshold (93.3 %, Table 1), while in the Upper Keys 1/3 H_TPC exceedances averaged 0.52 per bonefish

Table 3
Summary of the final GLM models for the number of pharmaceuticals per bonefish (Pharmaceutical Count) and the number of 1/3 H_TPC exceedances per bonefish.

Variable	Predictor	*p**	Null deviance	Residual deviance	AICc	χ^2	D^2
Pharmaceutical count	Region	3.2E−06***	125.4	94.5	471.4	30.9	0.20
1/3 H_TPC exceedances	Region	0.007**	107.1	93.0	260.7	14.1	0.08

*** *p*-Value < 0.001.
** *p*-Value < 0.01.
* *p*-Value < 0.05.

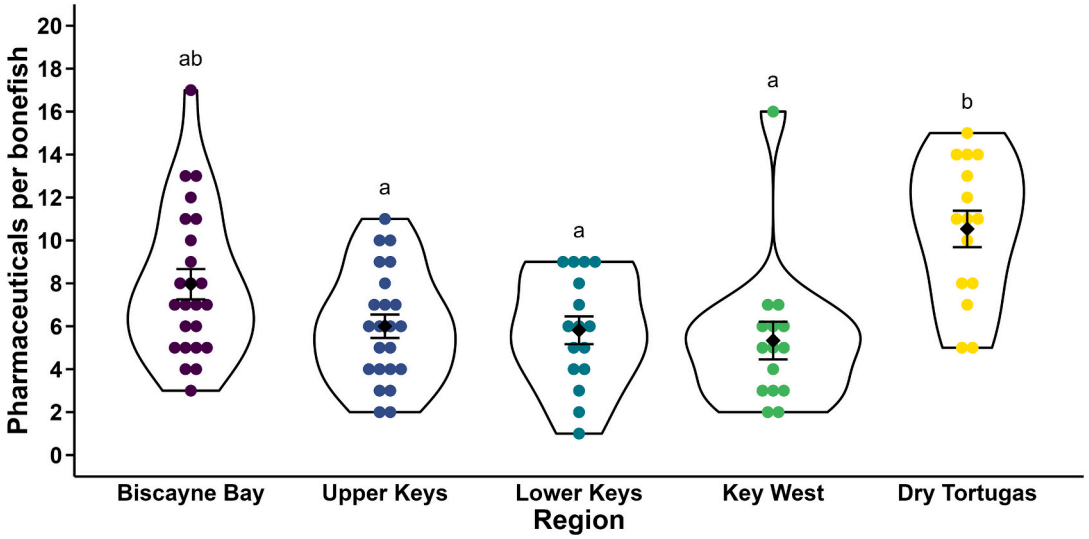

Fig. 2. Number of pharmaceuticals detected per bonefish across the five regions sampled. Violin plot outlines illustrate kernel probability density where width relates to frequency of bonefish with n-pharmaceuticals. Colored points represent individual bonefish samples, black diamonds denote regional means, and black bars show standard errors. Letters indicate significant regional differences per Tukey pairwise tests.

(Fig. 4).

In multivariate ordination space, region ($p = 0.001$, Fig. 5a) and spawning season ($p = 0.006$, Fig. 5b) drove dissimilarity in the 1/3 H_TPC pharmaceutical assemblage risk assessment (Table 4). In particular, Key West and the Dry Tortugas had the most distinct and less variable assemblages relative to the other 3 regions. Dry Tortugas differed from Key West and the Upper Keys (*adj.* $p = 0.01$), while Key West differed from Biscayne Bay (*adj.* $p = 0.01$), Lower Keys (*adj.* $p = 0.03$), and Upper Keys (*adj.* $p = 0.04$). Last, Biscayne Bay differed from the Upper Keys (*adj.* $p = 0.01$, Table S3). Similar to the pharmaceutical presence/absence assemblage, the 1/3 H_TPC pharmaceutical assemblage risk was more variable outside than inside the spawning season (Fig. 5b). Sixteen pharmaceuticals were found to influence the observed assemblage of pharmaceutical risk with a *p*-value ≤ 0.01: atenolol, paracetamol, ranitidine, venlafaxine, bupropion, naloxone, codeine, trimethoprim, bisoprolol, hydroxyzine, clindamycin, telmisartan, risperidone, pizotifen, irbesartan, rosuvastatin, in order of significance (Fig. 5a, b). In particular, the gradient of influence for codeine and naloxone was approximately opposite that of all other significant pharmaceuticals, largely contributing to the separation between Key West and the Dry Tortugas, Biscayne Bay, Lower Keys, and Upper Keys (Fig. 5a, b).

4. Discussion

4.1. Widespread pharmaceutical exposure in South Florida and its drivers

Pharmaceuticals were present across all five regions; however, there were significant differences. The results confirm our hypothesis that bonefish would be exposed to pharmaceuticals throughout the study area, but they do not support the hypothesis that frequency of pharmaceutical detections would positively correlate with the degree of urbanization and anthropogenic influence. Instead, the highest frequency of pharmaceutical detections was in the Dry Tortugas, the most remote and least populated of the five regions, followed by Biscayne Bay, the most populated and urbanized of the five regions, then the Upper Keys, Key West, and Lower Keys, respectively.

Previous research shows that the frequency of pharmaceutical detection can be highest in urbanized areas, where consistent wastewater effluent discharge and the presence of sewage lines, septic systems and livestock operations can all lead to pharmaceutical pseudo-persistence (Branchet et al., 2021; Brown et al., 2014; Daughton and Ternes, 1999; Fahlman et al., 2018). All of these potential sources are present in Biscayne Bay, the region with the second highest number of pharmaceuticals per bonefish. Urban areas surrounding Biscayne Bay have 120,000 septic systems, 9000 of which are considered vulnerable to failure, three wastewater treatment plants (WWTP) along its coast, and one ocean outfall ~4 km from the coast (Miami Dade Water and Sewar Department, 2020).

On the other hand, recent studies have documented pharmaceuticals in less urbanized and remote freshwater and marine ecosystems (Duarte et al., 2021; González-Alonso et al., 2017; Kallenborn et al., 2018; Wilkinson et al., 2022), but our study documents extensive pharmaceutical contamination at high concentrations in a remote tropical marine ecosystem with minimal human presence. The source of pharmaceuticals in the Dry Tortugas, which is ~110 km from Key West, the closest population center, although difficult to predict, is likely a result of multiple factors. It is important to note that every Dry Tortugas sample was collected during a 3-day period in December, while sampling in all other regions occurred throughout the year, possibly contributing to lower variability of the Dry Tortugas samples and their dissimilarity to all other regions. Previous research has documented the contamination of open ocean water via wastewater discharge from coastal sources as far away as 50 km (Lara-Martín et al., 2020), and by cruise ships and ocean current transport (Alygizakis et al., 2016, 2021; Brumovský et al., 2017). Point sources, such as onsite WWTP and septic systems, have been implicated in discharge of pharmaceuticals in remote Antarctic areas (Gunnarsdóttir et al., 2013; Perfetti-Bolaño et al., 2022). Although used by only the 15 on-site residents, a septic tank is present in the Dry Tortugas, which is a possible contributing source of pharmaceuticals. Caffeine and sucralose have both been detected in Dry Tortugas water samples (Ng et al., 2021), both of which are indicators of wastewater intrusion. Due to its relatively short half-life in water, caffeine is readily biodegradable and considered an indicator of recent wastewater intrusion (Henderson et al., 2020). The presence of paracetamol in 13 of 15 (86.7 %) Dry Tortugas bonefish and 17 of 24 (70.8 %) Biscayne Bay bonefish further suggests septic effluent as a source of contamination since WWTP removal rates are as high as 100 % ± 0.2 % (Du et al., 2014b; Henderson et al., 2020). With a high frequency of detection in both the most urban and remote regions, our results emphasize the necessity for further examination of pharmaceutical contamination at large spatial scales and support recent evidence for the widespread pharmaceutical contamination in marine and coastal environments, including seemingly remote and protected areas, e.g., no-take preserves.

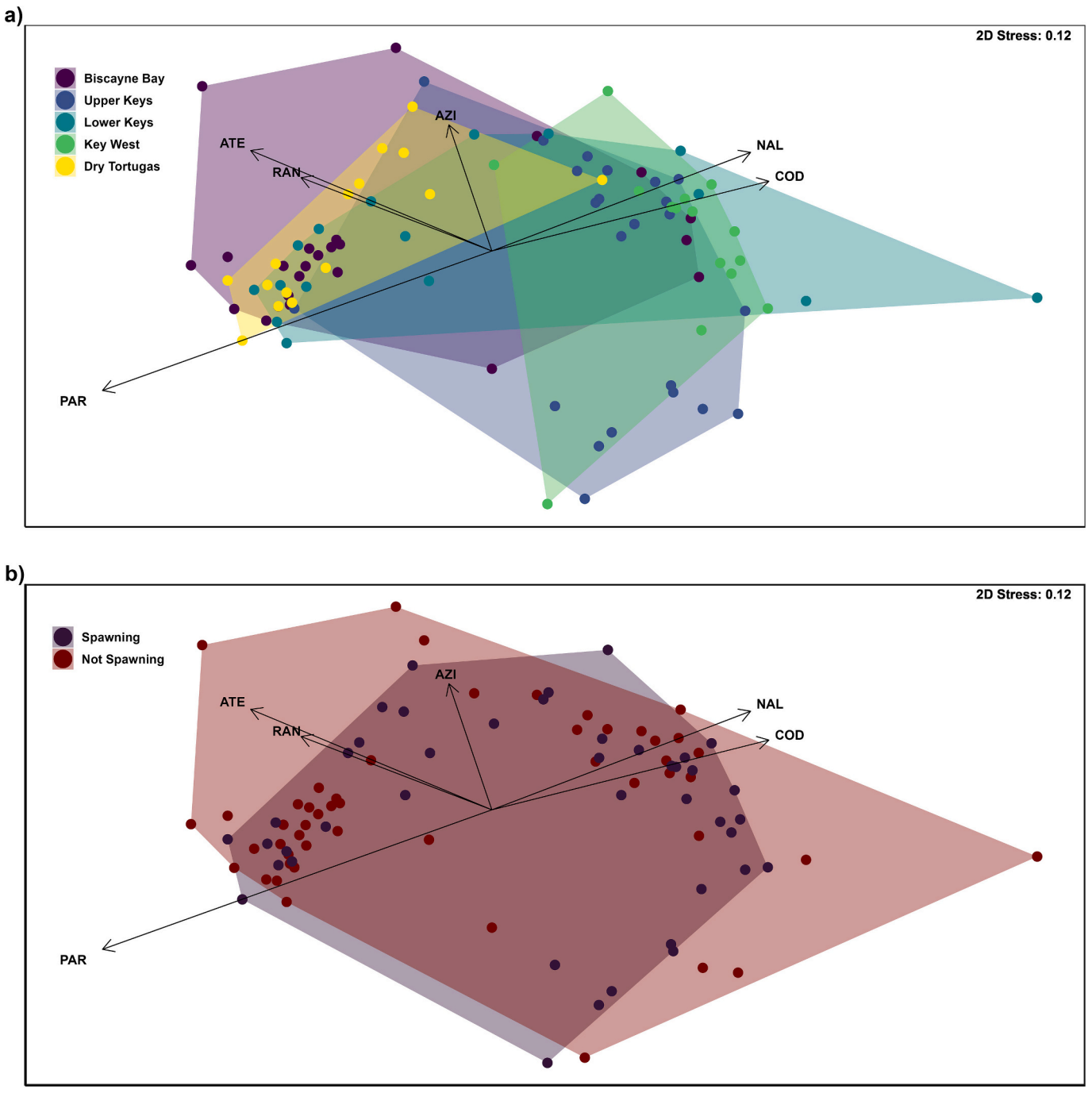

Fig. 3. nMDS plots showing the pharmaceutical assemblage (based on presence/absence) in multidimensional ordination space color coded by a) sampling region, and b) over time (spawning/not spawning season). Symbols represent individual bonefish, and polygons denote the boundaries of a) sampling regions and b) spawning season. Vector arrows show the relative direction and magnitude of pharmaceutical influence ($p \geq 0.01$). Abbreviations are as follows; ATE = atenolol, RAN = ranitidine, AZI = azithromycin, NAL = naloxone, COD = codeine, PAR = paracetamol.

In addition to regional differences, our analyses revealed temporal variation in the identity and concentrations of pharmaceuticals detected. Results indicated a lower degree of variability in pharmaceutical assemblage within spawning season (October through April). Seasonal variation in bonefish movement associated with spawning could explain the observed seasonal differences. Recent research has documented differences in pharmaceutical uptake and assemblage for highly mobile marine fish (Treu et al., 2022). Bonefish are mobile aggregating fish that migrate up to 70 km to spawn in the Bahamas (Boucek et al., 2019), and in South Florida spawning migrations could be much longer (Boucek et al., 2022). However, outside of spawning season, bonefish exhibit high site fidelity (Boucek et al., 2019; Griffin et al., 2022; Murchie et al., 2013; Perez et al., 2019; Pina-Amargós et al., 2023). Such variability in movement patterns could account for the significant differences in pharmaceutical composition between samples collected inside and outside the bonefish spawning season. In a recent study conducted in three of the five regions considered in our study (Biscayne Bay, Upper Keys, and Lower Keys), Castillo et al. (2024b) sampled bonefish prey items, including more sessile benthic invertebrates (e.g., mud crabs and various shrimp sp.), in order to determine potential pathways of pharmaceutical exposure. The researchers concluded that diet was a viable pathway of exposure, and emphasized the utility of using sessile species

Table 4
Summary of PERMANOVA main effects. Presence and absence refers to pharmaceutical composition in each bonefish and the resulting assemblage, calculated with Jaccard distance. Proportion refers to the detected pharmaceutical concentration divided by the 1/3 H_TPC threshold value, calculated with a square-root transformation and Bray-Curtis distance.

Model	Terms	df	Sum of sq	R^2	F model	*p*
Presence/ absence	Region	4	5.7	0.15	4.0	0.001***
	Spawning	1	0.7	0.02	2.0	0.02*
	Length	1	0.4	0.01	1.3	0.18
	Residual	86	30.2	0.82		
	Total	92	37.0	1.00		
Proportion	Region	4	4.1	0.14	3.7	0.001***
	Spawning	1	0.7	0.02	2.6	0.006**
	Length	1	0.3	0.01	1.1	0.33
	Residual	86	24.3	0.82		
	Total	92	29.5	1.00		

*** *p*-Value < 0.001.
** *p*-Value < 0.01.
* *p*-Value < 0.05.

to further elucidate regional variation when assessing pharmaceutical contamination on a large spatial scale and in mobile mesoconsumer species, such as bonefish. Lastly, seasonal fluctuations in climate, hydrological conditions and physio-chemical properties can also affect pharmaceutical assemblage (Bayen et al., 2013; Branchet et al., 2021).

Previous studies have demonstrated seasonal variation of pharmaceutical detections in freshwater tributaries (Burns et al., 2018; 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 quite varied. Seasonal tourism can influence the amount and composition of pharmaceutical contamination. South Florida experiences large seasonal fluctuations in the resident population and visitors, which could have influenced our results. In fact, annual visitation can exceed the resident population up to 90-fold (e.g., in the Upper Keys), with total annual visitation across all regions exceeding 6 million people. The effects of seasonal tourism on pharmaceutical contamination can be profound in both urban coastal ecosystems (Maasz et al., 2019), and in remote areas (Mandaric et al., 2017), especially when visitation is concentrated in a small area. As such, the ~80,000 annual visitors in the Dry Tortugas, which is <2 sq/km in total area, could have an effect on the pharmaceutical exposure found in our study. The observed seasonal differences in pharmaceutical assemblage, and the many possible drivers, necessitates additional research to parse out the main factor driving regional and seasonal variability in pharmaceutical composition across South Florida.

4.2. Widespread risk from pharmaceutical exposure and its drivers

Our results provide strong evidence supporting the ability of pharmaceuticals to accumulate in marine biota at concentrations high enough to elicit pharmacological effects. Pharmaceuticals in exceedance of the 1/3 H_TPC threshold were present in 52.7 % of all bonefish, indicating a ubiquitous and concerning threat throughout the entirety of South Florida. These results support our hypothesis that pharmaceuticals would be detected in bonefish at concentrations high enough to cause pharmacological effects, yet they do not support our hypothesis of greater risk of exposure to pharmaceuticals at concentrations capable of eliciting pharmacological effects closer to urbanized areas. While the second and third highest risks were present in more urbanized regions, the Lower Keys (56.3 % of samples), and Biscayne Bay (54.2 % of samples), the highest risk was present in the Dry Tortugas (93.3 % of samples), the most remote of the five regions. This indicates that the current view of a positive correlation between risk and urban population density (Hong et al., 2018; Letsinger et al., 2019; Nödler et al., 2014) is too simplified, and that other factors (e.g., tourism and currents) may contribute significantly to risk distribution (Dehm et al., 2021; Fonseca et al., 2020).

Similar to the frequency of pharmaceutical detections, the frequency of 1/3 H_TPC exceedances was best explained by spatial variation across regions, and not by fish length or spawning season. Experimental support for the increased bioaccumulation of pharmaceuticals with increasing fish size has been demonstrated (Arnot and Gobas, 2006). The primary mode of xenobiotic uptake in fish is through the gill epithelium (Stott et al., 2015), and past research supports the notion that an increased gill surface area can lead to an increase in organic chemical uptake (Sijm and van der Linde, 1995). However, negative correlations between fish weight and bioconcentration have also been observed (Heynen et al., 2016). Additionally, over 72 % of sampled bonefish were of spawning size (>480 mm; Larkin, 2011), suggesting similar diets and movement patterns (Boucek et al., 2019; Boucek et al., 2022; Campbell

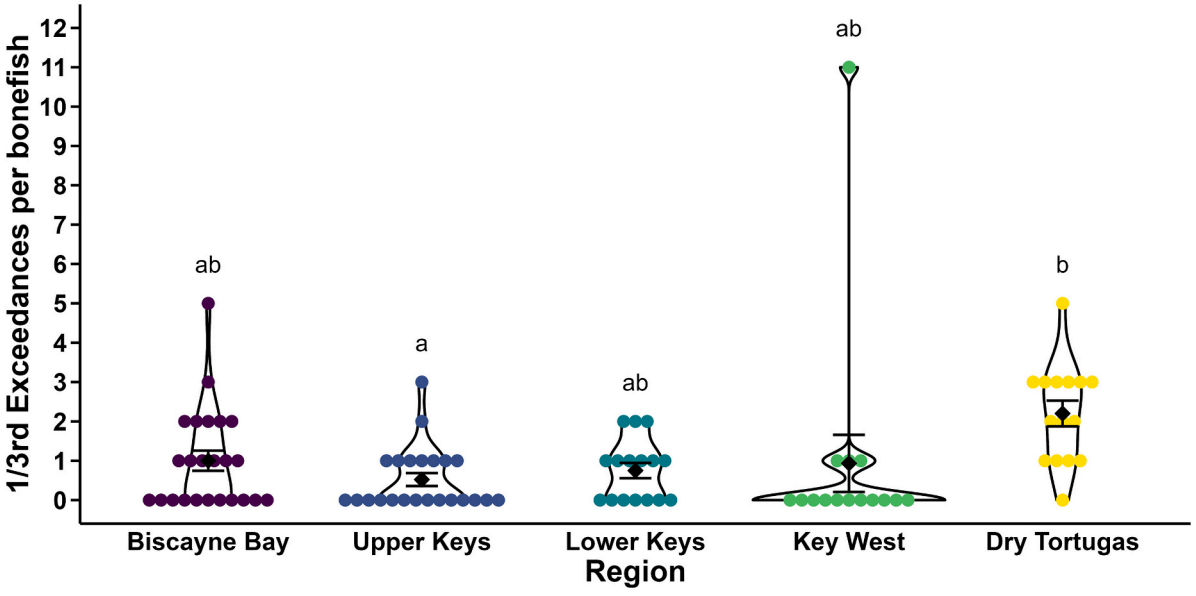

Fig. 4. 1/3 H_TPC exceedances across the five regions sampled. Violin plot outlines illustrate kernel probability density where width relates to frequency of bonefish with n-1/3 H_TPC exceedances. Colored points represent individual bonefish samples, black diamonds denote regional means, and black bars show standard errors. Letters indicate significant regional differences per Tukey pairwise tests.

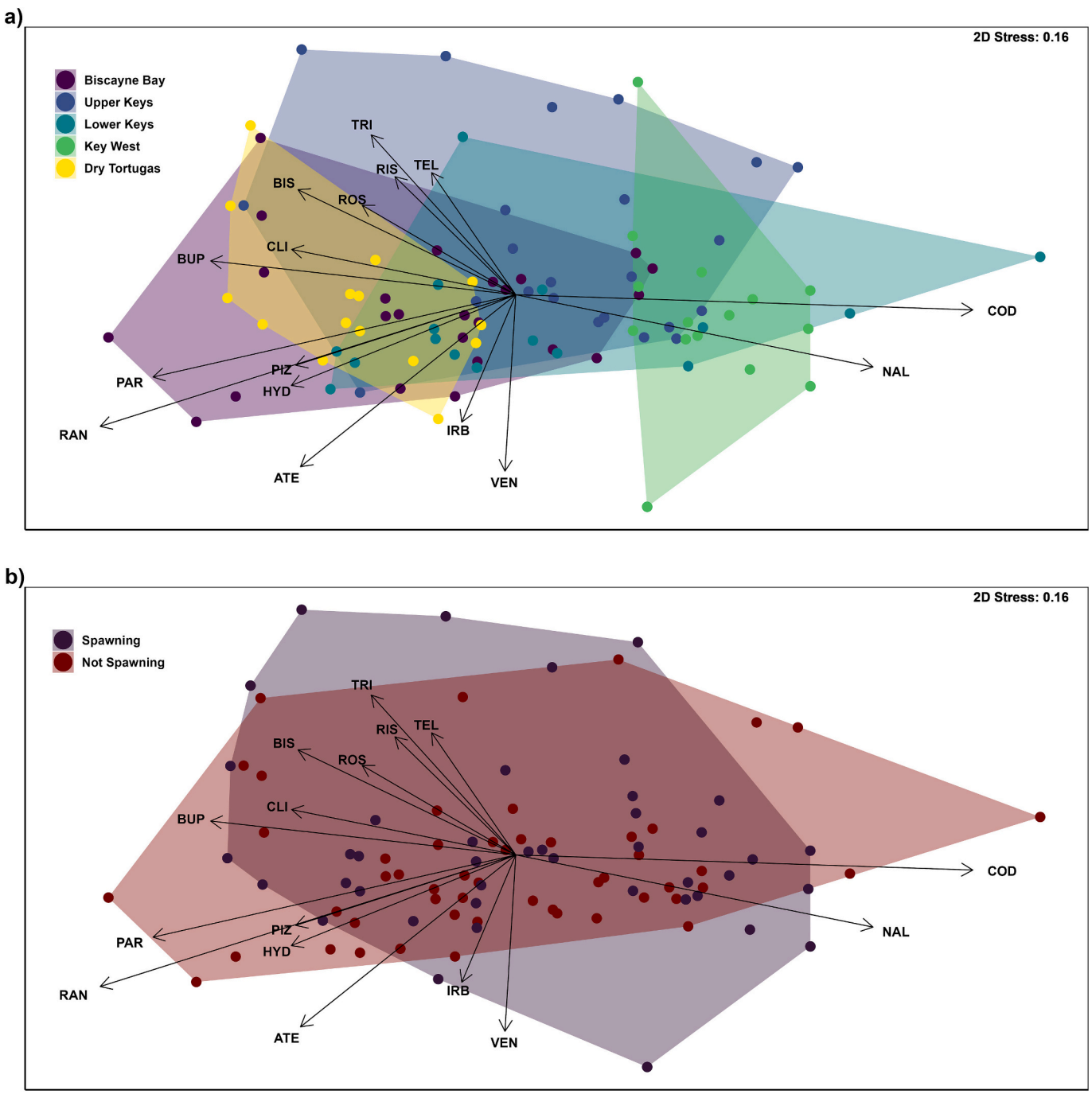

Fig. 5. nMDS plots of the assemblage in multidimensional ordination space of bonefish samples based on the proportion of the detected pharmaceutical concentration to the 1/3 H_TPC coded by, a) sampling region, and b) over time (spawning/not spawning season). Symbols represent individual bonefish, and polygons denote the boundaries of a) sampling region and b) spawning season. Vector arrows show the relative direction and magnitude of pharmaceutical influence ($p \geq 0.01$). Abbreviations are as follows; VEN = venlafaxine, IRB = irbesartan, ATE = atenolol, HYD = hydroxyzine, PIZ = pizotifen, RAN = ranitidine, PAR = paracetamol, BUP = bupropion, CLI = clindamycin, BIS = bisoprolol, ROS = rosuvastatin, RIS = risperidone, TRI = trimethoprim, TEL = telmisartan, COD = codeine, NAL = naloxone.

et al., 2022; Crabtree et al., 1998). These factors could have resulted in the lack of influence of bonefish length on exposure and risk.

Seasonal variation that can increase pharmaceutical diversity can also increase pharmaceutical concentrations in aquatic environments, in turn resulting in temporal variance (Branchet et al., 2021; Im et al., 2020; Lu et al., 2020; Tanabe and Ramu, 2012). Fluctuations in tourism, which in South Florida increases during the bonefish spawning season, as well as variation in climactic, hydrological, and marine water physiochemical properties can all influence pharmaceutical concentrations (Bayen et al., 2013; Branchet et al., 2021). Prevailing currents, gyres, and eddies fluctuate seasonally in South Florida and vary by region (Lee et al., 1994; Limouzy-Paris et al., 1997), which could contribute to both the spatial and temporal variance in exposure risk. In order to identify the most influential environmental variables for risk posed by exposure to pharmaceuticals at concentrations capable of eliciting pharmacological effects, additional research accounting for a greater breadth of variables is required.

5. Conclusion

Our results demonstrate that pharmaceuticals are present in bonefish in the South Florida marine environment at concentrations high enough to elicit pharmacological effects, establishing a pressing need for further investigation into pharmaceuticals as a threat to marine biota with the potential for ecosystem wide alterations. This study provides the first assessment of pharmaceuticals in marine fish on a large spatial scale in South Florida, contributing to the limited research on pharmaceuticals in marine environments. In addition to demonstrating a frequency of pharmaceutical exposure substantially higher than that currently documented in the literature (Madikizela et al., 2020; Prichard and Granek, 2016), our findings demonstrate the high likelihood of risk to exposed biota resulting from internal blood plasma concentrations exceeding a 1/3 H_TPC threshold of effect (over half of all bonefish had at least one pharmaceutical in exceedance). Further, environmental variability present in marine environments and the resulting effect on pharmaceutical contamination necessitates additional research on drivers of exposure and risk. The threat of pharmaceuticals posed to marine biota and environments should be incorporated into risk assessments and resulting fisheries and management practices. The presence of multiple pharmaceuticals in every bonefish and prevalence of pharmaceutical concentrations above a threshold of 1/3 the H_TPC is particularly concerning due to the unpredictable and often substantial effects of pharmaceutical cocktails on exposed organisms. Even transient acute exposure to such high concentrations can result in behavioral alterations threatening survivorship and population stability. An emphasis on upgrading WWTP infrastructure and septic to sewer conversion is needed to prevent pharmaceutical input because once pharmaceuticals are in the marine environment, remediation is not effective (Ojemaye and Petrik, 2019; Zandaryaa and Frank-Kamenetsky, 2021). We have established that pharmaceuticals are widespread contaminants present in subtropical coastal marine environments at concerning concentrations, and future research must extend beyond detection and explore the possible physiological and behavioral alterations resulting from exposure to fully understand the threat that they pose.

CRediT authorship contribution statement

N.A. Castillo: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **R.O. Santos:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **W.R. James:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **R. Rezek:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **D. Cerveny:** Writing – review & editing, Methodology, Formal analysis. **R.E. Boucek:** Writing – review & editing, Investigation. **A.J. Adams:** Writing – review & editing, Methodology, Conceptualization. **J. Fick:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **T. Brodin:** Writing – review & editing, Validation, 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 the following financial interests/personal relationships which may be considered as potential competing interests: Jennifer Rehage reports financial support and equipment, drugs, or supplies were provided by Bonefish & Tarpon Trust. Jennifer Rehage reports financial support was provided by United States Environmental Protection Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117143.

Data availability

Data will be made available on request.

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