Impacts of baseflow and flooding on microplastic pollution

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Environmental Science and Pollution Research (2021) 28:45375–45389
https://doi.org/10.1007/s11356-021-13724-w

RESEARCH ARTICLE

Impacts of baseflow and flooding on microplastic pollution in an effluent-dependent arid land river in the USA

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Received: 15 December 2020 / Accepted: 25 March 2021 / Published online: 17 April 2021

Abstract
Effluent discharge from wastewater treatment plants can be a substantial source of microplastics in receiving water bodies including rivers. Despite growing concern about microplastic pollution in freshwater habitats, the literature has not yet addressed effluent-dependent rivers, which derive 100% of their baseflow from effluent. The objective of this study was to document and explore trends in microplastic pollution within the effluent-dependent lower Santa Cruz River near Tucson, Arizona (USA). We examined microplastic concentrations in the water column and benthic sediment and microplastic consumption by mosquitofish (*Gambusia affinis*) at 10 sites along a ~40 km stretch of the lower Santa Cruz River across two time periods: baseflow (effluent only) and post-flood (effluent immediately following urban runoff). In total, across both sampling periods, we detected microplastics in 95% of water column samples, 99% of sediment samples, and 6% of mosquitofish stomachs. Flow status (baseflow vs post-flood) was the only significant predictor of microplastic presence and concentrations in our models. Microplastic fragment concentrations in the water column were higher post-flood, microplastic fiber concentrations in benthic sediment were lower post-flood, and mosquitofish were more likely to have consumed microplastics post-flood than during baseflow. The additional microplastics detected after flooding was likely due to a combination of allochthonous material entering the channel via runoff and bed scour that exhumed microplastics previously buried in the riverbed. Effluent-dependent urban streams are becoming increasingly common; more work is needed to identify microplastic pollution baselines and trends in effluent rivers worldwide.

Keywords Wastewater · Plastic · Sediment · Water column · Fish · Flood · Urban ecology

Introduction

Microplastic pollution is a ubiquitous phenomenon of the Anthropocene and is increasingly studied. Primary microplastics are those in their original form, such as microbeads in personal care products, and secondary microplastics are created from the breakdown of larger plastics, such as fibers from synthetic clothing, fragments from larger polymers, and film from plastic bags (Helm 2017). Rivers and streams, especially those in urban drainages, can be major transport pathways for primary and secondary microplastics originating from point sources (e.g., wastewater treatment plants) and nonpoint sources (e.g., urban runoff) (Moore et al. 2011; Rech et al. 2014; Horton et al. 2017a, 2017b; Rochman 2018). Microplastics have known associations with organic chemicals, which could facilitate the transport of toxic substances in aquatic environments (Wang et al. 2018). Once in freshwater and marine environments, microplastics can be ingested by fish and invertebrates, in some cases with negative individual- or population-level impacts as well as potential consequences for the food web as a whole (Foley et al. 2018; Rochman 2018; Simmerman and Coleman Wasik 2020).

Effluent discharge from wastewater treatment plants can be a substantial source of microplastics in receiving water bodies, despite the fact that the majority of microplastics are removed during the treatment process (Ziajahromi et al. 2016). A meta-

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analysis by Iyare et al. (2020) showed average microplastic reductions of 72, 88, and 94% after primary, secondary, and tertiary treatment stages, respectively. In mesic regions, baseflow in rivers helps to dilute effluent inputs and reduce the density of microplastics in receiving waters. However, in effluent-dependent rivers, which are common in arid and semi-arid regions, there is no dilution of incoming microplastics because baseflow is 100% treated wastewater (Hamdhani et al. 2020). Given this setting, the density of microplastics in effluent-dependent rivers could be much higher than in other types of streams. Despite this concern, the growing literature about microplastic in freshwater habitats has not yet addressed effluent-dependent rivers.

Microplastic concentrations in rivers are thought to be controlled not only by point and nonpoint source loading but also by a suite of factors including hydrological and geomorphological characteristics. For example, precipitation runoff is not only associated with nonpoint source microplastic loading (Zhang et al. 2017), but resulting flood flows can impact microplastic concentrations in the benthos (Hurley et al. 2018). Deposition and remobilization rates impact microplastic concentrations in both the water and benthic sediment and are controlled by a combination of flow velocity, distance traveled, and microplastic shape, among others (Ballent et al. 2016; Hoellein et al. 2019). Also, changes in substrate composition can impact microplastic retention in the benthos (Tibbetts et al. 2018; Blair et al. 2019).

The objective of this study was to document and explore trends in microplastic pollution within the effluent-dependent lower Santa Cruz River near Tucson, Arizona (USA). The lower Santa Cruz River is largely urban and is supported by two wastewater treatment plants, which provide 100% of the river's flow for large portions of the year. Along this effluent flow, the river has substantial changes to substrate composition and flow velocities. Additionally, heavy precipitation causes large amounts of urban runoff and flooding in the river channel during some parts of the year. Rivers with similar effluent-dependent baseflow and flashy runoff-derived floods are found in arid and semi-arid regions of the USA and around the world (Hamdhani et al. 2020), so we believe that the Santa Cruz River can serve as an informative case study for many other systems.

We examined microplastic concentrations in the water column and benthic sediment along effluent flow in the lower Santa Cruz River, which covers a relatively large spatial scale (~40 km). We sampled at 10 sites across two time periods: baseflow (effluent only) and post-flood (effluent baseflow immediately after urban runoff). We also examined microplastic consumption by western mosquitofish (*Gambusia affinis*), hereafter referred to as mosquitofish. Specifically, we explored how microplastic consumption by these fish differed between the two time periods. We expected that microplastic concentrations in water and benthic sediment would be influenced by distance from the effluent outfalls, flow velocity, and flow status (baseflow vs post-flood). Specifically, we hypothesized that (1) increasing distance from outfalls would reduce microplastic concentrations in both the water column and sediment due to longitudinal deposition and (2) increasing flow velocity would increase concentrations in the water column and decrease concentrations in sediment. We also hypothesized that (3) flooding would increase concentrations of microplastics in the water column due to mobilization of plastics from nonpoint sources and decrease concentrations in sediment due to scour and remobilization of plastics. Finally, we hypothesized that (4) consumption of microplastics by mosquitofish would be higher in the post-flood period and higher among larger fish, owing to feeding rates and gape size.

Materials and methods

Site description

The Santa Cruz River flows through a 22,000 km^2 basin in southern Arizona, USA, and northern Sonora, Mexico (Webb et al. 2014). The mean annual precipitation for this basin is relatively low (~300 mm), and rainfall is bimodal with monsoons in August and winter rains in December making them the wettest months. May and June are the driest months (data time range: 1948–2018, provided by NOAA NCDC Climate Data Online [http://ncdc.noaa.gov/]).

Although it historically supported sections with perennial flow, the present-day lower Santa Cruz River is ephemeral, and groundwater levels are 45 m below the surface near Tucson, Arizona (Carlson et al. 2011). However, effluent discharge from local water reclamation facilities (WRF; also known as wastewater treatment plants) supports perennial surface flow in two discrete reaches (Fig. 1). The upstream, shorter reach (~5 km long) is supported by the Agua Nueva WRF, which discharges ~30 million liters of effluent into the river each day (Sonoran Institute 2017). The longer downstream reach (~30 km long) is supported by the Tres Ríos WRF, which discharges ~115 million liters of effluent each day (Sonoran Institute 2017). Following upgrades in late 2013, both facilities have been producing and discharging tertiary-treated effluent into the river (Dong et al. 2015; Johnson et al. 2015). In addition to effluent baseflow, large floods often occur during the summer monsoon season (July–September) and less commonly during winter rains (December–March) (Fig. 2). The two study reaches are typically separated by ~1.5 km of dry riverbed but are connected during floods. During baseflow, the river typically varies in depth from 0.1 to 1 m and in wetted width from 4 to 10 m. These effluent-dependent reaches begin in urbanized areas and transition to rural and agricultural landscapes farther downstream (Fig. 1). Upstream from the study reaches, the

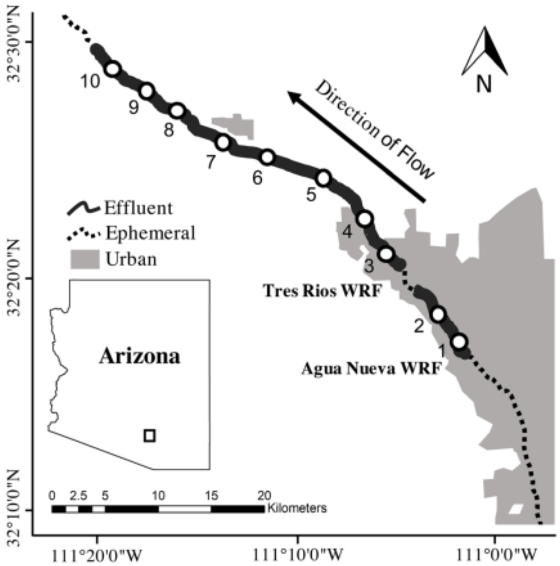


Fig. 1 Map of study area of the lower Santa Cruz River with the black dotted line indicating ephemeral reaches, the solid black line indicating effluent flow, and gray shading showing urban landscape. Sampling locations are denoted by open circles, and the locations of the water reclamation facilities (WRFs) are labeled

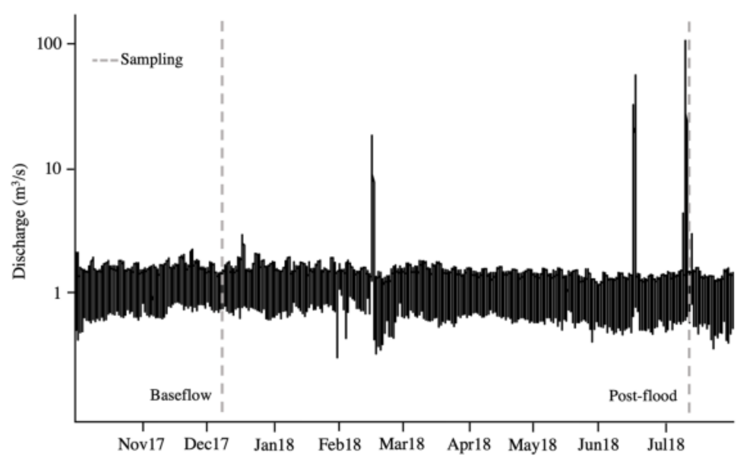
Santa Cruz River is ephemeral for > 80 km where perennial effluent flow is encountered again in the upper portions of the river basin. This ephemeral stretch is dry for the majority of the year but flows in response to heavy precipitation runoff.

Data collection

We sampled for microplastics and collected mosquitofish from 10 sites along the lower Santa Cruz River (Fig. 1) on December 9th, 2017, and July 12th, 2018. Sampling sites were spaced an average of 3.9 km (±1.1 SD) apart and were selected based on public land access. Sampling dates were selected to represent two distinct flow types: baseflow (December) and post-flood (July). Our baseflow sampling occurred after a long period of 100% effluent flow (110 days). In contrast, post-flood sampling occurred ~28 h after peak discharge from the first flood of the monsoon season that was large enough to mobilize sediment throughout the channel (≥100 m^3/s). Sampling occurred as soon as the flood receded to typical baseflow discharge and the river was safe to access (Fig. 2). Although day-to-day variation in microplastic loading can occur, Conley et al. (2019) found no seasonal variation in effluent microplastic concentrations at wastewater treatment plants.

To assess microplastic concentrations in the water column, we collected 1 L water grabs from the thalweg at 0.6× depth using distilled water-rinsed glass mason jars (Barrows et al. 2017; Green et al. 2018). Four replicate water grabs were collected at each site and date (total n=76; four jars were broken during transport). Drift net samples were collected but not included in this study due to concerns of organic matter clogging, which results in inaccurate estimates (Muehlbauer et al. 2017). The thalweg flow velocity at each site was recorded with a calibrated Marsh McBirney model 201D electromagnetic flow meter (Hatch Company, Loveland) by averaging three readings from the center of the channel measured at 0.6× total depth. To assess microplastics concentrations in benthic sediments, we collected ~0.23 L of sediment to an approximate depth of 5 cm using a Rickley Hydrological steel sediment core sampler (5 cm diameter). Five replicate sediment samples were taken at each site and date and spanned the river cross section to account for potential variation in benthic substrate size and composition (total n = 100). We measured cross-sectional flow velocity by taking 20 flow readings equally spaced across the width of the river measured at 0.6× total depth. Sediment samples were stored in distilled water-rinsed glass mason jars. Proportion of fine

Fig. 2 Lower Santa Cruz River hydrograph from October 2017 to August 2018 with discharge measured in cubic meters per second (log scale). Data is from USGS gage #09486500 at Cortaro Road (site 3). Vertical dashed lines indicate sampling dates on December 9th, 2017, and July 12th, 2018



substrate (grain size < 4.75 mm) in each core sample was measured in the lab. We also used field controls at each site to account for and quantify potential microplastic contamination from atmospheric deposition and handling practices. Controls consisted of a rinsed and distilled water-filled glass mason jar that was held open to the air for the same amount of time that microplastic samples from that site were open to the atmosphere (~20 seconds).

Finally, we sampled mosquitofish because they are the most abundant fish in the lower Santa Cruz River, and their abundances are high throughout the entirety of the flowing river. Mosquitofish have similar morphology, life history, and foraging ecology to the co-occurring endangered native fish, the Gila topminnow (*Poeciliopsis occidentalis*) (Minckley 1999; Pyke 2005). We collected mosquitofish by sampling all available habitats (e.g., riffles, pools) within 100 m of the water and sediment sampling locations at each site. We used 0.25-in. mesh seines and 0.25-in. mesh dip nets. The resulting mosquitofish were placed in a bucket for identification verification, and 80 mosquitofish per site and date were preserved in ~95% ethanol. Throughout the field campaign, care was taken to reduce potential plastic contamination. Whenever possible, glass and metal materials were used as substitutes for plastic, and all clothing worn was 100% cotton.

Laboratory processing

Microplastic sample processing protocols were adapted from Masura et al. (2015) and McCormick et al. (2014 and 2016). Water grabs were homogenized and poured into a graduated beaker to record the volume to the nearest 5 mL. Water samples were then vacuum pump filtered through a 47 mm diameter, 1.6 μm pore size Whatman glass fiber filter that was divided into quadrants to facilitate subsequent microplastic enumeration. Depending on silt content, multiple filters were used for some samples. Filters were removed using forceps, placed in tin weighing boats, covered with aluminum foil, and dried at 75 °C for 12 h.

Sediment samples were dried in their collection mason jars, covered with aluminum foil, at 75 °C for at least 12 h. Samples were considered dewatered when their weights stabilized, typically after 36 h. Weights were then recorded to the nearest 0.1 g using an Ohaus Scout electronic balance (Ohaus Corporation, Parsippany). Each sample was mixed to ensure homogenization, and a 50–70 g subsample was collected, which was fractionated using stacked sieves (4.75 mm and 0.33 mm) and rinsed thoroughly with distilled water. Material retained on the 4.75 mm sieve was dried and weighed to estimate the proportion of coarse sediment (specifically pebble) in the subsample. Material retained on the 0.33 mm sieve (fine sediment and microplastics from the entire subsample) and then underwent an 11.7 M zinc chloride ($ZnCl_2$; 1.6 g/mL) density separation in a glass funnel covered by aluminum foil (Zobkov and Esiukova 2017; Rodrigues et al. 2018b). Sediment particles were allowed to settle for at least 60 min. Solids were released and discarded from the separator, and the remaining solution and material was vacuum pump filtered through a 47 mm diameter, 1.6 μm pore size Whatman glass fiber filter. Depending on silt content, multiple filters were used for some samples. These filters were removed using forceps, placed in tin weighing boats, covered with aluminum foil, and dried at 75 °C for 12 h. Control samples underwent the same process described above. Water grab and sediment samples contained little organic matter, so we did not use a digestion stage in our processing.

After drying, filters from the water, sediment, and control samples were examined using dissecting microscopes at 10–45× magnification. The lower size threshold of microplastic detection in water samples was conservatively estimated as 200 μm as determined by identification training with known microplastic sizes. The lower threshold for sediment samples was 330 μm as determined by our sieve size. Observed microplastics were categorized into the four major types (fiber, fragment, film, and bead; Helm 2017) using identification protocols from Hidalgo-Ruz et al. (2012) and were enumerated. These identification methods, which utilized visual and tactile strategies, mitigate false positive identification in larger (> 100 μm) microplastics (Karlsson et al. 2020).

We randomly selected 20 mosquitofish per sampling site and date for diet analysis (N=400 fish analyzed). We identified sex based on gonopodia and classified the fish as female, male, or unknown, and gravid females were noted. We measured total length (TL) to nearest 0.5 mm and weight to the nearest 0.001 g for each fish. To account for length and weight changes from preservation in ethanol, we used a separate, random subset of mosquitofish (N=34) for comparisons of fresh TL and weight to preserved TL and weight after 4 months in ethanol (see Supplementary information). Mosquitofish used for diet analysis were preserved approximately 4 months. There are no known impacts of ethanol preservation on ingested microplastics (Courtene-Jones et al. 2017). We excised and opened the stomachs from preserved fish and used distilled water to rinse stomach contents into a glass Petri dish. Using a 10–45× dissecting microscope, stomach contents were identified and enumerated using previous described protocols for the water grabs and sediment samples. Microplastics found in the stomach were categorized into fiber, fragment, film, and bead. The lower size threshold of microplastic detection in fish stomachs was conservatively estimated as 200 μm.

Quality control

Controls were processed using the density separation and filtration protocols described previously. To minimize microplastic contamination in the laboratory, glass and metal materials were used as substitutes for plastic, the work space and materials were washed before each procedure, all density separations and filtering occurred under a laminar flow hood, filters were always covered except during readings, and all technicians wore white 100% cotton clothing. To limit the potential for contamination during the uncovered filter readings, white/clear fibers were not counted as they could have been cotton fibers shed from technician clothing. Similarly, microplastic counts from the controls (collected at each site) were subtracted from the counts of the water and sediment samples taken at those respective locations and dates. Four individuals read the filters (water, sediment, and control) and underwent the same training on the detection, identification, and enumeration of microplastics prior to beginning work on this study following guidance from Hidalgo-Ruz et al. (2012), Helm (2017), and Karlsson et al. (2020). Each filter was counted three times by at least two separate individuals on separate dates. These replicate readings were blind (no prior knowledge of previous counts) and aimed to reduce potential observer bias. Mean variance was 18%, and the median count for each microplastic type was selected as the final value for each filter. We acknowledge that our methods did not include plastic composition confirmation with spectroscopy, so potential for misidentification exists. However, our identification protocols (Hidalgo-Ruz et al. 2012; Helm 2017) were shown by Karlsson et al. (2020) to yield zero false positives in size ranges 300 μm or greater. We also acknowledge that given our sieve sizes, we excluded smaller microplastic sizes, which could not be reliably identified by our methods. Therefore, the estimates presented in this study do not reflect concentrations of smaller size ranges including nanoplastics (Enfrin et al. 2020).

Data analysis

We quantified the concentrations of microplastics by type (fiber, fragment, film, and bead) in both the water column (No./L) and benthic sediment (No./kg dry weight) and recorded microplastics found in the stomach of each mosquitofish. Microplastic pellets and beads are often combined into a "pellet" category (Helm 2017); however, in this study, no true pellets were observed. To test our hypotheses, we used Generalized Linear Mixed Models (GLMMs; Bolker et al. 2009) to determine (1) if distance from effluent outfall (distance), thalweg flow velocity (velocity), and flow status (baseflow vs post-flood) impacted microplastic concentrations in the water column; (2) if distance, cross-sectional flow velocity, and flow status affected concentrations in the benthic sediment; and (3) if microplastic concentrations in the water column, velocity, fish length, and flow status affected whether or not mosquitofish consumed microplastic. We included proportion of fine substrate (see "Laboratory processing") in our sediment models to account for potential differences in concentration based on grain size (Ling et al. 2017). We also included effluent source (WRF) as a predictor in the models to account for treatment differences between the Agua Nueva and Tres Rios WRFs. Agua Nueva utilizes 20 μm tertiary disk filtration prior to effluent discharge but Tres Rios does not (Molly Renner, Pima County Wastewater Reclamation, pers. comm.). Finally, our models also included sampling site as a random factor to account for potential bias from repeated measures. Models were run in the statistical program R (version 3.5.1: R Core Team 2019) with the package "glmmTMB" (Generalized Linear Mixed Model Thematic Model Builder, version 1.0.2.1; Brooks et al. 2017). To account for zero inflation, we used a Tweedie distribution log link family for both the water column and sediment microplastic concentrations, and we used a binomial logit link family for the presence/absence of microplastic consumption by the fish (Shono 2008; Bonat and Kokonendji 2017). We validated our models using R package "DHARma" (Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models, version 0.3.2.0, Hartig 2020) by testing and verifying zero inflation, overdispersion, outliers, and normality of residuals.

Results

Overview

We observed microplastics fibers, fragments, film, and beads in both water column and sediment samples, but only fibers, fragments, and film were found in mosquitofish stomachs (Fig. 3). In total, across both sampling periods, we detected microplastics in 95% of water column samples, 99% of sediment samples, and 6% of mosquitofish stomachs. Microplastic fragment concentrations in the water column were higher post-flood, fiber concentrations in benthic sediment were lower post-flood, and fish were more likely to have consumed microplastics post-flood than during baseflow (Fig. 4). Flow status (baseflow vs post-flood) was the only significant predictor of microplastic concentrations and presence. In sediment samples, we also inadvertently documented microplastics incorporated into the cases of caddisfly pupae (Fig. 1S; Hydropsychidae, *Smicridea*), which are benthic macroinvertebrates common in the river (Eppehimer et al. 2020). However, aquatic macroinvertebrates were not targeted in this study, so the prevalence of this phenomenon is unknown. Control samples only contained fibers, which ranged in abundance from 0 to 7 with a mean of 2.5 ± 0.4 SE (No./sample). These observed fiber counts from site- and date-specific controls were subtracted from the corresponding water and sediment samples.

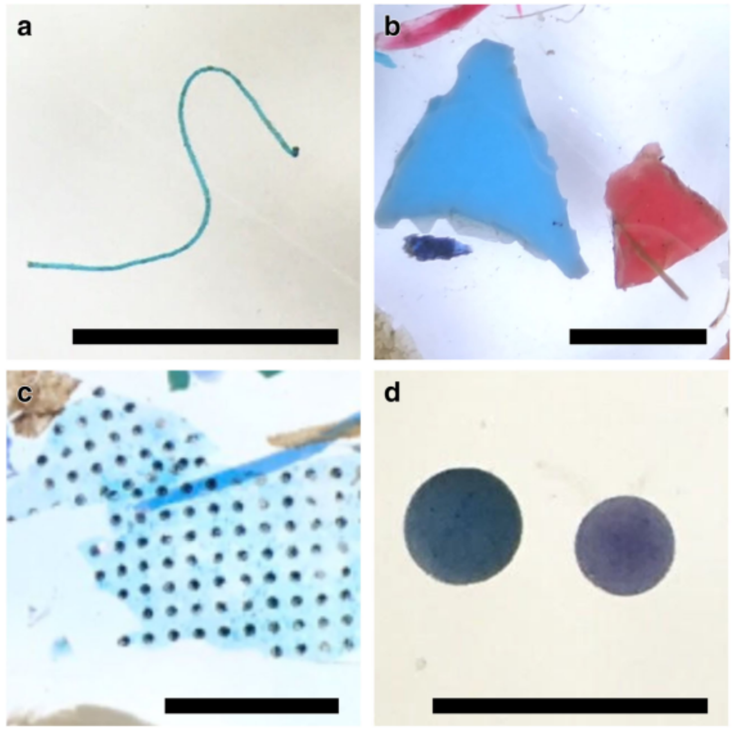


Fig. 3 Photos illustrating the observed microplastic types collected in the lower Santa Cruz River: **a** fiber, **b** fragment, **c** film, and **d** bead. Horizontal scale bar ≈ 0.5 mm

Water column microplastic

We observed an average microplastic concentration of 19.5 ± 2.2 SE (No./L) in the water column across all samples. Fibers were the most common type observed and were present in 88 and 100% of the samples collected during baseflow and post-flood, respectively. Fiber concentrations remained similar in both sampling periods averaging 18.4 ± 6.3 SE (No./L) [range: 0–99.9] during baseflow and 16.5 ± 1.9 SE (No./L) [range: 0.3–50.7] post-flood (Figs. 5a, 2S). Fragment concentrations increased from baseflow to post-flood from a mean of 1.2 ± 0.3 SE (No./L) [range: 0–6.8] to 3.6 ± 0.7 SE (No./L) [range: 0–19.0] (Figs. 5b, 3S). We observed fragments in 49% of baseflow water samples and 80% of post-flood samples.

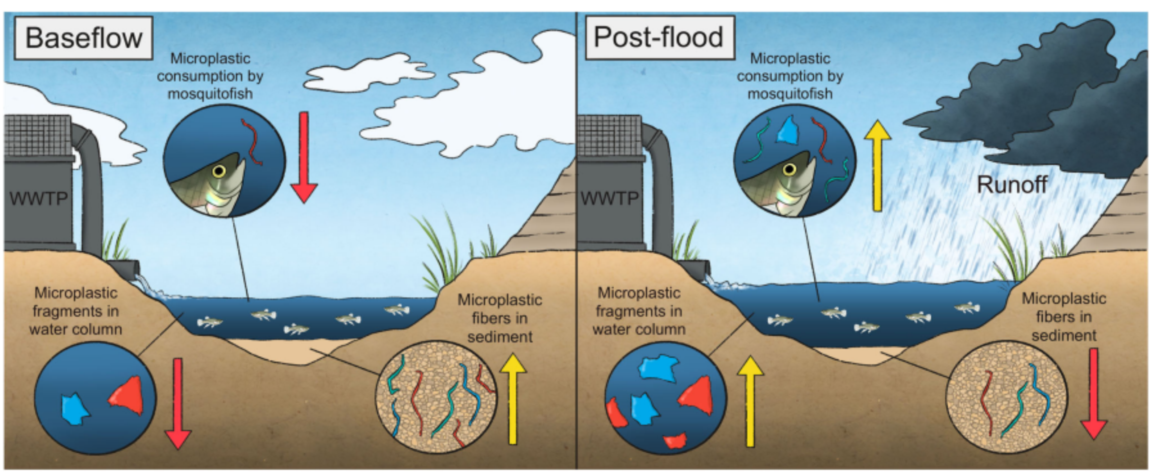


Fig. 4 Conceptual diagram illustrating significant microplastic abundance changes in the water column, benthic sediment, and mosquitofish consumption in the lower Santa Cruz River during baseflow and post-flood sampling

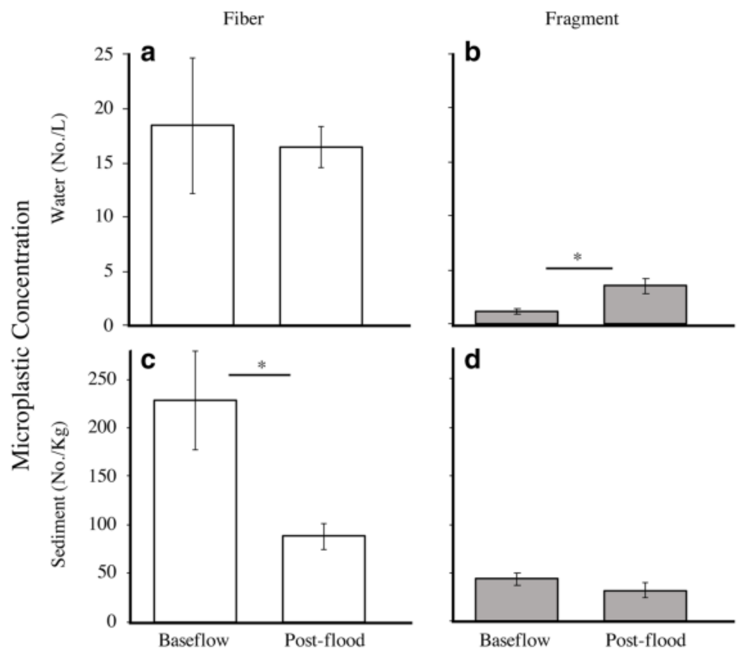


Fig. 5 Microplastic concentrations of **a** water column fibers (No./L), **b** water column fragments (No./L), **c** benthic sediment fibers (No./kg), **d** benthic sediment fragments (No./kg) averaged from all sites in the effluent baseflow and post-flood sampling periods, respectively. White denotes fibers, and gray denotes fragments. Error bars represent standard error, and asterisks show significant changes in concentration

Microplastic film and beads in the water column were rare in both baseflow and post-flood samples (Fig. 2SA). Film was observed in 10% of baseflow water samples (mean: 0.1 ± 0.1 SE (No./L) [range: 0–1.9]) and 26% of post-flood samples (mean: 0.3 ± 0.1 SE (No./L) [range: 0–2.0]). Finally, no beads were observed in baseflow water samples, but beads were present in 9% of post-flood samples at low concentrations (mean: 0.1 ± 0.1 SE No./L). Thalweg flow velocity at our sampling sites ranged from 0.12 to 1.30 m/s across both sampling periods with a mean of 0.55 ± 0.05 SE m/s in the baseflow sampling and 0.57 ± 0.04 SE m/s in post-flood sampling.

Concentrations of fibers, film, and beads in the water column were not related to any of our hypothesized predictors (distance from effluent outfall, thalweg flow velocity, effluent WRF source, or flow status). However, fragment concentrations were significantly higher in post-flood samples compared to baseflow (p = <0.001, 95% confidence interval: 0.76–1.78; Table 1; Fig. 5b). Fragment concentrations were not correlated with distance, thalweg flow velocity, nor WRF source.

Benthic sediment microplastic

We observed an average microplastic concentration of 246.9 ± 30.5 SE (No./kg) across all benthic sediment samples. Fibers were found in 94% of baseflow samples and 82% of post-flood samples. Fiber concentrations were higher in baseflow samples (mean: 228.3 ± 51.0 SE (No./kg) [range: 0–2394.4]) than post-flood samples (mean: 88.0 ± 13.9 SE (No./kg) [range: 0–392.2]) (Figs. 5c, 4S). Fragments concentrations were slightly higher in baseflow sediment samples (mean: 43.9 ± 6.9 SE (No./kg) [range: 0–151.2]) than in post-flood samples (mean: 32.3 ± 7.6 SE (No./kg) [range: 0–288.5]) (Figs. 5d, 5S). Fragments were observed in 74 and 62% of baseflow and post-flood sediment samples, respectively. Film and beads were relatively rare in the sediment samples (Fig. 2SB). During baseflow, film was observed in 14% of samples with a mean of 4.2 ± 1.5 SE (No./kg) [range: 0–99.4]. In post-flood conditions, film was observed in 18% of samples and averaged 4.7 ± 1.5 SE (No./kg) [range: 0–39.8]. Finally, beads were observed in 6% and 0% of baseflow and post-flood samples, respectively. During baseflow sampling, bead concentrations averaged 1.1 ± 0.6 SE (No./kg) [range: 0–19.0]. Across both periods, average cross-sectional flow velocity ranged from 0.10 to 1.10 m/s with a mean of 0.45 ± 0.04 SE m/s and 0.41 ± 0.03 SE m/s during baseflow and post-flood, respectively. Fractional proportion of fine sediment in benthic sediment samples ranged from 0.14 to 1.00 across both sampling periods with a mean of 0.74 ± 0.04 and 0.69 ± 0.03 during baseflow and post-flood, respectively.

Benthic sediment concentrations of fragments, film, and beads were not related to any of our hypothesized predictors (distance from effluent outfall, cross-sectional flow velocity, effluent WRF source, flow status, and fine sediment proportion). However, concentrations of microplastic fibers in benthic sediment samples were significantly lower post-flood than during baseflow (p = <0.001, 95% confidence interval:

Table 1 Summary of GLMM results explaining variation in microplastic concentrations (No./L) in the water column by type (fiber, fragment, film, and bead) with predictors distance from outfall (distance), velocity, water reclamation facility source (WRF), and sampling period: baseflow/post-flood (flow status) with sampling site as a random factor in the model. Results include beta estimates (estimates) with corresponding 95% confidence intervals (CI) and *p* values (*p*) as well as within site residual variance (σ^2), between site variance (τ_{00}), and intra-class correlation coefficient (ICC) for the random factor. Bold indicates significant *p* values of predictors ($\alpha \leq 0.05$)

	Fiber			Fragment			Film			Bead		
Predictors	Estimates	CI	*p*	Estimates	CI	*p*	Estimates	CI	*p*	Estimates	CI	*p*
(Intercept)	2.02	1.15 to 2.90	<0.001	0.10	−0.70 to 0.90	0.802	−3.21	−5.46 to −0.97	0.005	−32.54	$-3.1e^6$ to $3.1e^6$	1.000
Distance	0.01	−0.02 to 0.05	0.493	0.01	−0.02 to 0.04	0.347	0.03	−0.03 to 0.10	0.328	−0.01	−0.17 to 0.15	0.892
Velocity	0.18	−0.85 to 1.20	0.735	−0.82	−1.88 to 0.24	0.127	0.57	−1.78 to 2.93	0.632	−2.59	−9.37 to 4.20	0.455
WRF	0.46	−0.64 to 1.55	0.411	0.15	−0.74 to 1.04	0.740	0.35	−0.22 to 1.96	0.787	−0.94	−4.81 to 2.93	0.635
Flow status	0.05	−0.36 to 0.47	0.794	1.27	0.76 to 1.78	**<0.001**	0.87	−2.17 to 2.87	0.119	32.51	$-3.1e^6$ to $3.1e^6$	1.000
Random effect: site												
σ^2	0.57			0.71			1.87			3.23		
τ_{00}	0.13			0.00			0.00			0.00		
ICC	0.19			NA			NA			NA		
Non-zero observations	71			48			13			3		
Total observations	76			76			76			76		

1[*plastic concentration in water*]*~Distance + Velocity + WRF + Flow Status, random = Site, family = tweedie (link = log)*

−1.34 to −0.55; Table 2) (Fig. 5c). Fiber concentrations were not related to distance, flow velocity, WRF source, nor fine sediment.

Mosquitofish consumption of microplastics

Microplastic fiber, fragment, or film was present in 1.5% and 10% of mosquitofish stomachs during baseflow and post-flood, respectively. However, we never observed more than one microplastic piece per fish. During baseflow, only three individuals were found with fibers. Of these three mosquitofish, two were identified as female and one with unknown sex, and they had an average TL of 41.4 ± 8.2 SE mm. In post-flood samples, we observed 20 individuals with microplastics: 17 with fibers, 2 with film, and 1 with a fragment (Fig. 6). Of these 20 fish, 15 were identified as female (nine of which were gravid) and five male, and they had an average TL of 33.3 ± 1.2 SE mm. During post-flood sampling, gravid females and females in general were slightly overrepresented in microplastic consumption (75% female, of which 60% were gravid) when compared to their proportion in the sample population (see Supplementary information). Flow status was the only significant predictor of microplastic consumption ($p = < 0.001$, 95% confidence interval: 1.21–4.01; Table 3).

Discussion

Water column microplastic

As we predicted, microplastic fragment concentrations in the water column increased from baseflow to post-flood (Fig. 5b). Fragments represented 6.0% and 17.6% of total observed water column microplastics in the baseflow and post-flood periods, respectively. Surprisingly, however, we found that fragment concentrations were not predicted by distance from the effluent outfalls or flow velocity, nor did our models reveal significant relationships between these factors and any other type of microplastic. Microplastic fragments are secondary plastics and are most often associated with anthropogenic litter/debris (Helm 2017). These plastics are present in wastewater but also are transported into rivers during urban runoff events (Liu et al. 2019a; Pinon-Colin et al. 2020). For example, microplastic concentrations in the water column of a Chinese river were orders of magnitude larger following precipitation runoff (Zhang et al. 2017). In that study, the authors found that fragment concentrations increased over 17,000%. Our observed increased was much smaller (200%), but our smaller basin drainage area, land use/land cover, and sample timing are important covariates. For example, our post-flood samples were collected ~28 h after peak discharge, so any large, momentary increases in plastic concentration would

Table 2 Summary of GLMM results explaining variation in microplastic concentrations (No./kg) in benthic sediment by type (fiber, fragment, film, and bead) with predictors distance from outfall (distance), cross-sectional velocity (velocity), water reclamation facility source (WRF), sampling period: baseflow/post-flood (flow status) and fine sediment relative abundance (fine sediment) with sampling site as a random factor in the model. Results include beta estimates (estimates) with corresponding 95% confidence intervals (CI) and *p* values (*p*) as well as within site residual variance (σ^2), between site variance (τ_{00}), and intra-class correlation coefficient (ICC) for the random factor. Bold indicates significant *p* values of predictors ($\alpha \leq 0.05$)

	Fiber			Fragment			Film			Bead		
Predictors	Estimates	CI	*p*	Estimates	CI	*p*	Estimates	CI	*p*	Estimates	CI	*p*
(Intercept)	5.34	4.36 to 6.33	<0.001	3.92	2.76 to 5.08	<0.001	2.10	0.25 to 3.96	0.026	3.26	−1.54 to 8.05	0.183
Distance	0.02	−0.02 to 0.06	0.319	−0.03	−0.07 to 0.02	0.248	0.01	−0.05 to 0.08	0.721	−0.05	−0.41 to 0.31	0.782
Velocity	0.43	−1.00 to 1.87	0.553	−0.50	−2.08 to 1.09	0.539	−0.25	−2.91 to 2.42	0.856	−0.89	−10.85 to 9.07	0.862
WRF	−0.24	−1.23 to 0.75	0.629	−0.20	−1.40 to 0.99	0.740	−1.36	−2.93 to 0.21	0.090	−0.75	−8.20 to 6.71	0.845
Flow Status	−0.94	−1.34 to −0.55	**<0.001**	−0.31	−0.72 to 0.11	0.149	0.12	−0.72 to 0.97	0.779	−22.13	$-5.4e^4$ to $5.4e^4$	0.999
Fine sediment	-0.36	−1.29 to 0.57	0.446	0.66	−0.23 to 1.55	0.143	0.30	−1.55 to 2.15	0.752	−3.39	−8.49 to 1.72	0.194
Random effect site												
σ^2	0.64			0.70			1.75			3.52		
τ_{00}	0.10			0.24			0.00			0.00		
ICC	0.13			0.25			NA			NA		
Non-zero observations	88			68			16			3		
Total observations	100			100			100			100		

2[*plastic concentration in sediment*]~*Distance + Velocity + WRF + Flow Status + Fine Sediment, random = Site, family = tweedie (link = log)*

likely have moved through the system or been deposited in the flood plain prior to our sampling. Although we did not quantify concentrations in runoff, we assume that urban runoff contributed to the increased microplastic fragments we observed in the water column. Additionally, flood flows in ephemeral, upstream portions of the river may also remobilize microplastics deposited in dry stretches of the riverbed and transport them downstream to our perennial study sites. Research is needed to quantify and identify the proportional contributions of microplastics from point and nonpoint sources in effluent-dependent rivers.

Fiber concentrations in the water column were high during both of our sampling periods (Fig. 5a). Fibers are often the most abundant type of microplastic in rivers (Dris et al. 2015; Kapp and Yeatman 2018; Lenaker et al. 2019; Wang et al. 2021) and are usually associated with the washing of synthetic clothes (Prata 2018), which can shed large amounts of fibers into wastewater (Almroth et al. 2018). In our study, fibers comprised 93 and 80% of microplastics in the water during baseflow and post-flood sampling, respectively. These proportions are nearly double those reported from rivers in the northwestern and midwestern USA (45–58%; Kapp and Yeatman 2018; Lenaker et al. 2019). Unlike fragments, fiber concentrations did not appreciably change from baseflow to post-flood. The mechanisms behind this are unknown, but we speculate it could be due to loading rates from effluent. Given that baseflow in the lower Santa Cruz River is 100% effluent, which is derived in part from several hundred thousand urban residents washing their clothes, it is not surprising that fiber concentrations were consistently high in absolute and relative abundances (Conley et al. 2019). In conventional tertiary treatment, fibers have been shown to have a lower removal rate (71%) when compared to other types such as fragments (97%) (Ren et al. 2020), which could explain the dominance of fibers in our samples. Fibers are also the most common type mobilized and deposited by atmospheric transport (Liu et al. 2019b), which could influence concentrations in this urban river.

Contrary to our predictions, WRF source, flow velocity, and distance from effluent outfalls showed no effect on microplastic concentrations in the water column. Considering the Agua Nueva WRF utilizes tertiary disk filtration (20 μm pore size), we expected that microplastic concentrations at baseflow would be lower in samples collected below this WRF than in those collected below the Tres Rios WRF (Fig. 1). However, this was not the case and leads to speculation about additional benefits of disk filtration compared to traditional treatment methods, as well as speculation about the significant influence of urban nonpoint sources of microplastics, even during baseflow conditions. Future work identifying both influent and effluent concentrations with disk filtration as well as attempts to quantify various nonpoint sources (e.g., atmospheric deposition, tributary runoff) is needed.

Table 3 Summary of GLMM results explaining variation in observed microplastic consumption by mosquitofish (presence/absence) with predictors microplastic concentration in the water column (plastic concentration), velocity, mosquitofish TL (Fish TL), and sampling period: baseflow/post-flood (flow status) with sampling site as a random factor in the model. Results include beta estimates (estimates) with corresponding 95% confidence intervals (CI) and p values (p) as well as within site residual variance (σ^2), between site variance (τ_{00}), and intraclass correlation coefficient (ICC) for the random factor. Bold indicates significant p values of predictors ($\alpha \le 0.05$)

	Mosquitofish consumption		
Predictors	Estimates	CI	p
(Intercept)	−6.10	−8.71 to −3.48	<0.001
Plastic concentration	−0.03	−0.09 to 0.03	0.343
Velocity	0.96	−1.02 to 2.94	0.343
Fish TL	0.05	−0.00 to 0.10	0.068
Flow status	2.61	1.21 to 4.01	**<0.001**
Random effect: site			
σ^2	3.29		
τ_{00}	0.00		
ICC	NA		
Non-zero observations	23		
Total observations	400		

3[*fish plastic consumption*]~*Plastic Concentration* + *Velocity* + *Fish TL* + *Flow Status*, *random* = *Site*, *family* = *binomial* (*link* = logit)

Following floods, urban runoff enters the river from myriad locations, so longitudinal patterns downstream of WRF outfalls might be expected to be obscured. But during baseflow conditions, we expected that at least some microplastic deposition would occur over the 30 km of effluent flow. Indeed, experimental studies have shown that microplastics have different deposition rates determined by density, shape, and biofilm colonization, with fibers having longer transport lengths than denser fragments (Hoellein et al. 2019). So, it is surprising that we failed to find a longitudinal pattern in the effluent-dependent Santa Cruz River. It is possible that our spatial resolution (~4 km between sites) was too large to detect deposition gradients. However, work by Hoellein et al. (2017) examining microplastic abundance (>300 μm) in an urban stream receiving effluent found no distance trends on a smaller scale (0.8 km resolution, 2 km max distance). Our findings are in line with the studies from streams that receive effluent but are not dependent upon it for baseflow, which found no clear distance trends related to discharge locations (Estahbanati and Fahrenfeld 2016: particle size > 125 μm, fibers excluded; Rodrigues et al. 2018a: particle size > 55 μm). It is likely that a suite of complex, site- and reach-specific interactions influence deposition rates (Hoellein et al. 2019) and a complex network of nonpoint sources influence concentrations, thus obscuring broader longitudinal patterns. For example, effluent discharge in the Santa Cruz River has pronounced diurnal fluctuations (Eppehimer et al. 2020), which may alter concentrations and deposition rates on an hourly time scale (Watkins et al. 2019). Much more research, including field experiments, is needed to understand the factors controlling microplastic transport in the water column of rivers receiving effluent. This should include examination of plastics smaller than 330 μm with known compositions and densities.

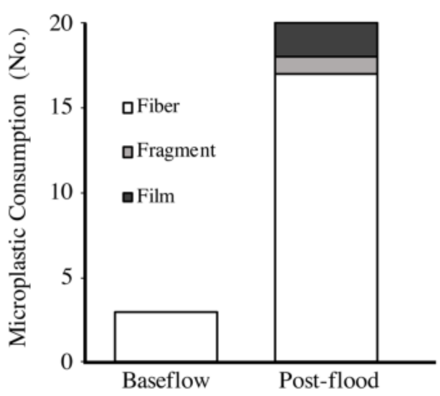


Fig. 6 Observed microplastic consumption by mosquitofish (number of individuals) in the effluent baseflow and post-flood sampling periods (n=200). White, gray, and black denote fiber, fragment, and film, respectively. No beads were observed

Benthic sediment microplastic

As we predicted, concentrations of microplastics in sediment were lower post-flood than during baseflow (Fig. 5c) but only for fibers, and no other explanatory factors were significant in the models. Microplastic fibers may be disproportionally subject to bed scour during floods. Fibers typically have longer transport lengths and are subject to less biofilm colonization, while fragments with jagged edges are more likely to resist remobilization (Hoellein et al. 2019). Bed scour can be quite significant in the Santa Cruz River during flooding, with transport and deposition of sediment documented at both reach (Duan et al. 2015) and river network (Meixner et al. 2009) scales during flooding. Furthermore, the amount of sediment mobilized increases exponentially with flow magnitude (Cheng 2002), and all of these factors suggest large-scale remobilization and long-distance transport of benthic microplastics during floods. For example, microplastic concentrations in the sediment of an English river decreased 70% due to bed scour during an extreme flood (Hurley et al. 2018). Additionally, Nel et al. (2018) reported greater concentrations of microplastics in sediment during low- versus high-flow conditions in a South African river with a flow regime similar to that of the Santa Cruz. In our study, sediment microplastic concentrations were 55% lower in post-flood compared to baseflow samples, but these sampling periods were separated by 6 months and a > 100 m^3/s flood, so we must be cautious in making direct comparisons with studies that occurred on different time scales with different flood magnitudes.

Given that microplastics exhibit different deposition rates (Hoellein et al. 2019), we were surprised that there was no correlation between microplastic concentrations in the sediment and either flow velocity or distance from effluent outfall, especially during our baseflow sampling following 110 days without runoff or floods. Other studies have found that higher flow velocities tend to result in lower microplastic concentrations in sediment (Ballent et al. 2016; Zhang et al. 2017), but it is possible that our baseflow velocities (range: 0.12–1.30 m/s) were not fast enough to dislodge microplastics in the sediment. Other studies have also failed to find any longitudinal patterns below effluent outfalls and speculated that complexities of nonpoint sources and variable hydrodynamics are likely responsible (Klein et al. 2015: particle size > 63 μm; Rodrigues et al. 2018a: particle size > 55 μm; Nel et al. 2018: particle size > 63 μm; Tibbetts et al. 2018: particle size > 63 μm). More research is needed to identify how plastics from a spectrum of shapes, sizes, and densities interact with the flow regimes specific to many effluent-dependent rivers that include daily fluctuations in discharge as well as seasonal flooding.

Finally, the proportion of fine sediment (particles between 0.33 and 4.75mm) in our benthic samples did not affect microplastic concentrations, despite the fact that changes in interstitial space could influence attenuation rates of microplastic. Tibbetts et al. (2018) and Blair et al. (2019) identified the potential for microplastic association with fine benthic sediment of UK rivers, and Ling et al. (2017) found a positive relationship between microplastic abundances and the proportion of fine sediment in a marine setting off the coast of Australia. However, other marine and coastal studies have observed no trends related to sediment grain size (Nor and Obbard 2014; Alomar et al. 2016). In streams that lose surface flow to infiltration like the Santa Cruz River (Webb et al. 2014), grain size and subsequent infiltration rates could potentially influence microplastic concentrations in river sediment and the depths to which they can travel. For instance, microplastics have been found below the subsurface in hyporheic zones of rivers (Frei et al. 2019). Future work is needed to identify concentrations at various depth strata in the sediment and to identify potential correlation with infiltration rates.

Mosquitofish consumption of microplastics

Flow status was the only significant predictor of microplastic consumption by mosquitofish, with higher consumption rates in post-flood compared to baseflow fish (Table 3; Fig. 6). The fact that microplastic consumption was higher post-flood but showed no relation to plastic concentrations in the water, flow velocity, or fish size indicates that other variables are at play. Although not measured in this study, we suspect that turbidity may have influenced consumption rates. Mosquitofish are visual predators that typically feed on aquatic insect larvae (Pyke 2005). Increased turbidity during and after floods could result in mosquitofish misidentifying larger microplastics as prey or incidentally consuming plastics via indiscriminate foraging behavior. The role turbidity plays in consumption trends remains unstudied and deserves further attention. Fibers were the most common type of microplastic observed in mosquitofish, similar to observations from goldfish in a Chinese lake (Yuan et al. 2019) and roach in the River Thames in England (Horton et al. 2018). But this raises the question: can fish see and intentionally consume fibers? We suspect that in rivers the answer is no and that fibers are consumed incidentally (Peters and Bratton 2016). However, given that we observed caddisflies with microplastics in their cases (Fig. S1), it is possible that at least some consumption by mosquitofish was secondary and reflected a trophic transfer of microplastics. Tibbetts et al. (2018) first documented plastics in caddisfly cases in a UK river and suggested that given the high biomass of caddisflies, they have the ability to impact microplastic retention and transfer rates. Recent studies have reported microplastics in 50–100% of macroinvertebrates sampled from US and UK streams (Windsor et al. 2019; Simmerman and Coleman Wasik 2020). Additionally, Nel et al. (2018) and Akindele et al. (2020) found that midge larvae in the genus *Chironomus* ingested microplastics. This genus is common in the Santa Cruz River (Eppehimer et al. 2020) and other effluent-fed streams (Gower and Buckland 1978; Boyle and Fraleigh Jr 2003; Arnon et al. 2015) and could serve as a conduit for food web transfers of plastics. Future studies on fish consumption of microplastics in effluent-dependent rivers would benefit from tandem aquatic invertebrate sampling to test for potential trophic connections.

Based on mosquitofish metabolic rates in warm water, the microplastic pieces we observed in mosquitofish likely pass through the fish within 3–4 h (Pyke 2005). As a result, our data show a snapshot of microplastic consumption, which at its highest was only observed in 10% of sampled mosquitofish. Numerous studies report much higher proportions of fish with microplastics, including 45% of sunfish in a Texas river (Peters and Bratton 2016), 72% of trout in an Irish river (O'Connor et al. 2020), and 98% of trout in a Wisconsin river (Simmerman and Coleman Wasik 2020). The relative lack of microplastics in mosquitofish could be due to the mosquitofish's small size (and corresponding feeding rates and gape limitation) when compared to larger bodied fishes. Peters and Bratton (2016) and Horton et al. (2018) found positive correlations between fish size and microplastic consumption, but other studies like ours have found none (Simmerman and Coleman Wasik 2020; O'Connor et al. 2020). Foraging ecology also is likely to influence microplastic consumption (Zhang et al. 2017; McNeish et al. 2018); mosquitofish typically feed at or near the surface (Pyke 2005). Benthic fishes in the lower Santa Cruz River, such as catfish or carp, may ingest more microplastic than mosquitofish. Finally, we visually quantified microplastic under 45× magnification, so we failed to detect very small (< 200 μm) microplastics in their stomachs. Future studies examining plastic consumption by small fish would benefit from spectroscopy identification and enumeration of plastic types and sizes to examine potential influences of plastic morphology on consumption and to detect small plastics potentially missed in this study.

Conclusions

To our knowledge, this is the first study on microplastic pollution in an effluent-dependent river. We found that microplastics are abundant in both the water column and benthic sediment and that mosquitofish do ingest microplastic, albeit at relatively low rates. The concentrations and occurrences of these plastics are strongly influenced by floods. Although we did not use spectroscopy to identify and quantify plastic composition (Song et al. 2015), we believe that our estimated microplastic concentrations are conservative due to our lower size thresholds. As stated previously, misidentification of nonplastics for microplastics in this study is possible. However, using the identification methods employed in our study, Karlsson et al. (2020) found zero false positives in pieces 300 μm or greater when confirmed with FTIR. Increasingly, recent studies are incorporating size classes much smaller than 330 μm in their analyses, and given the vast variability in the methods published in microplastic literature (Koelmans et al. 2017), care should be taken with direct comparisons of this study to future studies with different size classes. More work is needed to assess the nonpoint sources that many studies identify as significant contributors of microplastic pollution, and more work is needed to identify microplastic pollution baselines and trends in effluent rivers worldwide. As natural streams in arid and semi-arid climates become increasingly scarce, effluent-supported urban streams will become more common (Martí et al. 2009), and their value as conservation habitat will grow accordingly (Brooks et al. 2006; Bischel et al. 2013; Luthy et al. 2015). More information on microplastic pollution and its ecological impacts is needed to inform environmental managers and wastewater engineers about best strategies for utilizing effluent as a resource.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-13724-w.

Acknowledgements This study was completed as part of D.E. Eppehimer's PhD dissertation at the University of Arizona. We thank student technicians Chuck Landa and Emma Froelich for their field and lab contributions and Alton Livingstone for field support. Gary Deen and the Pima County Flood Control District provided access to field sites. Feedback from Susan Washko, Brian Gill, and anonymous reviewers improved previous versions of this manuscript. Maya Stahl created the conceptual art (Fig. 4). AZGFD scientific collecting license SP603606/SP62794 and IACUC protocol #17-270. The lower Santa Cruz River is the traditional homeland of the Tohono O'odham.

Author contribution DEE, MTB, and DMQ were responsible for the study design. DEE, HH, KDH, ZCN, and LNL were responsible for field work. DEE, KDH, and ZCN were responsible for lab work. DEE was responsible for data analysis, and all authors contributed to writing/editing the manuscript.

Funding Research was supported in part by a federal Water Resources Research Act Section 104b grant from the US Geological Survey through the University of Arizona Water Resources Research Center, a member of the National Institutes for Water Resources. This study was also supported in part by funding from the University of Arizona, the Graduate and Professional Student Council, Arid Lands Resource Sciences, National Park Service, WateReuse Arizona, Southern Arizona Environmental Management Society, and the Lincoln Institute's Babbitt Dissertation Fellowship Program.

Availability of data and materials The datasets generated and analyzed during this study are not currently publicly available because they are part of long-term datasets to be used in future studies but are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate Collection of mosquitofish was approved by AZ Game and Fish Department and the University of Arizona Institutional Animal Care and Use Committee: AZGFD scientific collecting license SP603606/SP62794 and IACUC protocol #17-270.

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

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