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Microplastic concentration and composition in surface waters and in stream-rearing Steelhead Trout (*Oncorhynchus mykiss*) in a rural coastal California stream

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ABSTRACT

Microplastic (particles < 5 mm) is an emerging environmental concern that threatens aquatic ecosystems worldwide. While extensive research has documented the ubiquity of microplastic in marine and coastal environments and its assimilation by organisms at all trophic levels, comparatively little is known about microplastic occurrence in rural riverine ecosystems. Even less information exists for salmon-bearing streams, where microplastic pollution may hinder salmonid conservation and recovery efforts. We examined the presence and concentration of microplastic in Scott Creek, a small coastal California (USA) watershed with minimal urban development. Our study quantified suspended microplastic in surface waters and in the gastrointestinal (GI) tracts of juvenile Steelhead Trout (*Oncorhynchus mykiss*). Microplastic was detected in 93 % of water samples, indicating that Scott Creek routinely transports and cycles microplastic. Concentrations were higher in late summer than in spring, although monthly spatial patterns were largely consistent among sites. In juvenile steelhead, 60.6 % of GI tracts contained one or more types of microplastic. For both surface waters and juvenile steelhead, microplastic was predominantly small (< 5 mm) dark blue, black, and transparent fiber-type particles, with fragments, shards and foams encountered less frequently. These findings suggest that fish ingest microplastic at low frequencies, or that ingested particles may pass rapidly through their digestive systems. This study provides one of the first assessments of microplastic in a minimally urbanized, salmon-bearing watershed. Our results serve as a baseline for evaluating the ecological implications of microplastic pollution in small coastal creeks and its potential to influence native salmonid populations.

Data Availability: The surface water and steelhead microplastic datasets generated and analyzed during the current study are publicly available via Zenodo (DOI: 10.5281/zenodo.18038857).

1. Introduction

Microplastic, plastic particles < 5 mm in diameter formed from the degradation of larger plastic debris, has emerged as a significant pollutant in aquatic ecosystems world-wide [1]. Over the past two decades, monitoring has revealed the ubiquitous occurrence of microplastic in

marine waters and sediments (coastal, pelagic, and deep sea), on coastal beaches and shorelines [2,3], and in freshwater lakes and rivers [4]. These plastics can leach toxic chemicals (e.g., polychlorinated biphenyls, PCBs), adsorb hydrophobic persistent organic pollutants [5], and serve as substrates for invasive microbes and novel bacterial assemblages [3,6,7]. A growing literature indicates that microplastic and

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associated chemical contaminants are frequently assimilated by marine [8–11] and freshwater organisms at all trophic levels [12]. While the long-term ecological and ecotoxicological consequences of microplastic remain uncertain, mounting evidence suggests they can adversely impact the fitness of individual animals [13,14] and more broadly the health and function of populations, communities and ecosystems [15,16].

It is well established that rivers and streams serve as conduits for the export of microplastic from terrestrial to estuarine ecosystems [17,18]. However, the persistence and cycling dynamics of microplastic within riverine ecosystems is poorly understood. Most research to date examining the prevalence and dynamics of microplastics in rivers has been conducted in large drainage networks with substantial urban development and complex land-use patterns [19–22]. In large watersheds, the bulk delivery of microplastic is often linked to myriad point sources including stormwater runoff from urban areas, wastewater treatment plant, household effluent, and industrial sewage sludge from agriculture and plastic mulching [20,23,24]. In contrast, small rural watersheds⁶ often lack salient point sources but may nevertheless contribute ecologically significant amounts of microplastic [25,26]. Microplastic introduction to small river systems is potentially driven by diffuse sources, including atmospheric deposition and agricultural runoff [27,28]. The presence of microplastics in small rural catchments warrants attention because such areas are of high conservation value, particularly for native and endemic aquatic species.

Microplastic may occur in several different forms, or ‘morphotypes’, in aquatic ecosystems. Common morphotypes include pre-production particles (nurdles), primary particles (microbeads), and secondary particles (fibers, fragments, and other particles broken off from larger plastic items), each occurring in an unlimited array of colors. In the gastrointestinal tracts of freshwater fishes, microplastic particles are often found in consistent yet low numbers [24,29]. Once present in the food web, trophic transfer of microplastic can occur through direct ingestion of contaminated prey [30], although persistent bioaccumulation in most aquatic animals appears limited [31,32]. Research indicates that fish consuming plant debris as part of their diet often ingest appreciable amounts of fiber-type microplastic, presumably because fibers are morphologically similar to many natural food items in terms of shape, color, and size [33]. Microplastic burden is typically lower in piscivorous fishes, however, suggesting a low transfer rate among species higher in the food web [34]. Nevertheless, it is believed that ingestion and assimilation of microplastic is relatively commonplace in fishes and that some particles can translocate to other organs via the intestines [29]. Nearshore oceanic studies of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) have demonstrated low concentrations of microplastic in gut contents [35], but studies focusing microplastic ingestion by salmonids during the freshwater phase of their life cycle are limited [36]. Pacific salmon and trout (*Oncorhynchus* spp.) may be particularly vulnerable to MP pollution due to their anadromous life history strategies (movement between freshwater, estuarine, and marine habitats during their life cycles) and their predatory roles in each ecosystem.

To address this knowledge gap, we quantified the concentration, morphotypes, and sources of microplastic in both the surface waters of a small coastal California stream and in the stomach contents of stream-rearing juvenile Steelhead Trout (*Oncorhynchus mykiss*). Our objectives were to: (1) determine the presence and characteristics of microplastic within surface waters; (2) identify spatiotemporal patterns in microplastic distribution and abundance during the spring and summer months; and (3) assess whether juvenile steelhead, the apex aquatic predator within the stream food web, were consuming microplastic particles.

2. Methods

2.1. Study area

Scott Creek is a small (drainage area = 70 km²) central California coastal watershed located approximately 90 km south of San Francisco (CA, USA, 37°02'26"N, 122°13'44"W; Fig. 1). The climate is cool Mediterranean and characterized by wet winters and a protracted dry period in summer and fall. Mean annual rainfall in the watershed is ~120 cm [37] and nearly all rain is delivered between November and April. Mean monthly air temperatures vary from 10°C in winter to 17°C in summer, with the latter moderated by recurrent coastal fog [38]. Stream discharge in the basin is highly dynamic and can exceed 65 m³/s during winter and spring storm events, whereas summer base flow generally decreases to < 0.1 m³/s in most years.

The Scott Creek watershed contains multiple sensitive aquatic species including populations of Coho Salmon (*Oncorhynchus kisutch*; Central California Coast evolutionarily significant unit) and steelhead (Central California Coast distinct population segment) which are listed as endangered and threatened, respectively, under the U.S. Endangered Species Act (ESA). The Scott Creek Coho Salmon population is of special management concern as it represents the southernmost persistent population of this species in North America, although abundance is generally low in most years. The creek also supports a native fish assemblage that includes Prickly Sculpin (*Cottus asper*), Coastrange Sculpin (*Cottus aleuticus*), and Threespine Stickleback (*Gasterosteus aculeatus*).

The Scott Creek watershed is representative of many small Pacific coastal salmon-bearing watersheds in that it is highly rural, with minimal urbanization and low population density. Housing density in the watershed is less than 1.8 homes/km² (Fig. 1)[39]. Predominant land uses within the Scott Creek watershed include small-scale timber harvest, agriculture and rangeland (Fig. 1) [40]. The headwaters of Scott Creek originate within the boundaries of Big Basin Redwoods State Park, which is a year-round destination for recreational activities, accommodating more than 200,000 visitors annually (Fig. 1) [39].

2.2. Surface water sampling and processing

We collected surface water samples at five sites in the Scott Creek watershed. Three sample sites, coded SC1, SC2, and SC3, were located on mainstem Scott Creek approximately 1.8, 7.0 and 7.9 km upstream (from the Pacific Ocean), respectively. We also sampled a single site in each of the two largest tributaries: Big Creek and Mill Creek (Fig. 1). Surface water samples were collected monthly in 2019 between April and July. During each sampling event, three replicate drift nets (363 µm mesh; mouth opening = 0.12 m²) were positioned side-by-side across the stream channel (perpendicular to stream flow) and deployed in the water column for 30 min. Wetted net area was quantified during each deployment and water depth (cm) and flow rate (m/s) at the mouth of each net were measured using a portable velocity meter attached to a top-setting wading rod (Marsh McBirney Flo-Mate model 2000, Loveland, Colorado, USA). Following each 30-min collection period, net contents were rinsed into the cod end using ambient site water and all retained material was transferred to pre-cleaned glass jars for transport and storage until laboratory processing.

In the laboratory, surface water samples were poured through Tyler nesting sieves to isolate and retain all solid material between 363 µm and 5.0 mm in size. Each sample jar was rinsed with distilled water over the sieve set until all residual solids were washed out. All material collected on the 5.0 mm sieve was discarded, whereas material retained by the 363 µm sieve was transferred into a pre-weighed 500 ml beaker using a metal spatula and distilled rinse water. Following the methods of Lippiatt et al. (2013) [41], samples were processed using a wet peroxide oxidation reaction to dissolve organic materials. However, we avoided heating samples above 65°C, as temperatures exceeding this value have been shown to breakdown some microplastic particles [42].

⁶ Minimally regulated river systems draining landscapes primarily allocated to low-intensity activities, such as forestry, agriculture, and livestock grazing

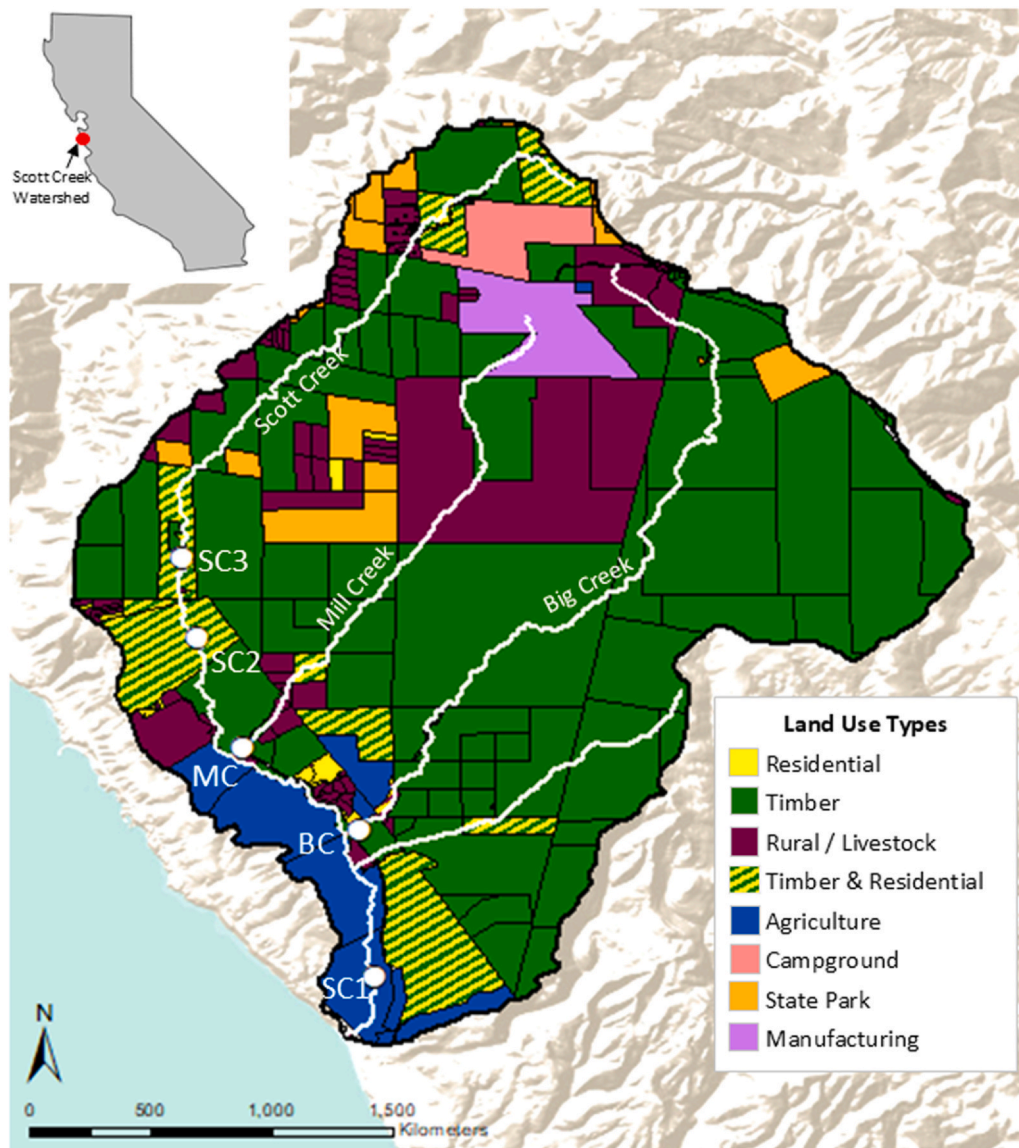


Fig. 1. Locations of surface water microplastic sampling sites (white dots) and the downstream migrant (smolt) trap where juvenile steelhead were collected (white rectangle). Sites 1, 4 and 5 were located on mainstem Scott Creek (lower, middle and upper, respectively). Sites 2 and 3 were tributary sites located on Big Creek and Mill Creek, respectively. Polygon colors denote major land use types. Maps created using land use data obtained from Santa Cruz County GIS (2022) (37°02'26"N, 122°13'44"W).

Sodium chloride was added to the remaining liquid to increase density (microplastic is less dense than saltwater), and a separatory funnel was used to isolate microplastic particles from heavier inorganic materials. The inorganic fraction was drained from the funnel, and all remaining contents were collected on glass fiber filters (Type A/E, 47 mm) using vacuum filtration. Individual filters were transferred to uniquely numbered aluminum sample boats, dried in a laboratory oven (60°C for ~24 h), and then stored in sterile 50-mm diameter Petri dishes with tight-fitting lids prior to microscopy.

2.3. Fish sampling and processing

To assess the presence of microplastic in riverine biota, we examined the stomach contents of juvenile steelhead trout, an apex predator in the Scott Creek aquatic food web. The juvenile steelhead used in this study were incidental mortalities that occurred at a downstream migrant trap operated by the National Marine Fisheries Service on the lower mainstem of Scott Creek (rkm 1.4) during April and May 2019. Fish mortalities were stored frozen until laboratory processing. The

juvenile steelhead used in this study ($n = 33$) ranged in size from 44 to 155 mm fork length and represented both sub-yearling (age-0 +) and yearling (age-1 +) age classes.

In the laboratory, we excised the entire gastrointestinal (GI) tract (esophagus to anus, extracted from the ventral surface) of each fish using pre-cleaned metal instruments (i.e., scalpel, scissors and forceps) and a glass dissection surface. Following excision, the GI tract was cut open and all contents were scraped onto a watch glass and then washed into a glass beaker using deionized water. All dissection instruments were also rinsed into the beaker. The contents of the beaker were collected on glass fiber filters (GF/A) using deionized water and vacuum filtration. Sample filters were subsequently transferred to pre-cleaned and numbered aluminum dishes and dried in a laboratory oven (60°C for ~24 h). We did not chemically digest GI tracts because the volume of stomach material in each fish was sufficiently low that all contents could be spread in a single, thin layer within a petri dish and examined thoroughly under a stereomicroscope without overlap. This allowed us to visually inspect all material directly and minimized the risk of particle breakdown via the chemical digestion process.

Table 1

Description of the morphotype categories used to characterize the presence of microplastic.

Category	Description
Fiber	Plastic threads or filaments longer than they are wide. Many enter the environment as a byproduct of washing synthetic clothing, or from the breakdown of fishing lines and nets.
Fragment	Broken off or separated piece of plastic with frayed or rounded edges. These plastic particles are thicker and bulkier than fibers, but do not have the hard edges of shards.
Shard	A piece of broken plastic with sharp edges.
Flexible Foam	Mass of bubbled plastic with trapped pockets of gas. Many plastic foams are made from polyurethane or polyethylene.
Rigid Foam	Expanded polystyrene in the form of a lightweight, durable and non-malleable foam.

2.4. Microplastic identification

Each filter containing dried sample material was examined for 10–15 min using a dissecting microscope (Leica model S91i; zoom range of $0.61\text{--}5.5\times$ for a total magnification of $6\text{--}55\times$ with $10\times/23$ eyepiece). For larger samples (i.e., those with high amounts of undissolved detritus), filters were wetted using deionized water to allow detrital material to be peeled off layer by layer, thus facilitating complete examination of the sample. All microplastic particles encountered were removed from filters using metal forceps and mounted on pre-cleaned glass microscope slides. Microplastic particles were counted in totality for each sample and classified by color and according to 5 different major morphotypes: (1) fiber, (2) fragment, (3) shard, (4) rigid foam (i.e., polystyrene/Styrofoam) and (5) flexible foam (i.e., polyurethane or low-density polyethylene foam) (Table 1). Polymer type was not determined because analytical instruments such as FTIR or Raman spectroscopy were not available for this study. Particle size was also not measured directly; all particles visible at the smallest magnification setting ($55\times$) were examined and recorded, but precise measurements were not obtained. As a result, our dataset includes all microplastics detectable at the lowest magnification setting but does not provide precise size classifications.

2.5. Contamination control

To minimize cross-contamination of microplastic in our samples, we avoided using plastic products or tools wherever possible during the study. All experimental work was carried out while wearing cotton laboratory coats and nitrile gloves and we deliberately avoided wearing synthetic polymer clothing in both the field and laboratory. Laboratory reagents were stored in air-tight containers and sample processing was completed in a laminar flow fume hood. Whereas microplastic has been shown to be ubiquitous in both water and air [43], we processed blank samples in the laboratory to account for potential ambient microplastic cross-contamination during sample collection and (or) processing. These blank samples were collected, processed, stored, and analyzed using the same methods as the surface water and steelhead GI tract samples. For all sample types (i.e., surface water and steelhead GI), we ran two sets of blanks: (1) ‘field blanks’ to control for ambient microplastic potentially present in the field gear used to collect samples, and (2) ‘laboratory blanks’ to identify possible ambient contamination present during filtering, chemical digestion, drying, and (or) microscope examination. We randomly dispersed the order of observation among all samples (i.e., water, fish, field blanks, and laboratory blanks). Prior to data analysis, we subtracted the mean number of microplastic particles present in the blanks from the surface water and steelhead totals with respect to color and morphotype but did not allow any microplastic count less than zero.

2.6. Data analysis

The total count of microplastic particles in each surface water sample, and combinations of morphotype and color, were standardized based on water volume to compare microplastic concentration across

sites and months. Microplastic concentration (particles/ m^3) was calculated using the equation:

$$\text{Concentration} = \frac{\left(M - \left[\frac{B}{N}\right]\right)}{(A \times V \times T)} \quad (1)$$

where M is the total number of microplastic counted per filter, B is the sum of the total number of microplastic counted in blank/control samples, and N is the total number of blank samples ($N = 6$). In the denominator, A is the cross-sectional area of flow entering each net (m^2), V is water velocity (m/s), and T is the duration of sampling. The value of A varied according to the depth of the water and streamflow; during low flow conditions, the net opening was not always fully submerged due to the shallow (depth $< 0.25\text{ m}$) water column. All additional analyses were conducted in R [44].

During each sample event the three drift nets employed at each site were treated as replicate samples. We compared microplastic concentration among surface water samples using two-way analysis of variance (ANOVA) to determine effects related to sample site and sample month. The significance level (α) was set a priori at 0.05. We identified sites and months with significant unique microplastic concentrations using Tukey multiple comparisons of means (95 % family-wise confidence intervals). Microplastic morphotypes and colors in water samples were compared among sites and months as an interaction and independently using multivariate analysis of variance tests (MANOVA).

Nonmetric multidimensional scaling (NMDS) ordinations were used to examine patterns in microplastic morphotypes and color assemblages among the four months and spatially across the five study sites. Using the ‘vegan’ package in R [45], we calculated Bray-Curtis similarities for surface water microplastic per water sample and used NMDS ordinations to visualize these relationships. In NMDS ordination plots, ellipses represent the 95 % confidence interval surrounding the mean score of each sample site or sample month in ordination space. Morphotypes with significant gradients on ordinations were determined by bootstrapping utilizing the ‘envfit’ function. Vectors represented statistically significant ($P < 0.05$) morphotypes associated with each NMDS axes. The direction and magnitude of each vector was proportional to the Spearman coefficient of correlation with each respective NMDS axis. We used a nonparametric multi-response permutation procedure (MRPP, permutations = 999) to test the null hypotheses of no spatial differences in microplastic composition among the sample sites. Euclidean distances were calculated for untransformed microplastic concentration data. We used Bonferroni-adjusted alpha levels of $\alpha = 0.010$ ($0.05/5$) and $\alpha = 0.013$ ($0.05/4$) to assess significant differences in microplastic compositions among sample sites and between months, respectively.

Whereas we had a limited number of juvenile steelhead GI samples, we converted microplastic count data to presence/absence prior to analysis. During initial data screening we tested for potential ontogenetic dietary shifts related to body size but found no significant differences in the abundance of microplastic in steelhead GI tracts based on fish fork length (one-way ANOVA, $P = 0.16$, data not shown). Preliminary Bray-Curtis similarity matrices and NMDS ordinations, and

multivariate analysis of variance tests (MANOVA) for microplastic color and morphotype in steelhead also showed no significant differences in composition. Consequently, we pooled all age and size classes of juvenile steelhead for subsequent analysis.

3. Results

3.1. Blanks

The blank (control) samples contained a small amount of short, mostly dark blue and black fibers, which we suspect originated from ambient particles floating in the air in our laboratory space. No other morphotypes of microplastic were observed on the control samples. Across all surface water blank samples, the number of microplastic fibers present averaged 4.17 (SD = 0.98, range = 2–5; [Figure S1](#)). In gastrointestinal tract samples, there was a mean of 2.17 (SD = 1.60, range = 1–5; [Figure S2](#)). Our ambient contamination rate was similar to rates reported for control/blank samples in similar studies [35]. Because no blank had a non-fibrous microplastic particle, we assume that all fragments, shards, rigid foam, and flexible foam particles were derived from sample sites. All reported results of water and GI samples reflect microplastic concentrations after subtracting the mean value of microplastic found in blanks from the sum of all microplastic particles counted on each filter.

3.2. Surface waters

Across the entire study, microplastic was present in 93 % of the surface water samples and represented by four plastic morphotype categories: fibers, fragments, shards, and rigid and flexible foam ([Fig. 2](#)). Fibers were the numerically dominant morphotype representing > 86 % of the microplastic recovered and were mostly black (43.4 %) or dark blue (21.0 %) in color ([Fig. 3](#)). The mean ($\pm$ SD) concentration of microplastic across all surface water samples from our study was

0.37 ± 0.58 microplastic/ m^3 , ranging between 0 and 3.6 microplastic/ m^3 . After adjusting for cross contamination, the fiber morphotype contained the widest array of colors: black (45.3 %), blue (37.8 %), transparent (i.e., clear and colorless; 8.0 %), red (4.0 %), pink (1.2 %), with green, white, teal, yellow and orange each contributing < 1 %. The fragment morphotype included eight colors: blue (44.8 %), pink (34.7 %), yellow (9.2 %), white (6.4 %), red (2.7 %), orange (1.3 %), black (0.5 %), and transparent (0.3 %). Shard samples were purple (40.0 %), white (40.0 %) or blue (19.9 %), while all flexible foam was yellow and all rigid foam was white.

Two-way ANOVA tests indicated the significant influence of both sampling month ($F_{(3, 51)} = 9.28$, $P < 0.01$) and site ($F_{(4, 51)} = 2.76$, $P = 0.03$) on microplastic concentration in surface water, but no significant influence of the interaction between the two ($F_{(12, 39)} = 1.29$, $P = 0.26$). There were significant temporal differences between the microplastic concentration in the water samples during the four months of the study ([Fig. 4](#)). Tukey comparisons indicated significant pairwise differences between July and all other sample months (July vs. April, $P < 0.01$; July vs. May, $P < 0.01$; July vs. June, $P < 0.01$). We found a higher concentration of microplastic during the low flow sample in July than in the higher flow samples collected during spring (April and May) ([Figs. 4, 6](#)). There was a significant difference in microplastic concentration between sites ([Fig. 4](#)). Post-hoc comparisons indicated pairwise differences between the major tributaries (Mill Creek and Big Creek, respectively) across months (Tukey HSD, $P = 0.02$; [Fig. 4](#)), although comparisons between other mainstem Scott Creek sites (SC1, SC2 and SC3) and these tributary sites were not significant. Multivariate analysis of variance tests indicated significant difference of microplastic color by month (MANOVA; $F_{(27, 135)} = 2.50$, $P < 0.01$, Wilks' $\Lambda = 0.23$). There was no statistically significant difference of color by site, or of morphotype by either month or site.

Nonmetric multidimensional scaling (NMDS) ordinations contrasting monthly microplastic assemblages based on color and morphotype revealed variation in the types of microplastic present in the

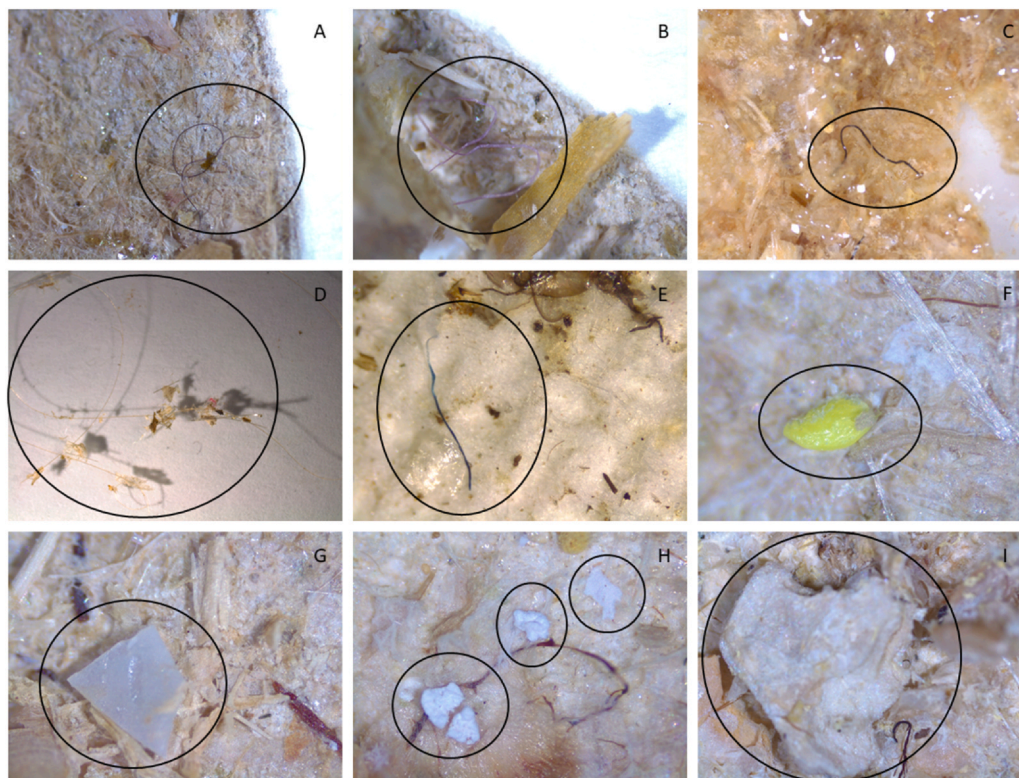


Fig. 2. Examples of microplastic observed in surface water and juvenile steelhead, including fibers (A–D), fragments (F), shards (G), rigid foam (H), flexible foam (I), and fibers recovered from steelhead GI tracts (E). All images taken at $55\times$ magnification.

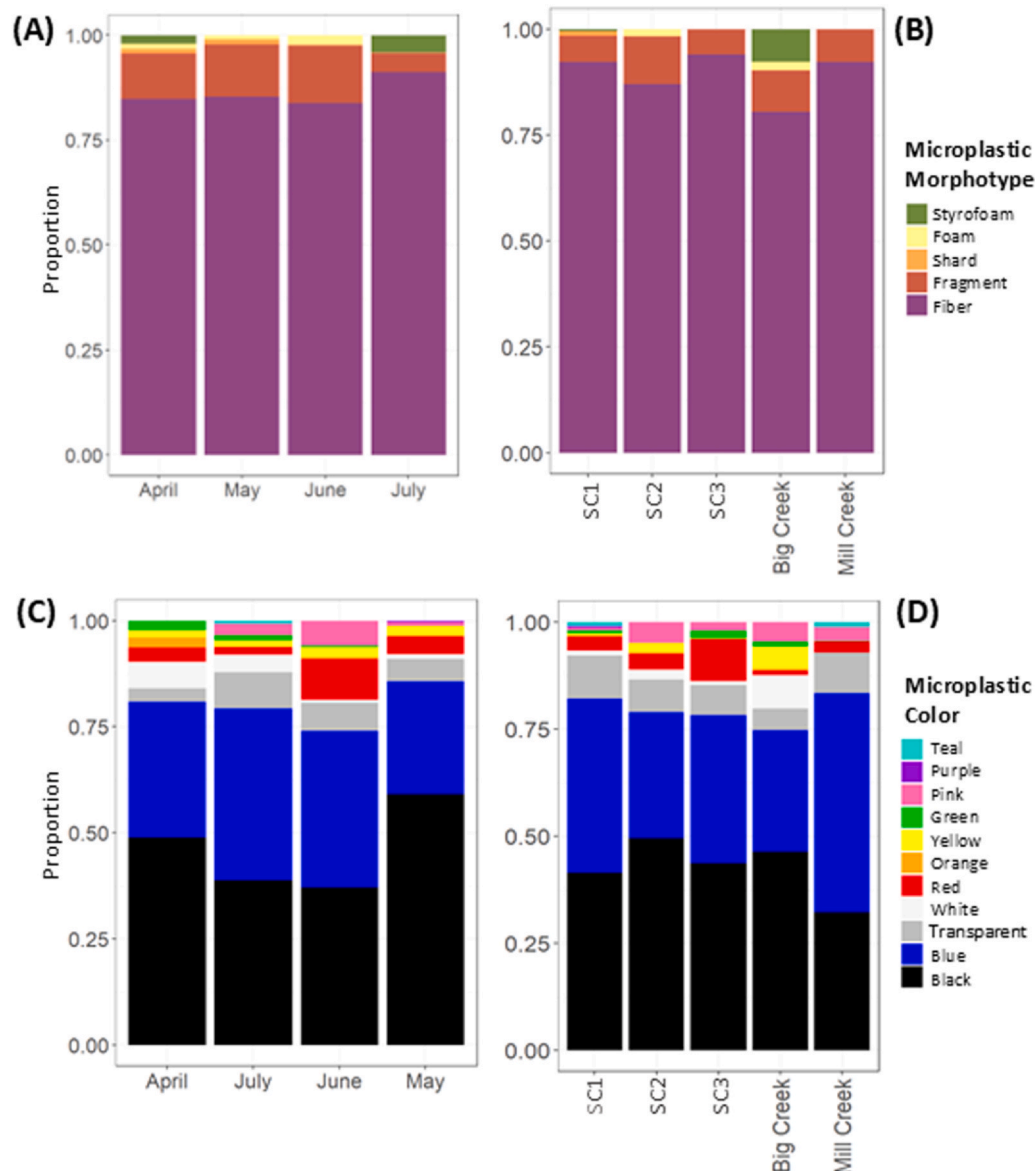


Fig. 3. Relative proportions of microplastic morphotypes (A, B) and colors (C, D) in surface water samples by month and sampling site. Fibers and blue/black particles were the dominant categories across all samples.

creek between spring (April) and summer (July) (Fig. 5). Differences in microplastic composition between months were due primarily to the presence of blue fragments, blue fibers and black fibers between sample months (Fig. 5). The final stress value of the ordination was 0.03 with two dimensions.

3.3. Fish gastrointestinal tracts

After adjusting for ambient contamination (mean microplastic per blank = 2.17), of the 33 juvenile steelhead GI tracts examined, 60.6 % ($n = 20$) contained at least one microplastic particle. Across all fish samples, individual steelhead contained an adjusted mean of $1.05 (\pm 1.29)$ microplastic particles in their GI tracts. No more than four unique particles of microplastic were found in any individual steelhead. With respect to morphotype, fibers made up 97.6 % of the microplastic recovered from steelhead GI tracts, and the most abundant microplastic were dark blue (42.1 %), transparent (26.0 %) and black fibers (11.6 %; Fig. 7).

4. Discussion

4.1. Microplastic in surface water

This study established the presence of microplastic particles in both the surface water of a small rural stream and in the stomachs of stream-rearing predatory fish. We documented five morphotypes of microplastic in the Scott Creek watershed: fibers, fragments, shards, and rigid and flexible foams (Figs. 2, 3). Most particles found in the surface water were fibers (88 %), while plastic fragments were found in lesser abundances, and foam occurred in only a few samples. Our high proportion of fibers is consistent with other studies of microplastic in fluvial ecosystems [33,46]. Microplastic in the Scott Creek watershed varied by color (Fig. 3) with blue, black and transparent fibers representing a majority of the assemblage. Fiber and fragment morphotypes typically represent secondary microplastic (Pico et al., 2019), supporting our expectation that secondary sources would predominate within the Scott Creek watershed. Given the rural setting and land use patterns, these particles likely originated from the breakdown of macroplastics (e.g.,

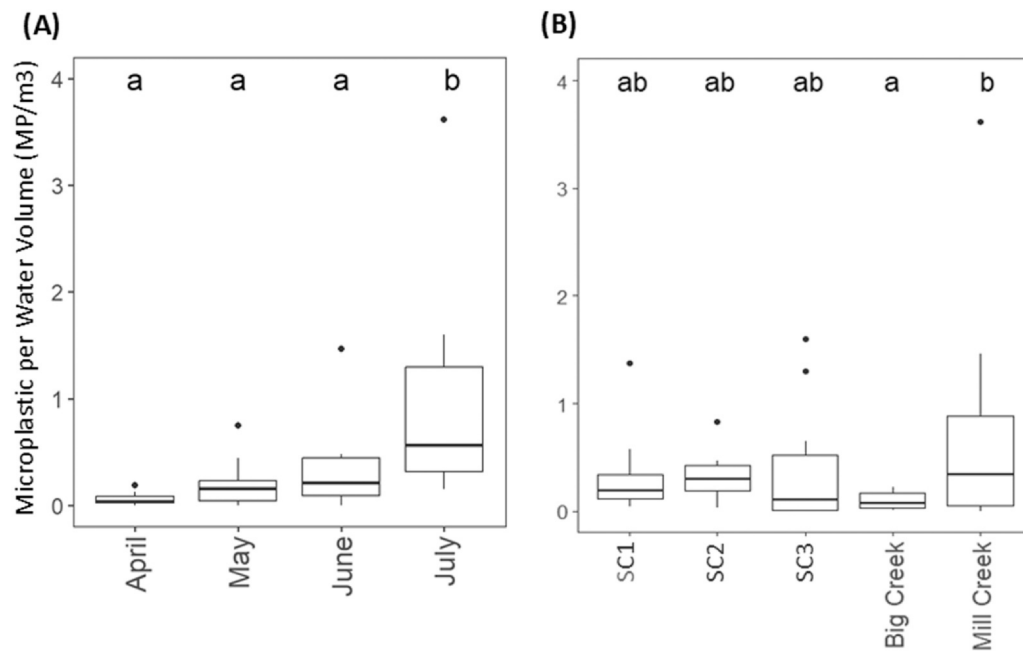


Fig. 4. Microplastic concentration (particles/m³) across (A) sampling months and (B) sample sites. Boxes show medians, interquartile ranges, and 5th–95th percentiles; points indicate outliers. Different lowercase letters denote significant differences (ANOVA with Tukey post-hoc tests).

tarps, pipes, tools and equipment) used for livestock ranching, timber harvest, and agriculture [47,48]. Additionally, the watershed's proximity to urban centers (e.g., San Francisco Bay area, Santa Clara valley and Santa Cruz mountain communities) makes it likely that some particles are entering the watershed as ambient environmental microplastic from air and (or) rainwater inputs [23,46]. Relatively minor land uses, including residential properties, manufacturing centers, and recreational areas, may also contribute secondary microplastic to the creek in the form of litter, wastewater, laundry effluent, and weathering of structures and facilities.

The NMDS ordinations revealed a temporal shift in microplastic assemblage composition between spring and summer sampling (Fig. 5). Specifically, the composition in April was distinct from that in July, driven by higher abundances of blue, transparent, pink, red and black microplastic fibers in the July samples. Despite this shift, monthly pairwise comparisons (MRPP) of microplastic assemblages indicated a relatively high degree of similarity across sample periods. We attribute the assemblage shift between April and July to seasonal land use practices, changing wind patterns that facilitate aeolian transport, and the scouring effect of spring high discharge events which likely 'reset'

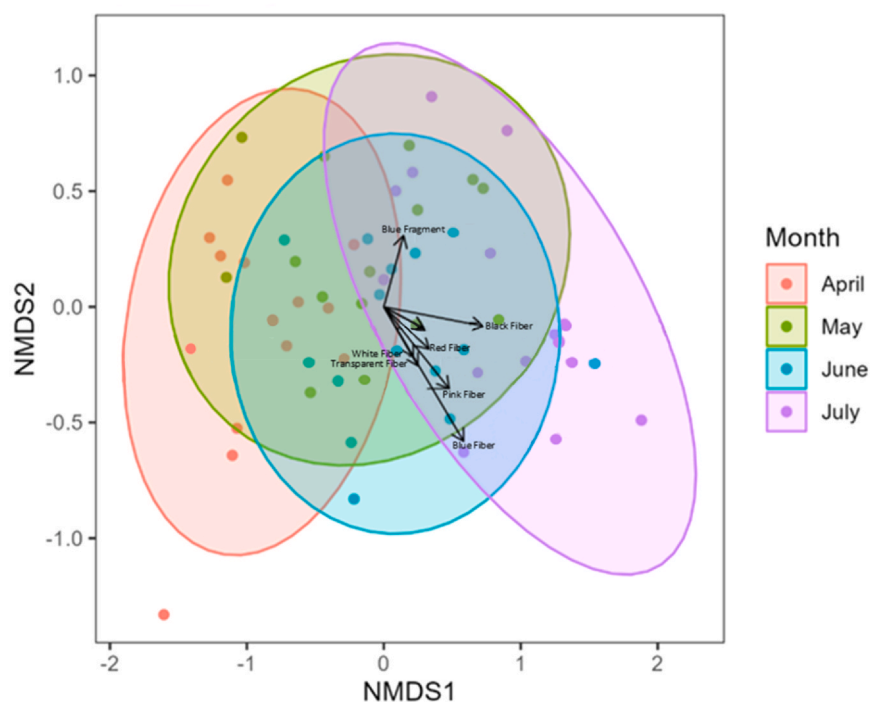


Fig. 5. Non-metric multidimensional scaling (NMDS) ordination illustrating monthly variation in microplastic composition (morphotype and color) in surface water. Points are individual samples, ellipses represent 95 % confidence intervals, and vectors indicate particle types with significant loadings ($p < 0.05$).

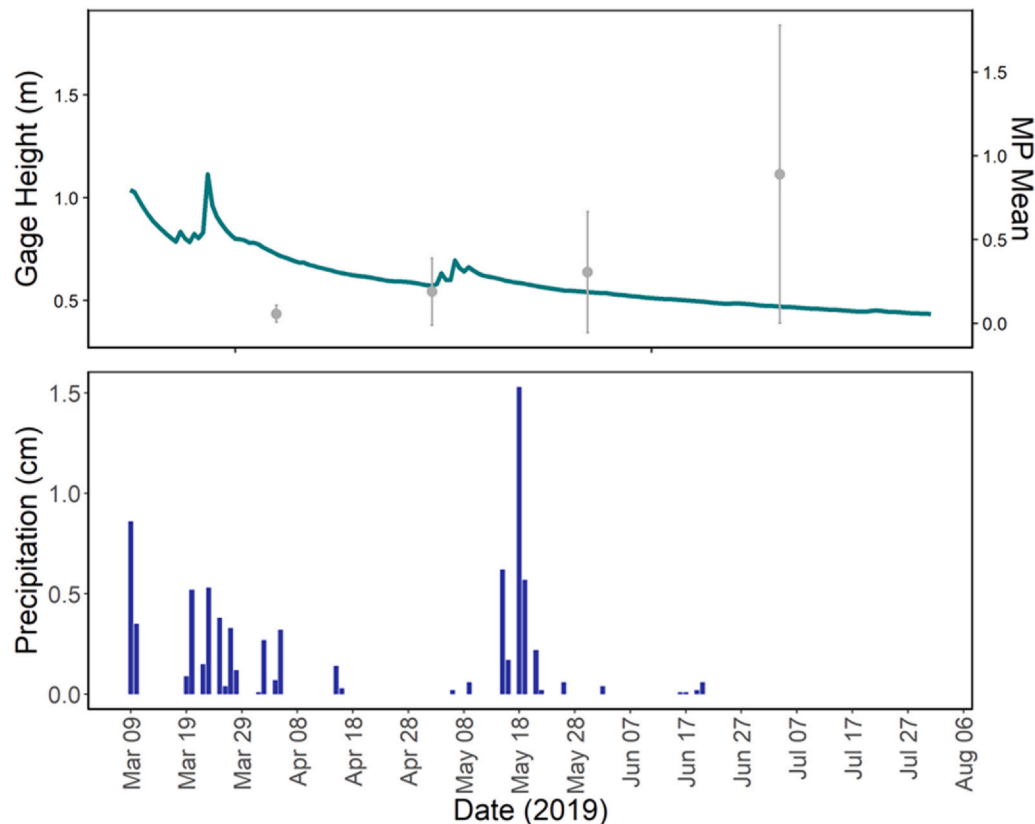


Fig. 6. Mean daily stream stage height (teal line) and precipitation (blue bars) in the Scott Creek watershed, with mean microplastic concentration (gray points $\pm$ SD) for the four sampling dates. Stream stage height was recorded at a gaging station in lower mainstem Scott Creek (river kilometer ~ 2.0), and precipitation data was sourced from the California Irrigation Management Information Center (Station 104, DeLavega gage; <https://cimis.water.ca.gov/WSNReportCriteria.aspx>).

the system and allow for the development of a novel microplastic assemblage during the summer.

Microplastic concentrations differed significantly between the two tributaries, Mill Creek and Big Creek. Averaged across all four months of the study, the Mill Creek sub-basin contributed more microplastic per water volume than the Big Creek sub-basin. This disparity likely reflects different land uses within each sub-watershed (Fig. 4); Mill Creek supports manufacturing, camping, and livestock farming, whereas Big Creek is primarily used for timber harvest (Fig. 1). Interestingly, microplastic concentration at the three mainstem Scott Creek sites (SC1, SC2, SC3) did not differ significantly from either tributary. This spatial homogeneity suggests that, in the absence of a singular point source, microplastic is distributed relatively evenly throughout the drainage network. Furthermore, the export of accumulated microplastic may be a protracted process, even in systems with highly dynamic hydrologic regimes. Regardless of the mechanism, these findings suggest that microplastic is continuously entering surface waters throughout this rural agricultural watershed.

Within the Scott Creek watershed, surface water samples collected during low flow conditions in July exhibited significantly higher microplastic concentration than those collected during higher stream flows in spring (April, May, and June) (Figs. 4 and 6). Our finding that microplastic concentration did not increase with increasing streamflow is contrary to some published works that reported a positive correlation between microplastic counts and discharge (e.g., [49,50]). We propose two mechanisms for this discrepancy. First, elevated stream flows during spring likely exert a dilution effect, reducing the overall concentration of microplastic per unit volume. Second, in hydrologically dynamic watersheds like Scott Creek, the ‘first flush’ associated with early season storms may export the bulk of accumulated microplastic during winter months, thereby reducing the amount of plastic material available for mobilization during spring runoff events. Conversely, the

high concentration of microplastic we observed in July could be a product of low-flow dynamics and the concentration of suspended plastic materials. Though speculative, it is plausible that longer residence times coupled with increased solar radiation during the summer accelerate the degradation and fragmentation of plastic debris (across size classes), thereby driving an increase in particle abundance in the water column [51]. The effects of summer low flows on instream accumulation and fragmentation of microplastic warrant additional research.

4.2. Microplastic in juvenile fish

We found very few microplastic particles in steelhead GI tracts, compared to ambient surface water concentrations. Of the microplastic quantified within juvenile steelhead, the overwhelmingly dominant morphotype was fibers; only a single individual contained a non-fiber morphotype (i.e., microplastic fragment) (Fig. 7). For individual steelhead containing microplastic in their GI tracts, ingestion likely occurred through one of three pathways: mistaking microplastic for natural prey items (e.g., invertebrates) [52], incidental ingestion while foraging [53], or trophic transfer from contaminated prey [54,55]. There are multiple plausible explanations for the relatively low microplastic occurrence in juvenile steelhead in Scott Creek. First, fish may actively reject non-food particles during drift feeding, or they may have few encounters with suspended microplastic as a result of benthic foraging [56]. Second, small particles may be egested (excreted) rapidly rather than accumulating within the GI tract [57,58].

We focused our efforts on juvenile steelhead because they are apex predators in the Scott Creek food chain. Studying this trophic level allowed us to assess both possible direct ingestion of microplastic and indirect exposure via the consumption of contaminated prey, offering insight into the potential for bioaccumulation of these pollutants within

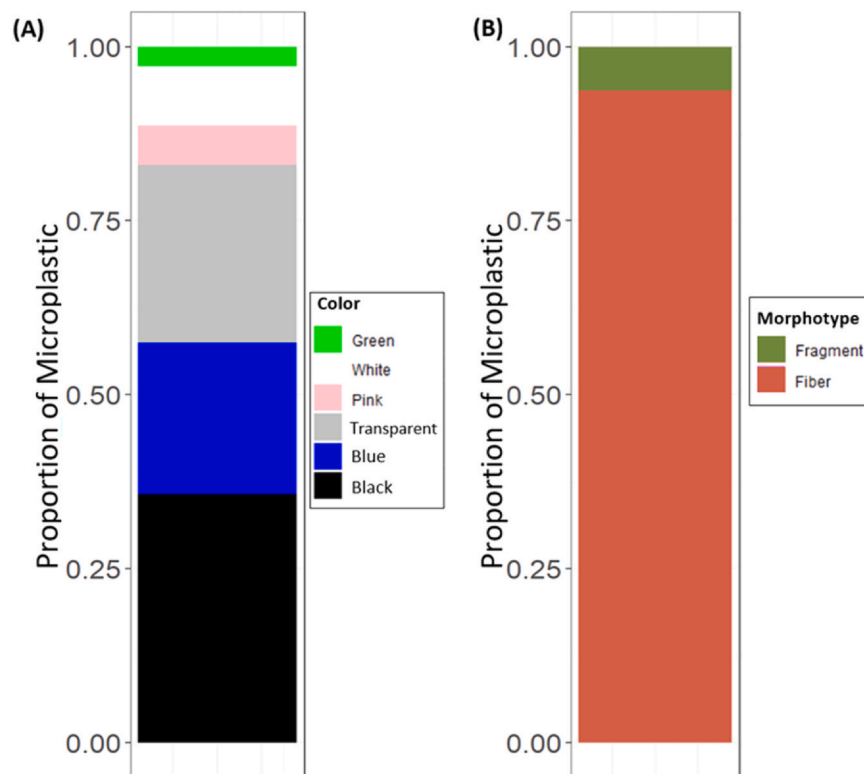


Fig. 7. Relative proportion on microplastic found in the gastrointestinal tracts of juvenile steelhead collected in the Scott Creek watershed by (A) color and (B) morphotype.

the food web. Furthermore, by focusing on juvenile steelhead during the freshwater phase of their life cycle, we confidently isolate exposure to sources of microplastic contamination occurring locally within the Scott Creek watershed. However, steelhead life history diversity adds both complexity and uncertainty to patterns of potential exposure. The seasonal lagoon at the mouth of Scott Creek serves as critical rearing habitat for juvenile steelhead, and substantial numbers of fish temporarily recruit to the lagoon during the spring and summer each year [38,40,59]. The lagoon is characterized by lentic conditions and known to be depositional habitat. Consequently, juvenile steelhead that rear in the lagoon could be exposed to elevated levels of microplastic relative to concentrations experienced by fish rearing higher in the drainage network. It is unknown whether any of the juvenile steelhead examined in our study had previously spent time rearing in the Scott Creek lagoon, which could potentially bias our results. The Scott Creek watershed is also home to several species of predatory fish that were not sampled in this study, including Coho Salmon, Threespine Stickleback and two species of sculpin. It is possible that the physiology or behaviors of these species make them more (or less) susceptible to ingestion and potential resultant accumulation of microplastic compared to juvenile steelhead. Even though microplastic counts in the GI tracts of juvenile steelhead were low, the presence of microplastic in the surface waters poses other potential threats. Chemical contaminants leached into the surface water from plastics can bioaccumulate in individual animals [60] or biomagnifying through the food web [32,61,62]. Additionally, microplastic ingestion by aquatic invertebrates has been linked to a decline in feeding behavior, growth, and fertility [63,64], which could potentially destabilize the trophic base that supports juvenile steelhead and other aquatic predators.

5. Conclusion

This study demonstrates that microplastic pollution is present even in small, rural, salmon-bearing coastal streams, underscoring the broad

reach of microplastic contamination in freshwater ecosystems. The consistent detection of microplastic in surface water samples in this study suggests that rural streams can act as conduits for microplastic transport despite limited local urbanization. While juvenile steelhead exhibited low incidence of microplastic ingestion, the occurrence of particles in their GI tracts confirms that microplastic is accessible to motile organisms and has the potential to enter animals at higher trophic levels. These results provide a baseline for future evaluations of microplastic dynamics in lotic systems that support native salmonids. Continued monitoring will be critical for determining long-term trends, identifying sources, and evaluating whether microplastic poses a measurable threat to native fish conservation and recovery in small coastal creeks.

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CRediT authorship contribution statement

Joseph D. Kiernan: Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Kern Cynthia:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Rosealea M. Bond:** Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Lindsay E. Hansen:** Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Fish Handling

The capture, handling and salvage of ESA-listed steelhead was authorized by the National Marine Fisheries Service under Section 10(a) (1)(A) permit No. 17292–2 A. All fish handling procedures were carried out according to approved protocols of the Institutional Animal Care and Use Committee at the University of California, Santa Cruz (Protocol No. KIERJ1604_A1).

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.epm.2026.02.001](https://doi.org/10.1016/j.epm.2026.02.001).

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