Title. Plastics in estuarine fish and sediments at the mouth of an urban watershed.

Authors.

Theresa Sinicrope Talley1*, Nina Venuti1, Rachel Whelan2

1California Sea Grant, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093-0232 USA.

2 Environmental and Ocean Sciences, University of San Diego, San Diego California 92110 USA.

*Corresponding Author, Email: tstalley@ucsd.edu, Address: California Sea Grant, Scripps Institution of Oceanography, La Jolla, CA 92037-0232 USA.

Abstract.

The extent to which small plastics and potentially associated compounds are entering coastal foodwebs, especially in estuarine systems, is only beginning to be realized. Using estuarine fish and sediment collected during June 2015 from urbanized Chollas Creek in San Diego, California, we tested the hypotheses that small plastic composition in sediments would be reflected in fish guts (non-selective consumption), and that semi-volatile organic compounds (SVOCs) would be present in all fish. Sediments contained about 10,000 small plastic pieces per m², consisting mostly (90%) of fibers, and hard and soft pieces. Nearly 25% of fish contained small plastics, but prevalence varied with size and between species. Of the 39 types of small plastics found in sediment, fish preferred 10 types (distinct colors and forms). Several SVOCs, both water soluble and sediment-associated compounds, were found in the two species of fish tested. We conclude that a species’ natural history may influence contamination levels with consequences, and lessons, for all consumers.
Keywords. Chollas Creek, ichthyofauna, plastics, San Diego Bay, water contamination, wetland fish

Introduction. Microplastics (plastic particles <5mm; Rochman 2018) are pervasive in our ocean and coastal ecosystems (Thompson 2015; Cole et al., 2011). These small plastics enter the environment as either primary microplastics (those manufactured as tiny pieces, such as microbeads) or secondary microplastics (those that form from the breakdown of larger plastic items) (de Sá et al. 2018; Auta et al. 2017). Microplastics are of concern not just because of their ubiquity, but because of the harmful effects they can have on biota. Microplastics can accumulate in the gut or gills of organisms, interfering with important life history processes such as feeding, growth, and reproduction (Chae and An 2017; Cole et al. 2015; Watts et al. 2014; Watts et al. 2015; Sussarellu et al. 2016). The monomers and additives that compose microplastics can be toxic to biota if they leach from their parent plastics into the environment (Smith et al. 2018; Thaysen et al. 2018; Teuten, et al. 2009). Furthermore, microplastics can sorb toxins such as metals, PCBs, PAHs, and DDT from the aquatic environment, and transmit these toxins to organisms, causing stress to internal organs, disruptions in normal bodily functions (e.g., enzyme inhibition, endocrine disruption), and reductions in organisms’ abilities to defend themselves against predators and other threats (Chae and An 2017; Smith et al. 2018; Rochman et al. 2013a,b; Browne et al. 2013; Barboza et al. 2018b; Rochman et al. 2014). Microplastics have been found to transfer between trophic levels (Welden et al. 2018; Farrell and Nelson 2013; Setälä et al. 2014), and thus may pose health risks to humans via consumption of contaminated seafood (e.g., Van Cauwenberghe and Janssen 2014; Rochman et al. 2015a; Barboza et al.)
2018a), though the impacts of microplastics on human health remain largely unknown (Wright and Kelly 2017; Smith et al. 2018).

One suite of contaminants of concern are semi-volatile organic compounds (SVOCs), which include additives commonly used in plastics manufacturing, such as phthalates, bisphenol A, and PBDEs, as well as PCBs, PAHs, and DDT (Lucatti et al. 2018; Weschler and Nazaroff 2008; Lusher et al. 2017b). SVOCs are susceptible not only to leaching out of plastics into the environment, but to resorbing to microplastics once present in the environment, due to their hydrophobic properties (Lusher et al. 2017b; Teuten et al. 2009; Cheng et al. 2013; Rochman et al. 2013a). SVOCs are a health concern for humans and wildlife because they are endocrine disrupting chemicals (EDCs) that have been linked with neurological, reproductive, metabolic, and behavioral abnormalities, as well as increased incidences of some forms of cancer (Koch and Calafat 2009; Xu and Zhang 2011; Gore et al. 2015). Microplastics facilitate the accumulation of SVOCs in organismal tissues (Rochman et al. 2013b; Besseling et al. 2013), however it is important to note that microplastics’ role as a conduit for SVOCs into coastal foodwebs may be relatively unimportant when compared with other vectors, such as contaminated water, prey, or sediments (Lusher et al. 2017b; Koelmans et al. 2016). It is also important to note that, while often associated with plastics, SVOCs found in the environment may stem from a number of different sources, including household cleaning products, cosmetics, and pesticides (Luccattini et al. 2018; Weschler and Nazaroff 2008).

Much of the research conducted on the ecological impacts of microplastics over the last two decades has focused on marine systems, only recently shifting to include terrestrial and...
freshwater systems (Rochman 2018; Chae and An 2017). This shift in focus upstream is an important one, given the fact that significant portions of marine plastic pollution come from land-based sources, and rivers are major conduits of debris from land to sea (Rochman 2018; Jambeck et al. 2015; Lebreton et al. 2017). The effects of microplastics in urbanized riverine ecosystems may be particularly acute (e.g., Peters and Bratton 2016), as rivers that flow through densely populated, urban areas have been shown to carry higher loads of debris (Lebreton et al. 2017; SCCWRP 2016; Yonkos et al. 2014). This study contributes to the growing body of research on microplastics upstream from marine ecosystems by investigating the presence of microplastics and associated contaminants in estuarine sediments and fish in a brackish stretch of urbanized Chollas Creek, which empties into San Diego Bay. Understanding the types, fates, and effects of small plastics in coastal watersheds is necessary to develop natural and social science-based solutions to marine debris and declining watershed health (Rochman 2018).

Knowledge of seasonal precipitation patterns in Mediterranean climates, such as that found in Southern California, result in accumulation and breakdown of photodegradable plastics during the sunny, dry season, followed by wet season pulses of debris inputs and transport through coastal watersheds (Anderson et al., 2012; Lee, 2011; Chandler, 2012; Moore et al., 2011). A recent regional survey of the Southern California Bight, to which this study contributed, revealed that 92% of the total stream length of urban watersheds in the Southern California Bight contained debris, while 48% of undeveloped watersheds did (SCCWRP, 2016). More than 60% of the debris found was plastic (SCCWRP, 2016). Many coastal wetland fishes, such as killifish, intensively forage on the substratum (West and Zedler, 2000) where small plastics and contaminants accumulate, putting them at particularly high risk of contamination. These fish may...
in turn be important vectors in transferring small plastics and contaminants to the broader coastal food web given their abundance, their roles in connecting intertidal with both subtidal and terrestrial ecosystems, and their roles as forage fish for many species (Trexler et al., 1994; West et al., 2003; Able et al., 2012; Kang et al., 2015).

**Project goals.** The goals of this study were to: a) determine the extent and magnitude of microplastics pollution in estuarine sediments and fish, and of SVOC contamination in estuarine fish, at the mouth of an urban coastal watershed, and b) determine whether sampled fish preferentially ingested certain types of microplastics, when compared with types and abundances of microplastics in sampled sediments.

**Materials and Methods.**

**Study location.** The Chollas Creek subwatershed (Fig. 1), considered one of the most impaired waterbodies in San Diego County, in part due to the large amounts of trash present in the creek (Anderson et al. 2012; State Water Board 2015), runs through a densely populated, urban section of the County and empties into San Diego Bay at one of the worst urban runoff sites of coastal San Diego (Pritchard 2014). In June 2015, we sampled sediments and fish along a 250-m long reach of intertidal Chollas Creek, located about 1 km upstream of the mouth (Fig. 1).

**Sample collection and processing.** Microplastics in sediment were sampled at low tide by collecting nine 10-cm diameter x 5-cm depth cores (393 cu cm) throughout the reach, placing cores in clean, airtight bags, and freezing cores until analysis in the lab. Plastics were sorted from sediments by placing a single layer of sediment at a time into a Petri dish (about 1 tablespoon or 15 cu cm) along with a squeeze of milliQ water to slightly liquefy the moist sediment. The dish...
with mud was systematically examined at 25-45x power using a dissection microscope; sorting of each dish took no more than 15 minutes. Particles that were clearly of anthropogenic origin, as determined by the shape and/or color of each particle (e.g., spherical microbeads, fibers with smooth surfaces and homogeneous thicknesses; often bright colors that stood out from the rest of the sample) were sorted out of the sample, classified according to the item from which they originated, when distinguishable (e.g., pieces of grocery bag, wrappers), or by hardness, shape and color (e.g., film, hard or soft pieces, fiber), and then counted and measured for maximum length. Particles that were not clearly of anthropogenic origin were examined using a compound microscope to check for lack of cell structure. Any particles that remained of uncertain origin were excluded from the analysis. It is important to note that because samples were only examined using microscopy (and not run through a spectroscope to chemically verify polymer types and particle counts), it is possible that the abundances of microplastics reported herein are either over- or underestimates of actual numbers, as both false positives and failures to identify very small plastic particles are relatively common when relying on microscopy to identify microplastics (Song et al. 2015; Lenz et al. 2015; Lusher et al. 2017a).

At the time this study was conducted, the risk of sample contamination from airborne plastics was just beginning to be realized, and protocols to control for such contamination followed soon after although such QA/QC protocols have not yet been standardized and debate remains about how best to control and account for contamination of samples (NOAA, 2015; but see Hidalgo-Ruz et al., 2012). We therefore attempted a post-hoc control of environmental plastics contamination by conducting three trials separated in time on 20 June, 20 July and 19 August 2016 to determine average levels of contamination in the lab used during the

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study. During each trial, six clean Petri dishes were set out for 15 minutes on the lab countertops. Three or four people were present in the lab each time (during sample sorting in 2015, two or three people were present at any one time). At 15 minutes, dishes were covered with clean, clear lids and examined for particle settlement using a dissecting microscope. Only fibers were found at an average of 0.5±0.3, 0.5±0.2 and 0.5±0.3 fibers per dish for the June, July and August trials, respectively (grand average = 0.5±0.0 fibers per dish per 15 minute time period). Fiber contamination for each fiber color (type) was then calculated using the following steps: The average number of fibers per dish (0.5) was multiplied by the number of dishes likely sorted for each sample core (393 cu cm core / 15 cu cm spoonful per dish = ~26 dishes per core) for an estimated total of 13.1 fibers contaminating each sample core. Since the color of fibers causing contamination in 2015 could not be determined after the fact, the estimate of 13.1 fibers per core was divided by the seven fiber color categories for an estimate of 1.87 fibers contaminating each fiber color category. This value (1.87 fibers) was then subtracted from each fiber color category of each core before analyses and summary statistics were calculated. If the result of the subtraction was a negative number, the value was assigned a 0.

Common wetland fish (Boerger, et al. 2010; Rochman et al., 2013b) were trapped using metal minnow traps baited with cat food placed in nylon sleeves (to prevent fish from consuming it), and set throughout the reach. Three species were captured: the native marsh residents California killifish (Fundulus parvipinnis; n = 68) and longjaw mudsucker (Gillichthys mirabilis; n = 4), and the introduced sailfin molly (Poecilia latipinna; n = 82). In the field, all fish collected for gut analysis were placed in ziplock bags (one bag per trap). Additionally, two composite samples (7 California killifish and 8 sailfin molly) were collected and immediately placed into clean glass jars for analysis of SVOCs. Only four longjaw mudsucker individuals were captured,
so all were used for gut analysis. The protocol of the AVMA Panel on Euthanasia (American Veterinary Medical Association, 2013) was followed, which recommends rapid chilling to euthanize warm-water fish. All fish remained frozen until analysis. Composite samples for SVOC analysis were analyzed for 67 SVOCs by a local analytical facility (Enviromatrix Analytical, Inc.) using EPA Method 8270C (EPA, 1996). Fish used for gut analysis were thawed, measured, weighed, and sexed in the lab. A ventral, longitudinal incision and two perpendicular ventral incisions (anterior and posterior) were made in each fish to expose the intact guts, and then the fish was placed in a clean glass dish under a dissecting microscope to complete the dissection and removal of gut contents. All contents were removed from inside the fish gut systematically as the gut was opened and analyzed a small section at a time for a total of ~15 min of exposure time (i.e., low risk of contamination). Since the post-hoc estimated contamination rates were ≤0.5 fibers per sample (0.5 fibers per dish divided by the one to four fiber color categories found in the fish samples equals 0.125-0.5 fibers contaminating each fiber color category) we did not use a correction but acknowledge that our fiber counts may be slight overestimates. Only materials drawn out of the gut were identified (or described) and counted. For items not feasibly counted (e.g., sand grains, organic debris, filamentous algae), presence in the gut was noted. Ten out of the 149 fish sampled had empty guts and were therefore excluded from further analyses. As with sediment samples, plastics were categorized by color and type (e.g., hard or soft pieces, fiber), then counted and measured for maximum length.

**Data analyses.** Descriptive statistics of sediment small plastics (average of all cores) and fish gut contents (average of small plastics and prey items for each species) were calculated to summarize findings. The concentrations of SVOCs, if present in at least one sample (at least one of the species), are reported. Fish diet preference was explored using Manly’s alpha (Chipps and
Garvey, 2007), which compares the abundances of the types of plastic found in the environment and consumed by the fish. Differences in size and sex ratios of all fish sampled to those that had consumed plastics were tested using t-tests (size variables) and Chi Square (sex ratios) in JMP 12.

Results

Small plastics in sediment. All sediment cores collected contained small plastics; the average abundance (±1SE) was 9,638±1,636 pieces m\(^2\) and average lengths (±1SE) of small plastics ranged from 1.8±0.3 to 4.6±1.1 mm. Common categories of plastics were plastic film pieces, such as film from bags and wrappers, polystyrene pieces, soft pieces, hard pieces, synthetic fibers, and a few miscellaneous items, such as strands of carpeting and synthetic turf (Fig. 2, Table 1). Synthetic fibers, hard pieces, and soft pieces were the most common types of plastics found across all sediment cores, together making up 90% of the fragments found (Fig. 2).

Fish gut contents.

Fish species present. Add short paragraph about ranges, and natural history characteristics of fundulus, sailfin molly, and mudsucker. Mention species captured here, put natural history in discussion.

Prey and non-plastics in guts. Half or more of these bottom feeding fish species had fed on sand or silt particles, and filamentous green algae (Table 2). Roughly 10% of both California killifish and sailfin molly also contained red algal filaments (Table 2). California killifish, primarily an
invertebrate predator contained remnants of small crustaceans (e.g., exoskeleton pieces, amphipod appendages), scales, unidentifiable fishes and insects, snails, tubificid oligochaetes, nematodes, and a sea cucumber (Table 2). The sailfin molly, although predominantly an herbivore, also commonly ingested tubificid oligochaetes and nematodes, and to a lesser extent, crustaceans and snails (Table 2). The predatory longjaw mudsucker contained scales, a digested fish, and nematodes (Table 2).

Characteristics of plastic eating fish. None of the longjaw mudsucker guts contained plastics, which may have been due to the small sample size of only four individuals. California killifish individuals that had plastics in their guts were, on average, 25% longer and 79% heavier than those free of plastics (p<0.03, Table 3). The ratio of males to females (to unknown sex) was similar between fish with and without gut plastics (Table 3). Neither size nor sex of sailfin molly individuals differed between those that contained plastics and those that did not (Table 3).

Small plastics in guts. Almost one quarter of fish examined contained small plastics, with 12% of California killifish (7 of 61) and 32% of sailfin molly (24 of 75) having consumed plastic (Table 3). Of the 39 types of plastic available in the environment, the California killifish and sailfin molly each consumed 10-11 different types of plastic items, mostly consisting of fibers and hard pieces (Fig. 2, Table 1). California killifish also ingested clear microbeads. The consumed items ranged in average length from 0.05±0.0 to 3.25±2.75 mm for California killifish and from 0.10±0.01 to 2.58±1.10 mm for sailfin molly.
Of the 10-11 types of small plastics that were consumed by the fishes, 7-8 types were selectively eaten (Manly’s alpha ≥0.025; Table 1), meaning the fishes’ guts contained higher proportions of these items than were found in the environment (Fig. 2). The items the fishes selected included blue, yellow, orange and/or red hard plastic pieces, and all colors of synthetic fibers (Table 1).

**SVOCs in fish.**

Three SVOCs of 67 tested were found in the tissues of these species. Both the California killifish and sailfin molly contained diethyl phthalate and benzyl alcohol, and the sailfin molly additionally contained 4-(3-) methylphenol (Fig. 3). The sources of these compounds in this creek are uncertain. The phthalate is a synthetic compound, while benzyl alcohol and 4-(3-) methylphenol have natural, albeit small, localized sources (PubChem, 2019 a,b,c). All three have common industrial applications, including additives in plastics, solvents, antiseptics, preservatives, pesticides, and/or additives in cosmetics and perfumes (WHO, 2003; Wade, 2019; Wiki, 2019; PubChem, 2019a,b,c).

**Discussion.**

**Small debris in sediment and fish.** This study, along with other recent studies (Moore et al., 2011; Rochman et al., 2015a; Rochman et al., 2016), illustrate the risk of plastic and plasticizer (SVOC) pollution to coastal food webs. A concurrent regional study of debris in the Southern California Bight found that sediments in embayments exhibited the highest abundance of small plastics, while continental shelf sediments exhibited the lowest abundance of small plastics (SCCWRP, 2016). Bay sediments, for example, were found to have a mean of 140 pieces/m², and continental shelf sediments were found to have ≤20 pieces/m² (SCCWRP, 2016). By

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While there is much knowledge about the presence of small plastics in coastal ecosystems, the extent to which pathways through which small plastics and potentially associated compounds are being integrated into coastal foodwebs remains an emerging area of research.
contrast, our study of the debris at the mouth of an urban stream emptying into San Diego Bay revealed a much denser concentration of small plastics, with almost 10,000 pieces/m². This may be because the mouth of Chollas Creek is in a highly urbanized area, in close proximity to roads, which are responsible for large inputs of debris (SCCWRP, 2016). Streams like Chollas Creek are conduits for plastics, carrying them into embayments and eventually to the ocean, where they accumulate at high densities in marine sediment (SCCWRP, 2016).

While we found a greater variety of small plastics in wetland fish than were observed in select species of pelagic and demersal fish sold in California for human consumption (Rochman et al., 2015a), both sets of fish predominantly ingested fibers (~60% of the plastics ingested by wetland fish, ~80% of market fish). Further, all of the small debris found in California-grown Pacific oysters were fibers (Rochman et al., 2015a). It is important to note that the fibers found within aquatic organisms, while undoubtedly anthropogenic, could be either synthetic or organic. We were unable to perform spectroscopic analysis to identify their composition.

**Natural history and contamination risk.** The extent that contaminants impact food webs depends upon the types of plastics and contaminants present, the environment (Renick et al. 2015), and the natural history of the organisms present (e.g., Renick et al. 2016), including feeding behavior, and changes associated with ontogeny and sex (Temming and Hammer, 1994; Smith et al., 2000; Talley, 2000; Borg et al., 2014).

The marsh fish in this area selectively fed on most of the small plastics found in their guts including all colors of fibers, blue and warm colors of hard pieces, and, in the case of California
killifish, microbeads. Anecdotally, these items often resembled prey, especially a similar

morphology between fish eggs and microbeads, and between synthetic fibers and filamentous

algae, oligochaetes and nematodes (e.g., Fig. 4), further raising the concern that fish may mistake

small plastics as food (Corley, 2014). The likelihood of plastics ingestion or the ability to pass

plastics may change throughout the life of an organism, as revealed by the higher incidence of

plastics ingestion in larger (older) California killifish individuals during this study. This is

consistent with other ontogenetic dietary shifts observed in California killifish, including changes

in prey type, prey size and different microhabitat use with time (Smith et al., 2000; Talley,

2000).

As with the patterns of plastics ingestion, contamination by SVOCs varied with fish species.

Diethyl phthalate, a water-insoluble, sediment-penetrating compound (PubChem 2019a), was

found in both species but was almost three times higher in the California killifish. Both species

ingest sediment while feeding (e.g., Table 3), so an explanation for the higher phthalate

concentration in California killifish is uncertain but may be linked to diet, with higher

abundances of benthic deposit feeders observed in the guts of killifish in this study (i.e.,

potentially more diethyl phthalate-laden sediment), or an artifact of small sample size (n=1). The

reasons underlying the presence of 4-(3-) methylphenol and the 3.5 fold greater benzyl alcohol

concentration in the sailfin molly compared with the California killifish are also uncertain. These

compounds, which are used as solvents, pesticides, antiseptics, anesthetics, and additives in

cosmetics and fragrances, are water soluble, have fairly rapid degradation rates in water, and do

not tend to accumulate in tissues (PubChem 2019b,c). Again, stomach contents (i.e., water

content) or small sample size could explain this observed difference. Although the sources and
pathways of exposure are uncertain (Weschler and Nazaroff, 2008), the presence of these compounds in our two samples reveals that transfer of contaminants, even those that are relatively transient, from the environment to food webs is a real risk. Further study is needed to understand the interactions among these contaminants, the species’ natural history, and other environmental stresses (e.g., predation, parasitism; Renick, et al., 2015; Renick et al. 2016).

**Health effects of small plastics and SVOCs.** The effects of small plastics and SVOC contamination on organisms may be complex (e.g., Renick et al., 2015) and remain largely uncertain, but knowledge of these effects is needed to understand the consequences of exposure to the organisms themselves and those that eat them. The acute and chronic effects of the three SVOCs found in this study have been observed on the growth, reproduction, enzyme activity, metabolic activity, respiration, kidney function and/or liver function in animals, while the effects on humans are less well known and are of concern (NIOSH, 1997; Lithner et al., 2011; Groshart and Okkerman, 2000; Ghoshpade et al., 2002; Okkerman and van der Putte, 2002; Gore et al., 2015; NIH, 2018; PubChem 2019a,b,c). Dietary guidelines and warnings about the risks of contamination in higher trophic level and longer-lived seafood species due to bioaccumulation are common, but studies like this illustrate that smaller and/or lower trophic level fish may have hazards of their own. There is increasing realization that plastics (Murray and Cowie, 2011; Van Cauwenberghe and Janssen, 2014; Rochman et al., 2015a; Sussarellu et al., 2016) and SVOCs (Windward Environmental, 2010) occur in species consumed by humans. Improved knowledge of the types and distributions of contaminants in an area, as well as of the biology and natural history of organisms, are needed to improve predictions of contamination risks of fish, shellfish and dependent higher trophic levels, including humans.
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Fig. 1. Study site located in lower Chollas Creek near the mouth with San Diego Bay, California, USA.
Fig. 2. Relative abundance and composition of microplastics found in sediments (0-5 cm depth) and the guts of two common wetland resident fish in lower Chollas Creek. N= 9 soils, 7 California killifish, 23 sailfin molly. Data are from June 2015.
Fig. 3. Semi-volatile organic compounds found in wetland resident fish in lower Chollas Creek, San Diego, California, USA. N=1 composite sample consisting of 3 California killifish and 2 sailfin molly individuals. Data are from June 2015.
Fig. 4. Similar looking microplastic fibers and prey items in guts of wetland resident fish.

Contents of California killifish guts included (A) a tubificid oligochaete and (B) a red plastic fiber. Contents of a sailfin molly guts included (C) a filament of green algae (top) and a green plastic fiber (bottom). Scale shown applies to all photos.
Table 1. Abundance of microplastics in surface sediments and the guts of common marsh resident fish, and Manly’s alpha where a ≥0.025 (in bold) indicates a dietary selective preference for microplastics compared to what were available in the environment. Only individuals with microplastics present in the gut were included in this summary. Samples were collected from lower Chollas Creek, San Diego, California, USA during June 2015.

<table>
<thead>
<tr>
<th>Type of microplastic</th>
<th>Abundance (%)</th>
<th>Abundance (no. gut)</th>
<th>California killifish</th>
<th>California killifish</th>
<th>Manly's alpha (q ≥ 0.026)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no. m⁻³)</td>
<td>(no. individual)</td>
<td>(no. individual)</td>
<td>(no. individual)</td>
<td>(no. individual)</td>
</tr>
<tr>
<td>Bag and packaging pieces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mylar wrapper</td>
<td>28 ± 19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear or opaque wrapper</td>
<td>33 ± 46</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grocery bag</td>
<td>36 ± 37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thick, opaque (plastic bag)</td>
<td>24 ± 24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thin translucent (produce bag)</td>
<td>44 ± 24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thick clear, soft (breakable zip bag)</td>
<td>23 ± 32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plastic film, crinkly (old, pastel bag)</td>
<td>24 ± 34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Packaging or packaging tape</td>
<td>67 ± 36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear hard plastic, shell packaging</td>
<td>28 ± 28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Styrofoam piece</td>
<td>67 ± 36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saltimbocca label</td>
<td>36 ± 28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rubber or foam piece</td>
<td>14 ± 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soft plastic pieces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear or white</td>
<td>467 ± 108</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Green</td>
<td>241 ± 109</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pink</td>
<td>467 ± 139</td>
<td>0.14 ± 0.14</td>
<td>0.13 ± 0.13</td>
<td>0.004 ± 0.004</td>
<td>0.012 ± 0.005</td>
</tr>
<tr>
<td>Red</td>
<td>109 ± 399</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Orange</td>
<td>46 ± 137</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hard plastic pieces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear or white</td>
<td>352 ± 109</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Green</td>
<td>108 ± 157</td>
<td>0.14 ± 0.14</td>
<td>0.04 ± 0.08</td>
<td>0.005 ± 0.005</td>
<td>0.020 ± 0.020</td>
</tr>
<tr>
<td>Pink</td>
<td>28 ± 28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blue</td>
<td>315 ± 205</td>
<td>0.20 ± 0.18</td>
<td>0.16 ± 0.12</td>
<td>0.107 ± 0.085</td>
<td>0.125 ± 0.08</td>
</tr>
<tr>
<td>Red</td>
<td>42 ± 133</td>
<td>0.14 ± 0.14</td>
<td>0.17 ± 0.15</td>
<td>0.005 ± 0.005</td>
<td>0.057 ± 0.044</td>
</tr>
<tr>
<td>Yellow</td>
<td>1013 ± 376</td>
<td>0.14 ± 0.14</td>
<td>0.13 ± 0.13</td>
<td>0.145 ± 0.14</td>
<td>0.046 ± 0.043</td>
</tr>
<tr>
<td>Black</td>
<td>14 ± 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Silver</td>
<td>14 ± 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Orange</td>
<td>59 ± 104</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear or white</td>
<td>2112 ± 1028</td>
<td>0.29 ± 0.18</td>
<td>0</td>
<td>0.006 ± 0.043</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>86 ± 36</td>
<td>0.39 ± 0.36</td>
<td>0</td>
<td>0.108 ± 0.04</td>
<td>0</td>
</tr>
<tr>
<td>Blue</td>
<td>57 ± 43</td>
<td>0.43 ± 0.30</td>
<td>0.45 ± 0.25</td>
<td>0.213 ± 0.14</td>
<td>0.239 ± 0.079</td>
</tr>
<tr>
<td>Green</td>
<td>14 ± 14</td>
<td>0.14 ± 0.14</td>
<td>0.13 ± 0.13</td>
<td>0.118 ± 0.13</td>
<td>0.130 ± 0.072</td>
</tr>
<tr>
<td>Red</td>
<td>75 ± 37</td>
<td>0.57 ± 0.30</td>
<td>0.22 ± 0.20</td>
<td>0.254 ± 0.17</td>
<td>0.121 ± 0.061</td>
</tr>
<tr>
<td>Salt or thick line</td>
<td>396 ± 314</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pieces of other items</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sponge</td>
<td>14 ± 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Boys, lego, bricks</td>
<td>28 ± 28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Artificial grass, turf</td>
<td>28 ± 28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Synthetic carpet</td>
<td>43 ± 30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total per core or gut</td>
<td>5618 ± 1638</td>
<td>2.86 ± 1.22</td>
<td>2.43 ± 0.69</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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Table 2. Abundance of prey and other non-plastics found in the guts of common marsh resident fish from lower Chollas Creek, San Diego, California, USA. Data are from June 2015.

<table>
<thead>
<tr>
<th>Gut content items</th>
<th>California killifish</th>
<th>sailfin molly</th>
<th>longjawed mudsucker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individuals</td>
<td>61</td>
<td>74</td>
<td>2</td>
</tr>
<tr>
<td>Items that could not be counted</td>
<td>n</td>
<td>Avg ± 1 SE</td>
<td>Avg ± 1 SE</td>
</tr>
<tr>
<td>sand or silt</td>
<td>48%</td>
<td>99%</td>
<td>50%</td>
</tr>
<tr>
<td>scales</td>
<td>5%</td>
<td>6%</td>
<td>26%</td>
</tr>
<tr>
<td>unknown exoskeleton pieces</td>
<td>13%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>unknown amphipod or shrimp pieces</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>unknown decapod pieces</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>unknown organics or digested pieces</td>
<td>18%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>green filamentous algae</td>
<td>75%</td>
<td>85%</td>
<td>50%</td>
</tr>
<tr>
<td>red filamentous algae</td>
<td>10%</td>
<td>31%</td>
<td>59%</td>
</tr>
<tr>
<td>Enumerated items</td>
<td>n (number of individuals)</td>
<td>Avg ± 1 SE</td>
<td>Avg ± 1 SE</td>
</tr>
<tr>
<td>snails (Barleeia californica, Assiminea californica)</td>
<td>0.49 ± 0.40</td>
<td>0.04 ± 0.03</td>
<td>0</td>
</tr>
<tr>
<td>tubificid oligochaetes, nematodes</td>
<td>1.66 ± 0.28</td>
<td>0.12 ± 0.05</td>
<td>1.75 ± 0.75</td>
</tr>
<tr>
<td>unknown whole digested fish</td>
<td>0.08 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.25 ± 0.25</td>
</tr>
<tr>
<td>unknown fish eggs or larvae</td>
<td>0.05 ± 0.05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sea cucumber (Leptosynapta sp.)</td>
<td>0.05 ± 0.03</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Comparison of fish that had and did not have small plastics in their guts with all fish analyzed in this study. Results of t-tests (fish morphological variables) and Chi square (sex ratios) are shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fish without plastics</th>
<th>Fish with plastics</th>
<th>t-test/Chi square results</th>
<th>Fish without plastics</th>
<th>Fish with plastics</th>
<th>t-test/Chi square results</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>61</td>
<td>74</td>
<td>23</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>standard length (cm)</td>
<td>4.34 ± 0.16</td>
<td>5.29 ± 0.32</td>
<td>0.003</td>
<td>2.52</td>
<td>4.05 ± 0.34</td>
<td>4.15 ± 0.29</td>
</tr>
<tr>
<td>weight (g)</td>
<td>2.35 ± 0.27</td>
<td>4.20 ± 0.32</td>
<td>0.000</td>
<td>2.41</td>
<td>1.96 ± 0.14</td>
<td>5.08 ± 0.34</td>
</tr>
<tr>
<td>sex: female / male / unknown</td>
<td>24 / 19 / 0</td>
<td>23 / 21 / 0</td>
<td>0.71</td>
<td>1.17</td>
<td>24 / 21 / 0</td>
<td>24 / 21 / 0</td>
</tr>
</tbody>
</table>