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# Natural history matters: Plastics in estuarine fish and sediments at the mouth of an urban watershed

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## Abstract

The extent to which small plastics and potentially associated compounds are entering coastal food webs, especially in estuarine systems, is only beginning to be realized. This study examined an estuarine reach at the mouth of urbanized Chollas Creek in San Diego, California to determine: 1) the extent and magnitude of microplastics pollution in estuarine sediments and fish, 2) the extent and magnitude of SVOC contamination in estuarine fish, and 3) whether fish preferentially ingested certain types of microplastics, when compared with the microplastic composition of creekbed sediments. Surface sediments (0–5 cm depth) contained about 10,000 small plastic pieces per $m^2$, 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 25 types of small plastics found in sediment, fish preferred about 10 types (distinct colors and forms). Several SVOCs, both water soluble and sediment-associated compounds, were found in the two species of fish tested. This study revealed that a species' natural history may influence contamination levels, and warrants further study to better understand the pathways of plastics and associated contaminants into and throughout coastal food webs, and the potential health risks for small and/or low-trophic level organisms.

## Introduction

Much research conducted over the last two decades has revealed that microplastics (plastic particles <5mm; [1]) are pervasive in marine systems around the world [2,3]. There has been a recent shift to examine the extent and magnitude of microplastics in terrestrial and freshwater systems [1,4], which is important given that significant portions of marine plastic pollution come from land-based sources and rivers, especially urbanized rivers, which are major conduits of debris from land to sea [5–13].

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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 [14,15]. These microplastics are of concern because they are ubiquitous, easily transported in flowing water, and harmful to biota [14,15]. Plastics may be inadvertently consumed by organisms, such as filter feeders (e.g., clams) or deposit feeders (e.g, earthworms) that feed relatively non-discriminately on appropriately sized prey, or intentionally consumed, such as when organisms cannot differentiate plastics from prey (e.g., fish, lobster) [14,15,16]. While some microplastics may pass through an organism's digestive tract, there are many risks associated with consumption. Consumption of plastics may reduce overall food intake, and therefore fitness, and may cause physical damage to an organism's digestive tract [15,17]. Further, microplastics can accumulate in the gut or gills of organisms, interfering with important life history processes such as feeding, growth, and reproduction [4,16–19]. The monomers and additives that compose microplastics can be toxic to biota if they leach from their parent plastics into the environment [20–22]. Furthermore, microplastics can sorb toxins such as metals, PCBs, PAHs, and DDT from the aquatic environment [23], and transmit these toxins to organisms [24], 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 [4,21,25–29]. Microplastics have been found to transfer between trophic levels [30–33], and thus may pose health risks to humans via consumption of contaminated seafood (e.g., [34,35], though the impacts of microplastics on human health remain largely unknown [21,36].

Additives commonly used in plastics manufacturing, such as phthalates, bisphenol A, PBDEs, PCBs, PAHs, and DDT [37–39] are all semi-volatile organic compounds (SVOCs). SVOCs are of 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 [40–42]. SVOCs are susceptible to leaching out of plastics into the environment and also resorbing to microplastics once present in the environment due to their hydrophobic properties ([20,26,38,43]. There are also many other sources of SVOCs in the environment, including household cleaning products, cosmetics, and pesticides [37,39]. Microplastics' role as a conduit for SVOCs into coastal food webs may be relatively unimportant when compared with other vectors, such as contaminated water, prey, or sediments [38,44]. Microplastics can, however, facilitate the accumulation of SVOCs in organismal tissues [27,45], making it important to understand the types, fates, and effects of small plastics and associated contaminants in coastal watersheds in order to develop natural and social science-based solutions to marine debris and declining watershed health [1].

This study, therefore, contributes to the growing body of research on microplastics upstream from marine ecosystems by examining a brackish reach of Chollas Creek, an urbanized creek that connects mid-city San Diego with San Diego Bay, to determine: 1) the extent and magnitude of microplastics pollution in estuarine sediments and fish, 2) the extent and magnitude of SVOC contamination in estuarine fish, and 3) whether fish preferentially ingested certain types of microplastics, when compared with the microplastic composition of creekbed sediments.

## Materials and methods

### Study location

The Chollas Creek subwatershed (Fig 1) is considered one of the most impaired waterbodies in San Diego County, due largely to nonpoint source pollution in runoff and significant inputs

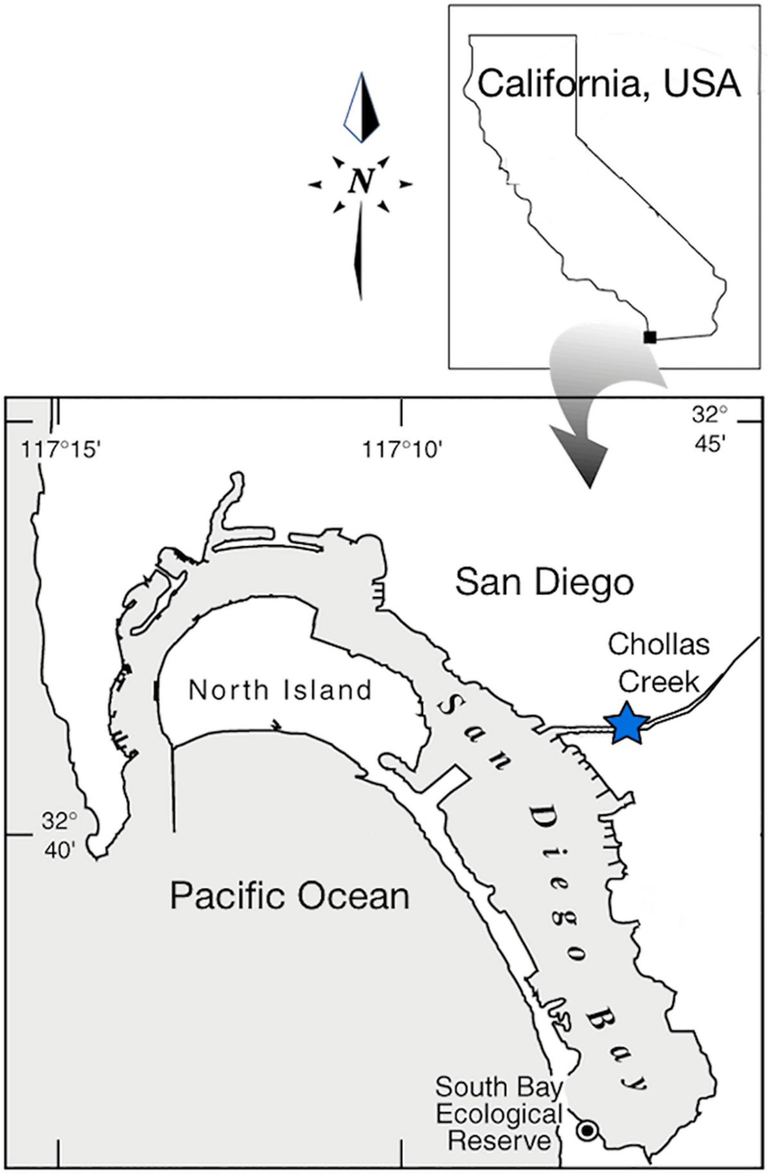

**Fig 1. Location of study site.** Study site located in lower Chollas Creek near the mouth with San Diego Bay, California, USA.

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of trash during both dry and wet seasons [46–49]. Chollas Creek originates from, and runs through, a densely populated, urban section of the county [50] and empties at one of coastal San Diego's most polluted runoff sites [51] in San Diego Bay, reported to be the second most polluted bay in the country [5]. In June 2015, sediments and fish were sampled along a 250-m long reach of tidal brackish Chollas Creek, located about 1.5 km upstream of the mouth (Latitude: 32.6953˚ N, Longitude:-117.1230˚ W; Fig 1).

## Sample collection

Estuarine sediments were collected at low tide throughout the reach of creek, in nine 10-cm diameter x 5-cm depth cores (393 cu cm). Cores were placed in clean, airtight zip-top bags, and frozen until analysis in the lab.

Common wetland fish 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 zip-top 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. All fish were frozen until analysis. A protocol of the AVMA Panel on Euthanasia [52], rapid chilling to euthanize warm-water fish, was followed to humanely euthanize the fishes. Use of AVMA protocols for euthanasia of these wetland fishes was approved by the University of San Diego Institutional Animal Care and Use Committee (IACUC), and IACUC training was received prior to the start of this research (report 3382698, TST). All collections were made in accordance with California Dept of Fish and Wildlife (Permit SC-5295), and no permissions were needed to access the field site, which is a City of San Diego public access point.

## Sample processing and analysis

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 dissecting 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 type (film, hard piece, soft piece, fiber) and color, 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 after being investigated through a compound microscope were excluded from the analysis. Anthropogenic particles removed from sediments ranged from 0.5-5mm in size.

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 Petri 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 approximately 15 minutes of exposure time. Only materials drawn out of the gut were identified (or described) and counted. As with sediment samples, anthropogenic particles were identified by shape, color, and/or lack of cell structure, categorized by type (film, hard piece, soft piece, fiber) and color, and then counted and measured for maximum length. Anthropogenic particles found in fish guts ranged from 0.05–6 mm in size, but only those particles $<5$ mm were included in analyses (one 6 mm-long red fiber was removed). Organic items found in fish guts were also counted, or, in the case of 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.

For SVOC analyses, composite fish samples were analyzed for 67 SVOCs by a local analytical facility (Enviromatrix Analytical, Inc.) using EPA Method 8270C [53].

## Limitations of microscopy

It is important to note that because samples were only examined for microplastics via 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 detect very small plastic particles are relatively common when relying on microscopy to identify microplastics [54–56].

## Contaminant control in sample processing

At the time this study was conducted, the risk of sample contamination from airborne plastics was just beginning to be realized [57,58], but see [59], and protocols to control for such contamination followed soon after (e.g., [35,60,61]), though such QA/QC protocols have not yet been standardized, and debate remains about how best to control and account for sample contamination [56,62]. Therefore, a post-hoc control of environmental plastics contamination was conducted using 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 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 sediment 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, then a 0 value was assigned.

As discussed above, fish gut contents were analyzed little by little, as the guts were drawn out of the fishes' bodies, resulting in only ~15 minutes of total exposure (and therefore, low risk of contamination). Since the post-hoc estimated lab contamination rates were $\leq$0.5 fibers per 15-minute sample (and 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), a contaminant correction was not used for fish samples, but it is acknowledged that fiber counts may be slight overestimates.

## Data analyses

Descriptive statistics of sediment microplastics (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 composite sample (at least one of the species), are reported. Fish diet preference was explored using Manly's alpha [63], which compares the abundances of the types of plastics 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

## Microplastics in sediment

All sediment cores collected contained microplastics; the average abundance (±1SE) was 9,544 ±1,413 pieces m$^{-2}$ and average lengths (±1SE) of small plastics ranged from 0.5±0.1 to 4.9±0.1 mm. Common categories of plastics were film pieces, polystyrene pieces, soft pieces, hard pieces, microbeads, and synthetic fibers (Fig 2, Table 1), with synthetic fibers, hard pieces, and soft pieces together making up 90% of the fragments found in sediments. Nearly half of all fragments were white or clear (Table 1).

## Fish and microplastics

**Prey and non-plastics in guts.** Half or more of the California killifish, longjaw mudsucker, and sailfin molly collected 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 [64], 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 [65], 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

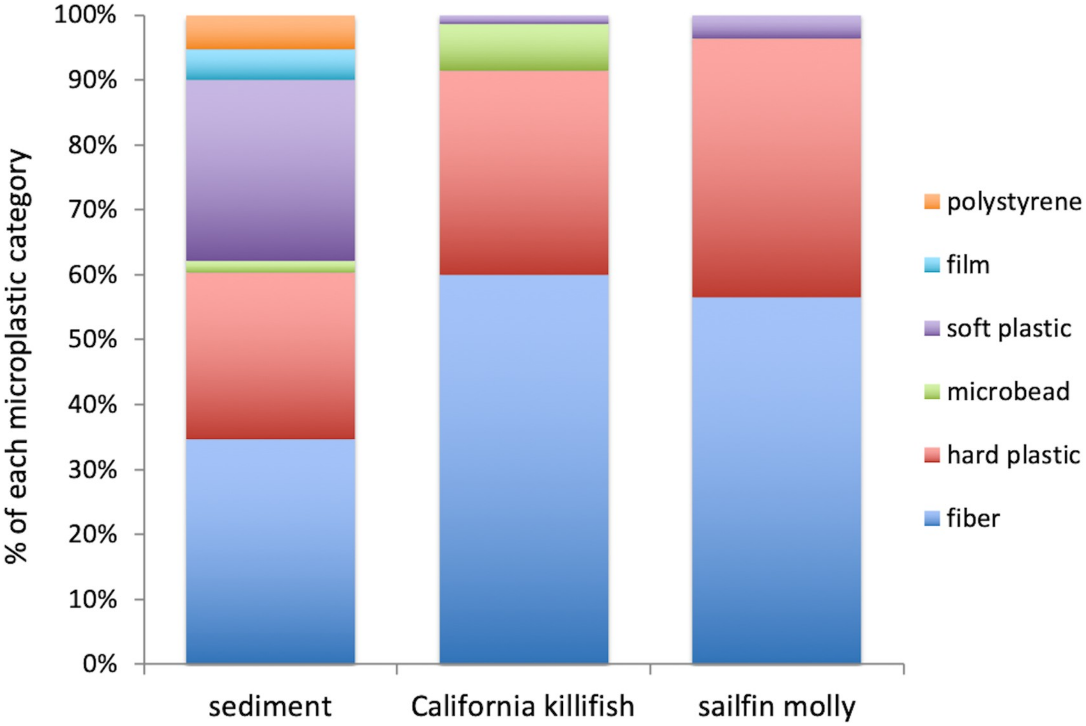

**Fig 2. Sediment and fish microplastics.** Relative abundance and composition of microplastics found in sediments (0–5 cm depth) and the guts of two common wetland resident fish in lower Chollas Creek. N = 9 soils, 7 California killifish, 23 sailfin molly. Data are from June 2015.

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**Table 1. Microplastics abundance in sediments and fish.**

| Type of microplastic | sediment | California killifish | sailfin molly | California killifish | sailfin molly |
|---|---|---|---|---|---|
| n = | 9 cores | 7 individuals | 23 individuals | 7 individuals | 23 individuals |
| | Abundance (no. $m^{-2}$) | Abundance (no. $gut^{-1}$) | | Manly's alpha ($\alpha \geq 0.040$) | |
| | Avg±1 SE | Avg ±1 SE | Avg± 1 SE | Avg± 1 SE | Avg± 1 SE |
| **Polystyrene piece** | 396 ±105 | 0 | 0 | 0 | 0 |
| **Rubber or foam piece** | 28± 28 | 0 | 0 | 0 | 0 |
| **Film** | | | | | |
| clear or white | 283±76 | 0 | 0 | 0 | 0 |
| silver or gray | 42±30 | 0 | 0 | 0 | 0 |
| **Soft plastic pieces** | | | | | |
| clear or white | 481±108 | 0 | 0 | 0 | 0 |
| green | 269±113 | 0 | 0 | 0 | 0 |
| blue | 467±192 | 0.14± 0.14 | 0.13± 0.13 | 0.004 ± 0.001 | 0.004± 0.002 |
| red | 1062±389 | 0 | 0 | 0 | 0 |
| yellow | 410±137 | 0 | 0 | 0 | 0 |
| orange | 99±59 | 0 | 0 | 0 | 0 |
| **Hard plastic pieces** | | | | | |
| clear or white | 524±212 | 0 | 0.04±0.08 | 0 | 0 |
| green | 368±157 | 0.14± 0.14 | 0.04±0.08 | 0.004± 0.004 | 0.002±0.002 |
| pink | 28±28 | 0 | 0 | 0 | 0 |
| blue | 311±150 | 0.29±0.18 | 0.65±0.52 | **0.100±0.076** | **0.181±0.178** |
| red | 424±133 | 0.14±0.14 | 0.17±0.19 | 0.002±0.002 | **0.066±0.049** |
| yellow | 1019±376 | 0.14±0.14 | 0.13±0.13 | **0.143±0.143** | **0.051±0.048** |
| black | 14±14 | 0 | 0 | 0 | 0 |
| silver | 14±14 | 0 | 0 | 0 | 0 |
| orange | 14±14 | 0 | 0.04± 0.08 | 0 | **0.044± 0.044** |
| microbead | 212±124 | 0.29± 0.18 | 0 | **0.099±0.099** | 0 |
| **Fibers** | | | | | |
| clear or white | 2923±961 | 0.57± 0.43 | 0 | **0.145±0.143** | 0 |
| black | 32±21 | 0 | 0.39±0.36 | 0 | **0.143±0.064** |
| blue | 44±22 | 0.43±0.30 | 0.43±0.25 | **0.173±0.141** | **0.227±0.080** |
| green | 16±16 | 0.14±0.14 | 0.13±0.13 | **0.181±0.138** | **0.143±0.078** |
| red | 32±28 | 0.57±0.30 | 0.22±0.20 | **0.153±0.075** | **0.133±0.131** |
| **Total per $m^2$ or gut:** | 9544±1413 | 2.86±1.37 | 2.43±0.68 | | |

Abundance of microplastics in surface sediments and the guts of common marsh resident fish, and Manly's alpha where a ≥0.040 (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.

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killifish individuals that had plastics in their guts were, on average, 25% longer and 79% heavier than those free of plastics ($p \leq 0.03$, Table 3). In fact, 24% of individuals that were 5.3 cm total length and weighed 2.6 g weight (the average total length and weight) and greater had plastics in their guts, while 3% of individuals shorter and lighter than the average had plastics. 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).

**Microplastics in fish 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

Table 2. Non-plastic fish gut contents.

| Gut content items | California killifish | sailfin molly | longjawed mudsucker |
|---|---|---|---|
| n (number of individuals) = | 61 | 74 | 4 |
| **Items that could not be counted** | Avg± 1 SE | Avg± 1 SE | Avg ± 1 SE |
| | % of fish with items present | | |
| sand or silt | 48% | 99% | 50% |
| scales | 5% | 0% | 25% |
| unknown exoskeleton pieces | 53% | 1% | 0% |
| unknown amphipod or shrimp pieces | 2% | 0% | 0% |
| unknown decapod pieces | 0% | 1% | 0% |
| unknown organics or digested pieces | 18% | 0% | 0% |
| green filamentous algae | 75% | 85% | 50% |
| red filamentous algae | 10% | 11% | 0% |
| **Enumerated items** | Avg ±1SE | Avg± 1SE | Avg ± 1SE |
| | (no. gut$^{-1}$) | (no. gut$^{-1}$) | (no. gut$^{-1}$) |
| snails (*Barleeia californica*, *Assiminea californica*) | 0.49±0.40 | 0.04±0.03 | 0 |
| tubificid oligochaetes, nematodes | 1.66±0.28 | 0.12±0.05 | 1.75±0.75 |
| unknown whole digested-fish | 0.02±0.02 | 0.01±0.01 | 0.25±0.25 |
| unknown fish eggs or larvae | 0.05±0.05 | 0 | 0 |
| unknown insect larvae or adult parts | 0.05±0.03 | 0 | 0 |
| sea cucumber (*Leptosynapta* sp.) | 0.05±0.03 | 0 | 0 |

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.

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plastic (Table 3). Of the 25 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) that ranged in length from 0.05 to 5 mm.

Table 3. Comparison of fish with and without plastics.

| | California killifish | | | | |
|---|---|---|---|---|---|
| | Fish without plastics | Fish with plastics | t-test/Chi square results | | |
| n = | 61 | 7 | | | |
| **Variable** | Avg ±1 SE | Avg± 1 SE | P | t/ Chi sq | df |
| standard length (cm) | 4.34±0.16 | 5.50±0.32 | 0.003 | 2.52 | 59 |
| total length (cm) | 5.12±0.18 | 6.40±0.40 | 0.005 | 2.41 | 59 |
| weight (g) | 2.35±0.27 | 4.20±0.32 | 0.005 | 2.25 | 59 |
| sex: female / male / unknown | 24/23/6 | 3/3/1 | 0.571 | 1.13 | 2 |
| | sailfin molly | | | | |
| | Fish without plastics | Fish with plastics | t-test/Chi square results | | |
| n = | 74 | 23 | | | |
| **Variable** | Avg± 1 SE | Avg± 1 SE | P | t/ Chi sq | df |
| standard length (cm) | 4.05±0.12 | 4.15±0.29 | 0.655 | 0.55 | 72 |
| total length (cm) | 4.96±0.14 | 5.08±0.34 | 0.624 | 0.58 | 72 |
| weight (g) | 2.05±0.19 | 2.39±0.38 | 0.466 | 1.04 | 72 |
| sex: female / male / unknown | 28/21/2 | 15/7/1 | 0.677 | 0.78 | 2 |

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

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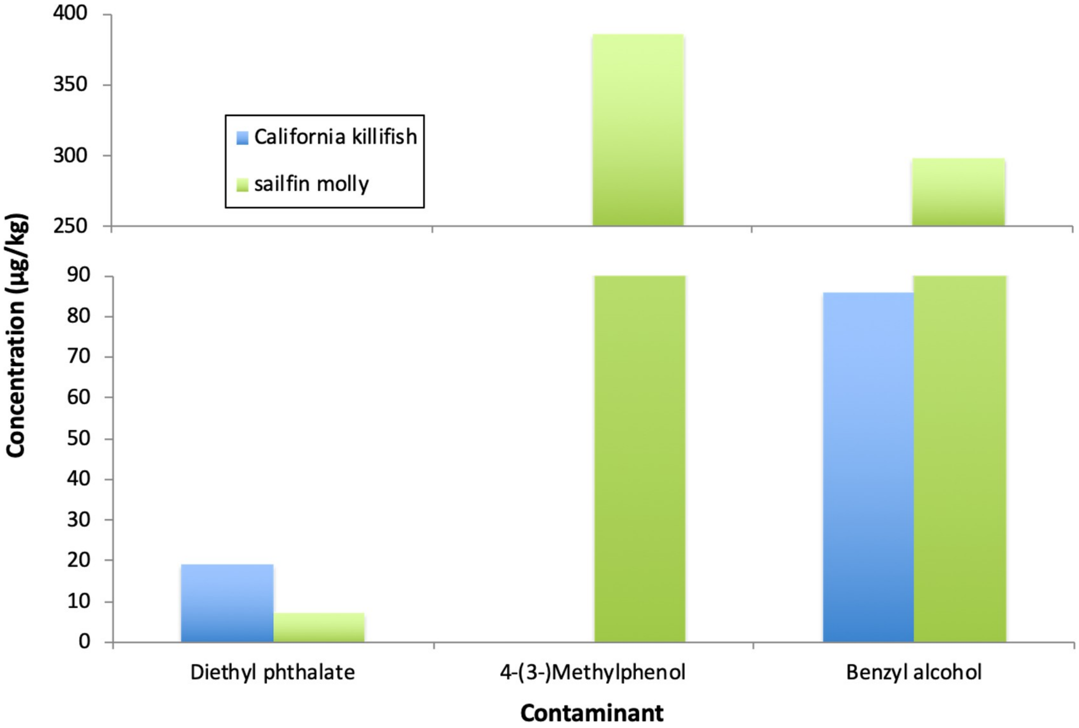

**Fig 3. SVOCs in fish.** Semi-volatile organic compounds found in wetland resident fish in lower Chollas Creek, San Diego, California, USA. N = 1 composite sample per species (3 California killifish and 2 sailfin molly individuals). Data are from June 2015.

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Of the 10–11 types of small plastics that were consumed by the fishes, 7–8 types were selectively eaten (Manly's alpha $\geq$0.040; 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 blue, green, red, black, white and/or clear fibers (Table 1). California killifish additionally preferentially consumed microbeads (Fig 2, 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 strictly a synthetic compound, while benzyl alcohol and 4-(3-) methylphenol have both synthetic and natural, albeit localized, sources [66,67,68]. All three have common industrial applications as additives in plastics, solvents, antiseptics, preservatives, pesticides, and/or additives in cosmetics and perfumes [66–71].

## Discussion

### Microplastics in upstream sediments

Examining the extent and magnitude of plastics pollution upstream of marine systems provides insights into the land-based sources and transport mechanisms of marine debris, and the potential dynamics and impacts of these pollutants throughout watersheds. This study, particularly, provides key insight into microplastics loads and dynamics in the Chollas Creek subwatershed, as it revealed densities of microplastics at the mouth of urbanized Chollas Creek that were 70–500 times greater (~10,000 pieces/m$^2$) than those concurrently found in San Diego bay sediments (avg. of 140 pieces/m$^2$) and continental shelf sediments (avg. of $\leq$20 pieces/m$^2$) [10,72]. In contrast, the microplastics densities found at the mouth of Chollas Creek were lower than those found further upstream; around the same time this study was conducted, an average of 13,000 microplastic pieces/m$^2$ was found in a freshwater riparian reach of Chollas Creek about 3 km upstream from the study site, and averages of 18,000 and 46,000 pieces/m$^2$ were found 5.5–6 km farther upstream in two seasonal tributaries [73]. This suggests heavy inputs from the land uses surrounding and upstream of the creek mouth region.

As stated previously, this subwatershed is highly urbanized, replete with land uses and activities commonly linked to trash inputs, including industrial and high-density residential areas, a large homeless population, and illegal dumping [47,50,74], all crisscrossed by a dense network of roads, which are themselves linked to large inputs of debris and contaminants [10,75]. The abundance of large trash and debris in this region undoubtedly contributes to the accumulation of microplastics by trapping and transporting microplastic particles, and by degrading into small plastic pieces, especially given the sun exposure in this area [76–78]. Large plastics (0.5–50 cm) concurrently found in this and nearby subwatersheds [73,74,79] commonly consisted of types of plastics similar to those observed in this microplastics study, namely hard and soft plastics (e.g., whole and broken containers, flexible packaging), film plastics (e.g., bags, wrappers), synthetic fibers (e.g., clothing, blankets, furniture stuffing), and foamed plastics (polystyrene, rubber), suggesting that the breakdown of larger trash items may be a potentially significant source of microplastics in this watershed. Additionally, microplastics ($\leq$0.5 cm) found in the upstream reaches of this same subwatershed (3, 5.5, 6 km upstream) were similar to those found in the mouth region, namely hard and soft pieces, film, fibers, and microbeads [73], suggesting that sources, accumulations, and, potentially, transport of both primary and secondary microplastics exist throughout the watershed. While it remains important to study the transport and fate of microplastics in bays and oceans [10,12,80], the impacts of these high microplastics densities in upstream seasonal creeks also need to be explored [1,4,81].

## Natural history and contamination risk in fish

The extent that plastics and their associated contaminants impact food webs depends upon the types of plastics and contaminants present [21], the environment [82], and the natural history of the organisms (e.g., [83]), including feeding behavior, and changes associated with ontogeny and sex [16,84–88]. While the California killifish is primarily a predator and the sailfin molly is primarily an herbivore, both, as with other estuarine species, intensively forage on the substratum for food items and inadvertently ingest detritus and sediment [86,89]. The substratum is also where dense small plastics and contaminants accumulate, putting these species at particularly high risk of contamination [15]. Omnivorous fishes have also been observed to have four to six times higher abundances of gut plastics than more selective herbivores or predators [88]. Both the predatory and herbivorous estuarine fishes in this study selectively fed on many of the small anthropogenic particles 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, with similar morphologies observed 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 and other animals may mistake plastics as food due to similar visual or olfactory cues [90,91]. The likelihood of plastics ingestion or the

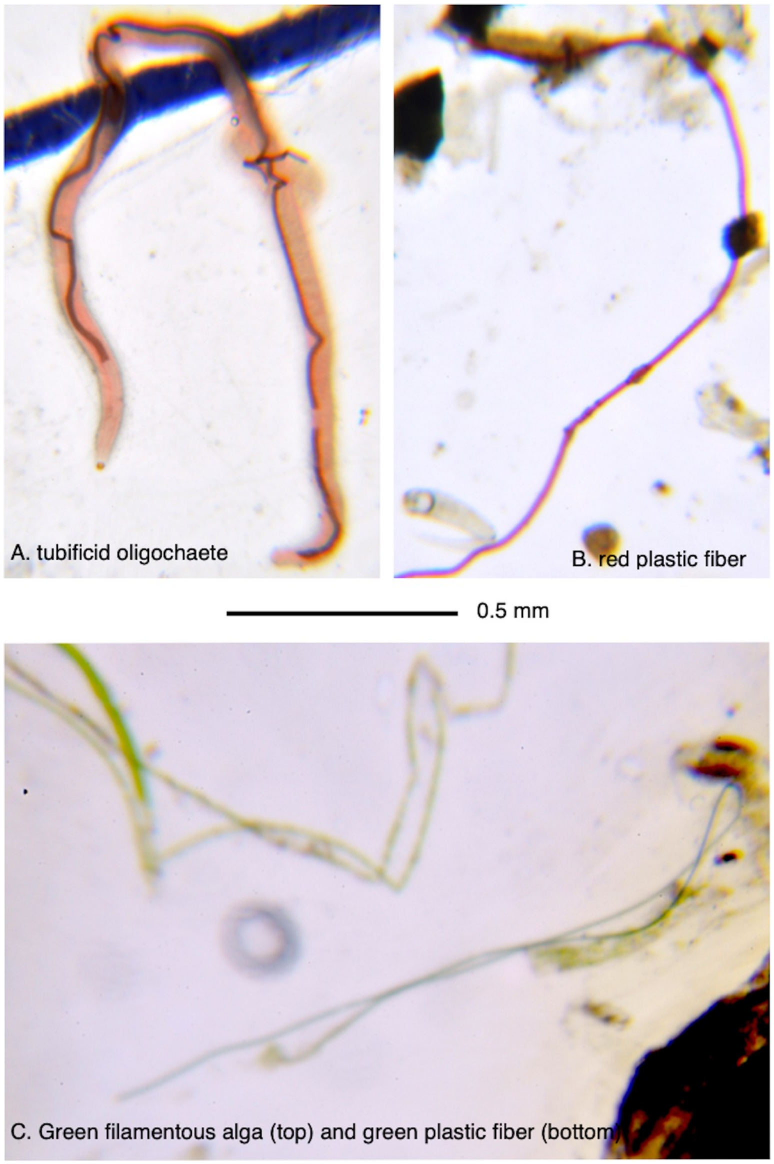

**Fig 4. Plastics and food in fish guts.** Similar looking microplastic fibers and prey items in guts of wetland resident fish. Contents of California killifish guts included (A) a tubificid oligochaete and (B) a red plastic fiber. Contents of sailfin molly guts included (C) a filament of green algae (top) and a green plastic fiber (bottom). Scale shown applies to all photos.

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ability to pass plastics may change throughout the life of an organism, as revealed by the higher incidence of plastics in the guts of larger (older) California killifish individuals during this study. This is consistent with other ontogenetic dietary shifts observed in California killifish, such as changes in prey types, prey sizes, prey abundance, and microhabitat use with time [85,86]. These fish may, in turn, be important vectors for 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 [92–95]. While examples of the transfer of plastics between trophic levels are on the rise [30–33], needed is a better understanding of the mechanisms underlying the pathways of microplastics into and through food webs, and the subsequent consequences for these food webs, so that outcomes of contamination may be better predicted.

As with the patterns of plastics ingestion, contamination by SVOCs varied with fish species. Diethyl phthalate, a water-insoluble, sediment-penetrating compound [66], was found in both the California killifish and sailfin molly, 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 the 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, alternatively, may be an artifact of small sample size (n = 1 composite sample). The reasons underlying the presence of 4-(3-) methylphenol and the 3.5-fold greater benzyl alcohol concentration in the sailfin molly, as compared to 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 [67,68]. 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 [37], the presence of these compounds in two samples in this study reveals that transfer of contaminants, even those that are relatively transient, from the environment to food webs is a real risk.

## Health implications of small plastics and SVOCs

The effects of small plastics and SVOC contamination on organisms may be complex (e.g., [82]) and remain largely uncertain, but knowledge of these effects is needed to understand the consequences of exposure to the organisms themselves and to others in the food web, including humans. While plastics are thought to largely pass through the guts of consumers, the extent of retention, degradation, interactions with gut microbiota, and subsequent health effects are only beginning to be realized [96–100], as are the effects of the plastic-associated contaminants (e.g., [100,101]). 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 [42,66–68,102–108].

The list of marine life, including seafood items consumed by humans, that contain plastics and associated contaminants is growing [19,21,34,69,109–111]. Therefore, it is not only important to continue to improve our understanding of contaminant dynamics and subsequent health effects throughout food webs, but to translate this information into consumption guidelines. While such guidelines exist to reduce consumer exposure to bioaccumulated contaminants in large, longer-lived, and/or higher trophic level seafood species, this and other recent studies (e.g, [34,97,110,112]) illustrate that smaller and/or lower trophic level fish and shellfish may have hazards of their own linked with plastics and associated compounds.

## Recommendations

This study is one more example in the burgeoning literature on the entry of plastics and associated contaminants into food webs through ingestion. Multiple examples are valuable for documenting the breadth of contexts under which this, and other processes, can occur. Needed now are more efforts to find generalities among examples, such as how trophic level(s), life history strategy, or life style (e.g., benthic, demersal, pelagic) correlate with the incidence of ingestion, and types and levels of contamination. From these patterns, more exploration into the underlying mechanisms is needed so that we may better predict outcomes of contamination (i.e., risk and vulnerability) and effectiveness of proposed solutions.

## Supporting information

**S1 Data.**
(DOCX)

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## References

1. Rochman CM. Microplastics research: From sink to source. Science 2018; 360(6384):28–29. https://doi.org/10.1126/science.aar7734 PMID: 29622640
2. Cole M, Lindeque P, Halsband C, Galloway TS. Microplastics as contaminants in the marine environment: A review. Mar Pollut Bull. 2011; 62(12):2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025 PMID: 22001295
3. Thompson RC. Microplastics in the marine environment: Sources, consequences and solutions. In: Bergmann M, Klages M, editors. Marine Anthropogenic Litter. Heidelberg: Springer; 2015. p. 185–200. https://link.springer.com/book/10.1007/978-3-319-16510-3
4. Chae Y, An Y-J. Effects of micro- and nanoplastics on aquatic ecosystems: Current research trends and perspectives. Mar Pollut Bull. 2017; 124(2):624–32. https://doi.org/10.1016/j.marpolbul.2017.01.070 PMID: 28222864
5. Long ER. Spatial extent of sediment toxicity in U.S. estuaries and marine bays. Environ Monit Assess. 2000; 64:319–407.

**6.** Hirai H, Takada H, Ogata Y, Yamashita R, Mizukawa K, Saha M, et al. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Mar Pollut Bull. 2011; 62 (8):1683–1692. https://doi.org/10.1016/j.marpolbul.2011.06.004 PMID: 21719036

**7.** Moore CJ, Lattin GL, Zellers AF. Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. J Integr Coast Zone Manag. 2011; 11(1):65–73. https://doi.org/10.5894/rgci194

**8.** Yonkos LT, Friedel EA, Perez-Reyes AC, Ghosal S, Arthur CD. Microplastics in four estuarine rivers in the Chesapeake Bay, U.S.A. Environ Sci Tech. 2014; 48(24):14195–202.

**9.** Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. Plastic waste inputs from land into the ocean. Science 2015; 347(6223):768–71. https://doi.org/10.1126/science.1260352 PMID: 25678662

**10.** Moore S, Sutula M, Von Bitner T, Lattin G, Schiff K. Southern California Bight 2013 Regional Monitoring Program Volume III: Trash and Marine Debris. Costa Mesa (CA): Southern California Coastal Water Research Project; 2016 Jun. Technical Report No.: 928. http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/928_B13_Debris.pdf

**11.** Peters CA, Bratton SP. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. Environ Pollut. 2016; 210:380–87. https://doi.org/10.1016/j.envpol.2016.01.018 PMID: 26807984

**12.** Lebreton LCM, van der Zwet J, Damsteeg J-W, Slat B, Andrady A, Reisser J. River plastic emissions to the world's oceans. Nat Commun. 2017; 8:15611. https://doi.org/10.1038/ncomms15611 PMID: 28589961

**13.** Schmidt C, Krauth T, Wagner S. Export of plastic debris by rivers into the sea. Environ Sci Tech. 2017; 51:12246–12253. https://doi.org/10.1021/acs.est.7b02368 PMID: 29019247

**14.** Auta HS, Emenike CU, Fauziah SH. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environ Int. 2017; 102:165–76. https://doi.org/10.1016/j.envint.2017.02.013 PMID: 28284818

**15.** de Sá LC, Oliveira M, Ribeiro F, Rocha TL, Futter MN. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? Sci Total Environ. 2018; 645:1029–39. https://doi.org/10.1016/j.scitotenv.2018.07.207 PMID: 30248828

**16.** de Sá LC, Luís LG, Guilhermino L. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ Pollut. 2015; 196:359–362. https://doi.org/10.1016/j.envpol.2014.10.026 PMID: 25463733

**17.** Lei L, Wu S, Lu S, Liu M, Song Y, Fu Z, et al. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 2018; 619–620:1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

**18.** Watts AJR, Urbina MA, Corr S, Lewis C, Galloway TS. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ Sci Tech. 2015; 49 (24):14597–14604.

**19.** Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet MEJ, et al. Oyster reproduction is affected by exposure to polystyrene microplastics. PNAS 2016; 113(9):2430–2435. https://doi.org/10.1073/pnas.1519019113 PMID: 26831072

**20.** Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Bjorn A, et al. Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc B: Biol Sci. 2009; 364 (1526):2027−2045. https://doi.org/10.1098/rstb.2008.0284 PMID: 19528054

**21.** Smith M, Love DC, Rochman CM, Neff RA. Microplastics in seafood and the implications for human health. Curr Environ Health Rep. 2018; 5(3):375–86. https://doi.org/10.1007/s40572-018-0206-z PMID: 30116998

**22.** Thaysen C, Stevack K, Ruffolo R, Poirier D, De Frond H, DeVera J, et al. Leachate from expanded polystyrene cups is toxic to aquatic invertebrates (*Ceriodaphnia dubia*). Front Mar Sci. 2018; 5:71. https://doi.org/10.3389/fmars.2018.00071

**23.** Rios LM, Moore C, Jones PR. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar Pollut Bull. 2007; 54:1230−1237. https://doi.org/10.1016/j.marpolbul.2007.03.022 PMID: 17532349

**24.** Mato Y, Isobe T, Takada H, Kanehiro H, Ohtake C, Kaminuma T. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ Sci Tech. 2001; 35(2):318–324. https://doi.org/10.1021/es0010498 PMID: 11347604

**25.** Browne MA, Niven SJ, Galloway TS, Rowland SJ, Thompson RC. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr Biol. 2013; 23:2388–2392. https://doi.org/10.1016/j.cub.2013.10.012 PMID: 24309271

**26.** Rochman C.M, Hoh E, Hentschel BT, Kaye S. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. Environ Sci Tech. 2013a; 47(3):1646–1654. https://doi.org/10.1021/es303700s PMID: 23270427

**27.** Rochman CM, Hoh E, Kurobe T, Teh SJ. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci Reports 2013b; 3:3263. https://doi.org/10.1038/srep03263 PMID: 24263561

**28.** Rochman CM, Kurobe T, Flores I, Teh SJ. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci Total Environ. 2014; 493:656–61. https://doi.org/10.1016/j.scitotenv.2014.06.051 PMID: 24995635

**29.** Barboza LGA, Vieira LR, Branco V, Figueiredo N, Carvalho F, Carvalho C, et al. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquat Toxicol. 2018b; 195:49–57. https://doi.org/10.1016/j.aquatox.2017.12.008 PMID: 29287173

**30.** Farrell P, Nelson K. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ Pollut. 2013; 177:1–3. https://doi.org/10.1016/j.envpol.2013.01.046 PMID: 23434827

**31.** Setälä O, Fleming-Lehtinen V, Lehtiniemi M. Ingestion and transfer of microplastics in the planktonic food web. Environ Pollut. 2014; 185: 77–83. https://doi.org/10.1016/j.envpol.2013.10.013 PMID: 24220023

**32.** Nelms SE, Galloway TS, Godley BJ, Jarvis DS, Lindeque PK. Investigating microplastic trophic transfer in marine top predators. Environ Pollut. 2018; 238:999–1007. https://doi.org/10.1016/j.envpol.2018.02.016 PMID: 29477242

**33.** Welden NAC, Abylkhani B, Howarth LM. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, *Pleuronectes plastessa*, and spider crab, *Maja squinado*. Environ Pollut. 2018; 239:351–58. https://doi.org/10.1016/j.envpol.2018.03.110 PMID: 29674213

**34.** Van Cauwenberghe L, Janssen CR. Microplastics in bivalves cultured for human consumption. Environ Pollut. 2014; 193:65–70. https://doi.org/10.1016/j.envpol.2014.06.010 PMID: 25005888

**35.** Rochman CM, Kross SM, Armstrong JB, Bogan MT, Darling ES, Green SJ, et al. Scientific evidence supports a ban on microbeads. Environ Sci Tech. 2015a; 49:10759−10761. https://doi.org/10.1021/acs.est.5b03909 PMID: 26334581

**36.** Wright SL, Kelly FJ. Plastic and human health: A micro issue? Environ Sci Tech. 2017; 51:6634–6647.

**37.** Weschler CJ, Nazaroff WW. Semivolatile organic compounds in indoor environments. Atmos Environ. 2008; 42(40):9018–9040. https://doi.org/10.1016/j.atmosenv.2008.09.052

**38.** Lusher AL, Hollman PCH, Mendoza-Hill JJ. Microplastics in fisheries and aquaculture: Status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Report. Rome (Italy): Food and Agriculture Organization of the United Nations; 2017b. Report no.: 615.

**39.** Lucattini L, Poma G, Covaci A, de Boer J, Lamoree MH, Leonards PEG. A review of semi-volatile organic compounds (SVOCs) in the indoor environment: Occurrence in consumer products, indoor air and dust. Chemosphere 2018; 201:466–82. https://doi.org/10.1016/j.chemosphere.2018.02.161 PMID: 29529574

**40.** Koch HM, Calafat AM. Human body burdens of chemicals used in plastic manufacture. Philos Trans Roy Soc B: Biol Sci. 2009; 364(1526):2063–2078. https://doi.org/10.1098/rstb.2008.0208 PMID: 19528056

**41.** Xu Y, Zhang JS. Understanding SVOCs. ASHRAE J. 2011; 53(12):121–125. https://surface.syr.edu/cgi/viewcontent.cgi?article=1009&context=mae

**42.** Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015; 36(6):593–602. https://doi.org/10.1210/er.2015-1093 PMID: 26414233

**43.** Cheng Z, Nie X-P, Wang H-S, Wong M-H. Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. Environ Int. 2013;57–58:75–80. https://doi.org/10.1016/j.envint.2013.09.007

**44.** Koelmans AA, Bakir A, Burton GA, Janssen CR. Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. Environ Sci Tech. 2016; 50(7):3315–26.

**45.** Besseling E, Wegner A, Foekema EM, van den Heuvel-Greve MJ, Koelmans AA. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ Sci Tech. 2013; 47(1):593–600.

**46.** Schiff K, Bay S, Diehl D. Stormwater toxicity in Chollas Creek and San Diego Bay, California. Environ Monit Assess. 2003; 81:119–132. PMID: 12620010

**47.** Roeland K. Dry Season Urban Runoff in the Chollas Creek Watershed and San Diego Bay [MAS thesis]. La Jolla (CA): Center for Marine Biology and Conservation, Scripps Institution of Oceanography, University of California San Diego; 2009. Available at: https://escholarship.org/uc/item/7f32x77m

**48.** Anderson B, Phillips B, Markiewicz D, Stillway M. Toxicity in California Waters: San Diego Region. San Diego (CA): California Water Board Surface Water Ambient Monitoring Program; 2012. 37 p. Available from: http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/reglrpts/rb9_toxicity_2012_new.pdf

**49.** State Water Board. Final 2012 California Integrated Report, Category 5 California 303(d) list of water quality limited segments: Chollas Creek. Sacramento (CA): State Water Resources Control Board; 2015. Available at: https://www.waterboards.ca.gov/water_issues/programs/tmdl/2012state_ir_reports/category5_report.shtml

**50.** Solutions Weston. Chollas Creek TMDL Source Loading, Best Management Practices, and Monitoring Strategy Assessment. Final Report. Carlsbad (CA): Weston Solutions; 2006. Sponsored by City of San Diego. Available at: https://water.ca.gov/LegacyFiles/irwm/grants/docs/Archives/Prop84/Submitted_Applications/P84_Round1_Implementation/San%20Diego%20County%20Water%20Authority/Chollas%20Creek%20Integration/Chollas%20Creek%20TMDL%20-%20Final%20Report%20(September%202006).pdf

**51.** Pritchard T. These ten locations featuring urban runoff pollution will shock you. San Diego (CA): San Diego Coastkeeper; 2014. Available from: https://www.sdcoastkeeper.org/blog/urban-runoff/these-ten-locations-featuring-urban-runoff-pollution-will-shock-you

**52.** American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals. Schaumburg (IL); 2013. 102 p. Available from: https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

**53.** EPA (Environmental Protection Agency). Method 8270C Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS). Washington DC; 1996. Report No.: SW-846 Ch 4.3.2. Available at: http://www.caslab.com/EPA-Methods/PDF/8270c.pdf

**54.** Lenz R, Enders K, Stedmon CA, Mackenzie DMA, Nielsen TG. A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. Mar Pollut Bull. 2015; 100(1):82–91. https://doi.org/10.1016/j.marpolbul.2015.09.026 PMID: 26455785

**55.** Song YK, Hong SH, Jang M, Han GM, Rani M, Lee J, et al. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. Mar Pollut Bull. 2015; 93(1–2):202–09. https://doi.org/10.1016/j.marpolbul.2015.01.015 PMID: 25682567

**56.** Lusher AL, Welden NA, Sobral P, Cole M. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal Methods 2017a; 9:1346–60.

**57.** Masura J, Baker J, Foster G, Arthur C. Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments. Technical Memorandum. Silver Spring (MD): NOAA Marine Debris Program, National Oceanographic and Atmospheric Administration; 2015 Jul. Report No.: NOS-OR&R-48. Available at: https://marinedebris.noaa.gov/sites/default/files/publications-files/noaa_microplastics_methods_manual.pdf

**58.** Gago J, Carretero O, Filgueiras AV, Viñas L. Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. Mar Pollut Bull. 2018; 127:365–76. https://doi.org/10.1016/j.marpolbul.2017.11.070 PMID: 29475673

**59.** Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Tech. 2012; 46:3060–3075.

**60.** Lusher AL, Burke A, O'Connor I, Officer R. Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling. Mar Pollut Bull. 2014; 88:325–33. https://doi.org/10.1016/j.marpolbul.2014.08.023 PMID: 25224764

**61.** Woodall LC, Gwinnett C, Packer M, Thompson RC, Robinson LF, Paterson GLJ. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. Mar Pollut Bull. 2015; 95:40–46. https://doi.org/10.1016/j.marpolbul.2015.04.044 PMID: 25936572

**62.** Rochman CM, Brookson C, Bikker J, Djuric N, Earn A, Bucci K, et al. Rethinking microplastics as a diverse contaminant suite. Environ Toxicol Chem. 2019; 38(4):703–11. https://doi.org/10.1002/etc.4371 PMID: 30909321

63. Chipps SR, Garvey JE. Assessments of diets and feeding patterns. Guy CS, Brown ML, editors. Analysis and Interpretation of Freshwater Fisheries Data. Bethesda (MD): American Fisheries Society; 2007. p. 473–514.

64. Fritz ES. The life history of the California killifish, *Fundulus parvipinnis* Girard, in Anaheim Bay, California. Calif. Fish Game Fish Bull. 1975; 165:91–106.

65. Nico L, Schofield PJ, Neilson M. Nonindigenous Aquatic Species Database: *Poecilia latipinna* (Lesueur, 1821). Gainesville (FL): U.S. Geological Survey; 2016 [cited 28 July 2019]. Available from: https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=858

66. PubChem. Compound Summary: Diethyl phthalate. Bethesda (MD): National Center for Biotechnology Information, U.S. National Library of Medicine; 2019a. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/6781

67. PubChem. Compound Summary: m-Cresol. Bethesda (MD): National Center for Biotechnology Information, U.S. National Library of Medicine; 2019b. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/m-cresol

68. PubChem. Compound Summary: Benzyl alcohol. Bethesda (MD): National Center for Biotechnology Information, U.S. National Library of Medicine; 2019c. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/244

69. WHO (World Health Organization). Concise International Chemical Assessment Document 52: Diethyl phthalate. Geneva: World Health Organization; 2003. Sponsored by United Nations Environment Programme, International Labour Organization, World Health Organization. Available at: http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf?ua=1

70. Wade LG. Cresol: Chemical Compound. In Augustyn A, Bauer P, Duignan, Eldridge A, Gregersen E, McKenna A, et al., editors. Encyclopedia Britannica. Online; 2019 [cited 2019 May 10]. Available from: https://www.britannica.com/science/cresol

71. Wiki (Wikipedia.org) [Internet]. Benzyl alcohol. 2019 [cited 2019 Aug 25]. Wikipedia Foundation, Inc. Available at: https://en.wikipedia.org/wiki/Benzyl_alcohol

72. Miller-Cassman T, VonBitner T, Boerger C, Bredvig J, Pritchard T, Kuhn T (San Diego Bay Debris Study Workgroup). Status of the bay: assessment of plastic debris in San Diego Bay. San Diego Bay Debris Study: Special Study Plastic Debris Monitoring Report. San Diego (CA): Surface Water Ambient Monitoring Program, State Water Resources Control Board and Southern California Bight 2013 Regional Monitoring Survey Bight '13 Debris Planning Committee; 2016a Oct. p. 23–45. Available at: https://www.waterboards.ca.gov/sandiego/water_issues/programs/sdbay_strategy/doc/FINAL_San_Diego_Bay_Debris_Study_Oct2016.pdf

73. Talley TS, Goodwin L, Mothokakobo R, Ruzic R, Venuti N. Testing the sources and pathways of trash through an urban watershed. Citizen Science Program Final Report. San Diego (CA): California Sea Grant, Ruzic Consulting Inc, Ocean Discovery Institute; 2016. Award No. 1324962. Sponsored by the National Science Foundation Advancing Informal Science Learning Program. Available at: https://caseagrant.ucsd.edu/sites/default/files/NSF-AISL-PlasticsFinalReport-Oct2016.pdf

74. Talley TS, Venuti N, Adams C, Barkan J, Bowlby E. Building Climate Resilience of Urban Waters, Ecosystems, and Communities. Final Report. San Diego (CA): California Sea Grant, San Diego Canyonlands, Ocean Discovery Institute; 2018 Jul 31. Project No.: 3760-101-6083007. Publication No. CASG-18-024. Sponsored by the California Coastal Conservancy. Available at: https://caseagrant.ucsd.edu/publication/building-climate-resilience-of-urban-waters-ecosystems-and-communities

75. Hwang H-M, Fiala M, Wade TL, Park D. Review of pollutants in urban road dust: Part II. Organic contaminants from vehicles and road management. Int J Urb Sci. 2018. https://doi.org/10.1080/12265934.2018.1538811

76. Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans Roy Soc B: Biol Sci. 2009; 346(1526):1985–98. https://doi.org/10.1098/rstb.2008.0205 PMID: 19528051

77. Andrady AL. Microplastics in the marine environment. Mar Pollut Bull. 2011; 62(8):1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030 PMID: 21742351

78. Weinstein JE, Crocker BK, Gray AD. From macroplastic to microplastic: Degradation of high-density polyethylene, polypropylene, and polystyrene in a salt marsh habitat. Environ Toxicol Chem. 2016; 35:1632–1640. https://doi.org/10.1002/etc.3432 PMID: 26992845

79. Miller-Cassman T, VonBitner T, Talley TS, Goodwin L, Mothokakobo R (San Diego Bay Debris Study Workgroup). Riverine debris across San Diego Bay watersheds: An assessment of plastics in Otay River, Sweetwater River and Chollas Creek tributaries. San Diego Bay Debris Study: Special Study Plastic Debris Monitoring Report. San Diego (CA): Surface Water Ambient Monitoring Program, State Water Resources Control Board and Southern California Bight 2013 Regional Monitoring Survey Bight '13 Debris Planning Committee. 2016b Oct. p. 1–22. Available at: https://www.waterboards.ca.gov/

sandiego/water_issues/programs/sdbay_strategy/doc/FINAL_San_Diego_Bay_Debris_Study_Oct2016.pdf

80. Choy CA, Robison BH, Gagne TO, Erwin B, Firl E, Halden RU, et al. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Sci Reports 2019; 9:7843. https://doi.org/10.1038/s41598-019-44117-2 PMID: 31171833

81. de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biol. 2018; 24:1405–1416.

82. Renick VC, Anderson TW, Morgan SG, Cherr GN. Interactive effects of pesticide exposure and habitat structure on behavior and predation of a marine larval fish. Ecotoxicology 2015; 24(2):391-400. https://doi.org/10.1007/s10646-014-1388-2 PMID: 25421633

83. Renick VC, Weinersmith KL, Vidal-Dorsch DE, Anderson TW. Effects of a pesticide and a parasite on neurological, endocrine, and behavioral responses of an estuarine fish. Aquat Toxicol. 2016; 170:335-343. https://doi.org/10.1016/j.aquatox.2015.09.010 PMID: 26454718

84. Temming A, Hammer C. Sex specific food consumption of dab (*Limanda limanda* L.) based on a 24th fishery. Arch Fish Marine Res. 1994; 42(2):123–36.

85. Smith KJ, Taghon GL, Able KW. Trophic linkages in marshes: Ontogentic changes in diet for young-of-the-year mummichog, *Fundulus heteroclitus*. In: Weinstein MP, Kreeger DA, editors. Concepts and Controversies in Tidal Marsh Ecology. The Netherlands: Kluwer Academic Publishers; 2000. p. 221–237.

86. Talley DM. Ichthyofaunal utilization of newly-created versus natural salt marsh creeks in Mission Bay, CA. Wetlands Ecol Manag. 2000; 8:117–132. https://doi.org/10.1023/A:1008436301041

87. Borg JPG, Westerbom M, Lehtonen H. Sex-specific distribution and diet of *Platichthys flesus* at the end of spawning in the northern Baltic Sea. J Fish Biol. 2014; 84(4):937–51. https://doi.org/10.1111/jfb.12326 PMID: 24641237

88. Mizraji R, Ahrendta C, Perez-Venegas D, Vargasa J, Pulgara J, Aldanac M, et al. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar Pollut Bull. 2017; 116:498–500. https://doi.org/10.1016/j.marpolbul.2017.01.008 PMID: 28063703

89. West J, Zedler JB. Marsh-creek connectivity: Fish use of a tidal salt marsh in Southern California. Estuaries 2000; 23(5):699–710. http://www.jstor.org/stable/1352896

90. Corley C. Why those tiny microbeads in soap may pose problem for Great Lakes. National Public Radio. 2014 May 21. Available at: www.npr.org/2014/05/21/313157701/why-those-tiny-microbeads-in-soap-may-pose-problem-for-great-lakes

91. Savoca MS, Wohlfeil ME, Ebeler SE, Nevitt GA. Marine plastic debris emits a keystone infochemical for olfactory foraging seabirds. Sci Adv. 2016; 2(11):e1600395. https://doi.org/10.1126/sciadv.1600395 PMID: 28861463

92. Trexler JC, Tempe RC, Travis J. Size-selective predation of sailfin mollies by two species of heron. Oikos 1994; 69(2):250–258. https://doi.org/10.2307/3546145

93. West J, Williams GD, Madon SP, Zedler JB. Integrating spatial and temporal variability into the analysis of fish food web linkages in Tijuana Estuary. Environ Biol Fish. 2003; 67(3):297–309.

94. Able KW, Vivian DN, Petruzzelli G, Hagan SM. Connectivity among salt marsh subhabitats: Residency and movements of the mummichog (*Fundulus heteroclitus*). Estuar Coast. 2012; 35: 743–53. https://doi.org/10.1007/s12237-011-9471-x

95. Kang CK, Park HJ, Choy EJ, Choi KS, Hwang K, Kim JB. Linking intertidal and subtidal food webs: Consumer-mediated transport of intertidal benthic microalgal carbon. PLoS One. 2015. https://doi.org/10.1371/journal.pone.0139802 PMID: 26448137

96. Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ Sci Tech. 2008; 42 (13):5026−5031. https://doi.org/10.1021/es800249a PMID: 18678044

97. Murray F, Cowie PR. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar Pollut Bull. 2011; 62(6):1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032 PMID: 21497854

98. Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka M, Watanuki Y. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar Pollut Bull. 2013; 69:219–222. https://doi.org/10.1016/j.marpolbul.2012.12.010 PMID: 23298431

99. Welden NAC, Cowie PR. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ Pollut. 2016; 218:895–900. https://doi.org/10.1016/j.envpol.2016.08.020 PMID: 27524255

100. Lu L, Luo T, Zhao Y, Cai C, Fu Z, Jin Y. Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health. Sci Total Environ. 2019; 667:94–100. https://doi.org/10.1016/j.scitotenv.2019.02.380 PMID: 30826685

**101.** Devriese LI, De Witte B, Vethaak AD, Hostens K, Leslie HA. Bioaccumulation of PCBs from microplastics in Norway lobster (*Nephrops norvegicus*): An experimental study. Chemosphere 2017; 186:10–16. https://doi.org/10.1016/j.chemosphere.2017.07.121 PMID: 28759812

**102.** NIOSH (U.S. National Institute of Occupational Safety and Health). Registry of Toxic Effects of Chemical Substances (RTECS online database). Bethesda (MD): National Toxicology Information Program, National Library of Medicine (US); 1997 [cited April 19 2019]. Available from: https://www.cdc.gov/niosh/docs/97-119/default.html

**103.** Groshart C, Okkerman PC. Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption—preparation of a candidate list of substances as a basis for priority setting. Annex 15: List of 66 substances with categorisation high, medium or low exposure concern. Final report. Delft (The Netherlands): European Commission DG Env; 2000 Nov 10. Report No.: M0355008/1786Q/10/11/00. Available at: http://ec.europa.eu/environment/archives/docum/pdf/bkh_main.pdf and http://ec.europa.eu/environment/archives/docum/pdf/bkh_annex_15.pdf

**104.** Ghorpade N, Mehta V, Khare M, Sinkar P, Krishnan S, Rao CV. Toxicity study of diethyl phthalate on freshwater fish *Cirrhina mrigala*. Ecotoxicol. Environ Safety 2002; 53(2):255–258. https://doi.org/10.1006/eesa.2002.2212 PMID: 12568461

**105.** Okkerman PC, van der Putte I. Endocrine disrupters: Study on gathering information on 435 substances with insufficient data. Annex 13: The summary profiles of (41) Category 1 chemical groups. Final Report. Delft (The Netherlands): European Commission DG Env; 2002 Nov 15. Report No.: M0355037. Available at: http://ec.europa.eu/environment/chemicals/endocrine/pdf/bkh_report.pdf#page=148

**106.** Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: Toxicology and exposure. International J. Hyg Environ Health 2007; 210(5):623–634. https://doi.org/10.1016/j.ijheh.2007.07.011 PMID: 17889607

**107.** Lithner D, Larsson A, Dave G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ. 2011; 409(18):3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038 PMID: 21663944

**108.** NIH (National Institute of Health). Hazardous Substances Data Bank (HSDB). Bethesda (MD): National Toxicology Information Program, National Library of Medicine (US); 2018 [cited April 15 2019]. Available from: http://toxnet.nlm.nih.gov

**109.** Windward Environmental. East waterway operable unit supplemental remedial investigation feasibility study: Fish and shellfish tissue collection. Final report. Seattle (WA): Windward Environmental LLC; 2010. Sponsored by U.S. Environmental Protection Agency, Region 10. Available at: hwww3.epa.gov/region10/pdf/sites/harborisland/east_waterway/fish_shellfish_data_report_0410.pdfs

**110.** Rochman CM, Tahir A, Williams SL, Baxa DV, Lam R, Miller JT, et al. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci Reports 2015b; 5. https://doi.org/10.1038/srep14340 PMID: 26399762

**111.** Barboza LGA, Vethaak AD, Lavorante BRBO, Lundebye A-K, Guilhermino L. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar Pollut Bull. 2018a; 133:336–48. https://doi.org/10.1016/j.marpolbul.2018.05.047 PMID: 30041323

**112.** Boerger CM, Lattin GL, Moore SL, Moore CJ. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar Pollut Bull. 2010; 60(12):2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007 PMID: 21067782