To Sink or Swim

A Snapshot Evaluation of the Fate and Types of Microplastics in Lake Tahoe

A collaborative original research study conducted by the
University of California Davis Tahoe Environmental Research Center and One Health Institute
with funding by the Nevada Division of Environmental Protection and the Tahoe Water
Suppliers Association

ACKNOWLEDGEMENTS

Report prepared for:
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Final Report Date: Feb. 6, 2023

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ACKNOWLEDGEMENTS

We would like to acknowledge the assistance and advice provided by members of the TERC field collection team (Brant Allen, Brandon Berry, Raph Townsend, Kian Bagheri) and laboratory analysis team (Helen Fillmore, Anne Liston).

Additionally, we would like to thank the Gjeltema HEAL Lab volunteers (Lena Wigger, Dylan Lin, and Madison Li) who assisted with laboratory processing and particle characterization for this project.

Some of the findings of our study have already been included in displays at the UC Davis Tahoe Science Center in Incline Village, NV, and are building awareness of microplastic pollution in the general public. We acknowledge the role of Heather Segale, Alison Toy, and our AmeriCorps members in that effort, as well as the philanthropic donors who have made that possible.

We wish to thank the Nevada Division of Environmental Protection for the funding to undertake this project, and Jason Kuchnicki for his work as NDEP project manager. We also thank the Tahoe Water Suppliers Association for their financial support of this project as well as Madonna Dunbar, Patrick McKay and Kristine Lebo for their assistance with the collection of municipal water samples. Our sincere gratitude to Captain Bob of Reel Deal Sportfishing Lake Tahoe for the collection of the kokanee salmon for this project.

PROJECT OVERVIEW

ENVIRONMENTAL MICROPLASTIC POLLUTION

Due to the ubiquitous and largely unrestricted use of plastics, its frequent unintended release into the environment, and increasing recognition of potentially harmful effects; there is an urgent need to better understand the current levels, environmental fate, as well as the hazards posed to human, wildlife, and ecosystem health in order to appropriately assess risks associated with its presence.

Plastics are composed of synthetic (human-made) polymers typically derived from petroleum oils. These synthetic polymers are made up of repeating identical molecular sub-units (monomers) that are chemically linked together into long chains. The characteristics of a plastic are determined by the particular sub-unit's chemical properties that can be augmented with additive chemicals (like plasticizers, flame retardants, other polymers, or dyes) that are mixed into the plastic to adjust specific properties including rigidity, flexibility, durability, melting point, color, and clarity. Table 1 describes the characteristics and uses of the most commonly produced plastics are outlined below.

Table 1. Characteristics and uses of the most commonly produced plastics worldwide.

Commonly Produced Plastics						
Synthetic Polymer (Plastic)	**Abbr**	**Density g/cm^3**	**Sink or float**	**Global Plastic Product**	**Key Characteristics**	**Typical uses**
Polyethylene	PE	0.91 - 0.97	Float	36%	Durable Easily molded Lightweight	Bottles/food containers/bags Pipes Fishing gear/nets
Polypropylene	PP	0.9 - 0.91	Float	21%	Rigid and tough Fatigue resistant Susceptible to solvents Heat resistant	Food packaging Automotive parts Medical supplies Upholstery, consumer goods
Polyester & Polyethylene Terephthalate	PES PET	1.23 - 2.3 1.37 - 1.45	Sink	10%	Strong and stiff Resistant to shatter Lightweight	Clothing and textiles Bottles/food containers
Polyvinyl chloride	PVC	1.16 - 1.58	Sink	12%	Hard and durable Tensile strength Rigid or flexible forms	Constructions materials Pipes/Flooring/wiring Packaging
Polystyrene	PS	1.04 - 1.1	Sink	10%	Hard Rigid/brittle Forms plastic mixtures	Foam food containers Disposable cutlery Building insulation
Polyurethane	PU	1.2	Sink	10%	Abrasion resistant Rigid or flexible forms Bonds well	Building insulation Insulating foams/mattresses
Polyamide (Nylon)	PA	1.02 - 1.05	Sink	-	Tensile strength Low friction Resists abrasion Dries quickly	Clothing Industry/construction Fishing gear/nets Electronics/machine parts

PROJECT OVERVIEW

The term 'microplastic' is colloquially used to refer to any small piece of plastic and are generally defined as synthetic polymers measuring between 1 µm and 5 mm in size. Table 2 defines the more precise terminology is used in the scientific communities to refer to different size classes of plastic, however, debate continues as to the exact size encompassed by each term (Padervand et al. 2020).

Table 2. Size class definitions and descriptions of common "microplastic" terminology.

Commonly Terminology used for Microplastic Research	
Term	**Description**
Particle	General term referring to any small piece of matter with physical and chemical properties that may be used when the underlying composition is plastic, non-plastic, or unknown
Suspected Plastic Particle	Term referring to a particle that has characteristics consistent with or similar to plastic but that has not been definitively identified or confirmed to be composed of plastic.
Nanoplastic	A piece of plastic commonly measured in nanometers (usually 1-1000nm) by its longest dimension
Microplastic	A piece of plastic commonly measured in micrometers (usually 1-1000µm) by its longest dimension
Mesoplastic	A piece of plastic measuring 1-10mm by its longest dimension
Macroplastic	A piece of plastic measuring larger than 1cm by its longest dimension

Plastic is refractory to biodegradation, which makes it a resilient and durable material that is useful for many applications. Its chemical resilience means that pieces of plastic often physically break into smaller pieces long before it can chemically degrade. Because chemical degradation tends to occur at a much slower rate than physical break-down into smaller pieces, an accumulation of ever-smaller pieces of plastic (microplastics) may persist in contaminated environments for many decades to centuries or even millennia after being released.

While harmful effects of microplastic exposure have been researched and described, there is currently only a rudimentary understanding of the hazards posed by microplastic pollution. Much remains unknown about how microplastic characteristics and composition may contribute to harmful effects, how environmental fate of microplastics may affect exposure pathways, and at what environmental level harmful effects occur for different environmental matrices.

Although risk to human health from drinking water is considered low at this time, this conclusion assumes drinking water undergoes standard treatment and is based on currently understood health effects. This conclusion may not be appropriate to extrapolate to untreated water sources, other routes of exposure, and wildlife and ecosystem health.

PROJECT OVERVIEW

MICROPLASTICS IN LAKE TAHOE

Since 2016, three independent studies have confirmed the presence of microplastics in Lake Tahoe's surface waters, stormwater inflows, and beach. The confirmation of microplastics in Lake Tahoe gives great urgency to understanding their fate once in the lake as this could have future impacts on water quality, human health, and local wildlife.

RESEARCH GOALS AND SCOPE

The primary goal of this research project was to examine and document the current status of microplastic pollution within Lake Tahoe. Plastic particles obtained from lake surface water, different water depths, and from lake sediment were quantified, identified, and characterized to provide insight into the abundance, types, shape, and sizes of plastics found. A preliminary investigation of the fate of microplastic particles within the lake was also evaluated for a limited number of biota from within the lake that serve different ecological roles, exist at different trophic levels, and that have different microhabitat predilection. Additionally, municipal water samples sourced from the lake were also evaluated for the presence of microplastic particles.

Note that the tow net data are constrained to be microplastics larger than 335 microns by the mesh size used, thus making them considerably larger than the size range that directly impacts lake clarity. This also grossly underestimates the true number of microplastics in the lake, although as this mesh size is widely utilized, it does allow comparisons with other aquatic systems.

PROJECT OVERVIEW

Table 3. Detailed project overview of sample collections, laboratory methods, spectral analysis and size detection limits.

Project Overview Summary of Samples and Analysis					
Sample Type & Collection Method	**Collection Method**	**Number**	**Sample Preparation**	**Analysis Methods**	**Particle Size Limits**
Surface Water (0m Depth)	Tow Net (335µm mesh)	12 monthly tows over 1 year	Digestion of organic material Filtration Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>335µm
Subsurface Water (15m avg. Depth)	Tow Net (335µm mesh)	12 monthly tows over 1 year	Digestion of organic material Filtration Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>335µm
Vertical Water (0, 15, 50, 250, 450m)	Van Dorn Grab Sample	4 quarterly samples over 1 year	Sample Filtering Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>20µm
Lake Sediment	Box Core Grab Sample	1 collection	Digestion of organic material Filtration Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>20µm
Asian Clams	Ponar Sediment Sampler	30 clams from single collection	Digestion of organic material Filtration Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>20µm
Kokanee Salmon	Procured by local fishermen	3 salmon stomachs	Digestion of organic material Filtration Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>20µm
Municipal Water	Procured at two municipal sources	4 quarterly samples over 1 year	Filtration Hand Selection and Mounting	Raman microspectroscopic Analysis Visual Particle Characterization Digital imaging and measurement	>20µm

PROJECT OVERVIEW

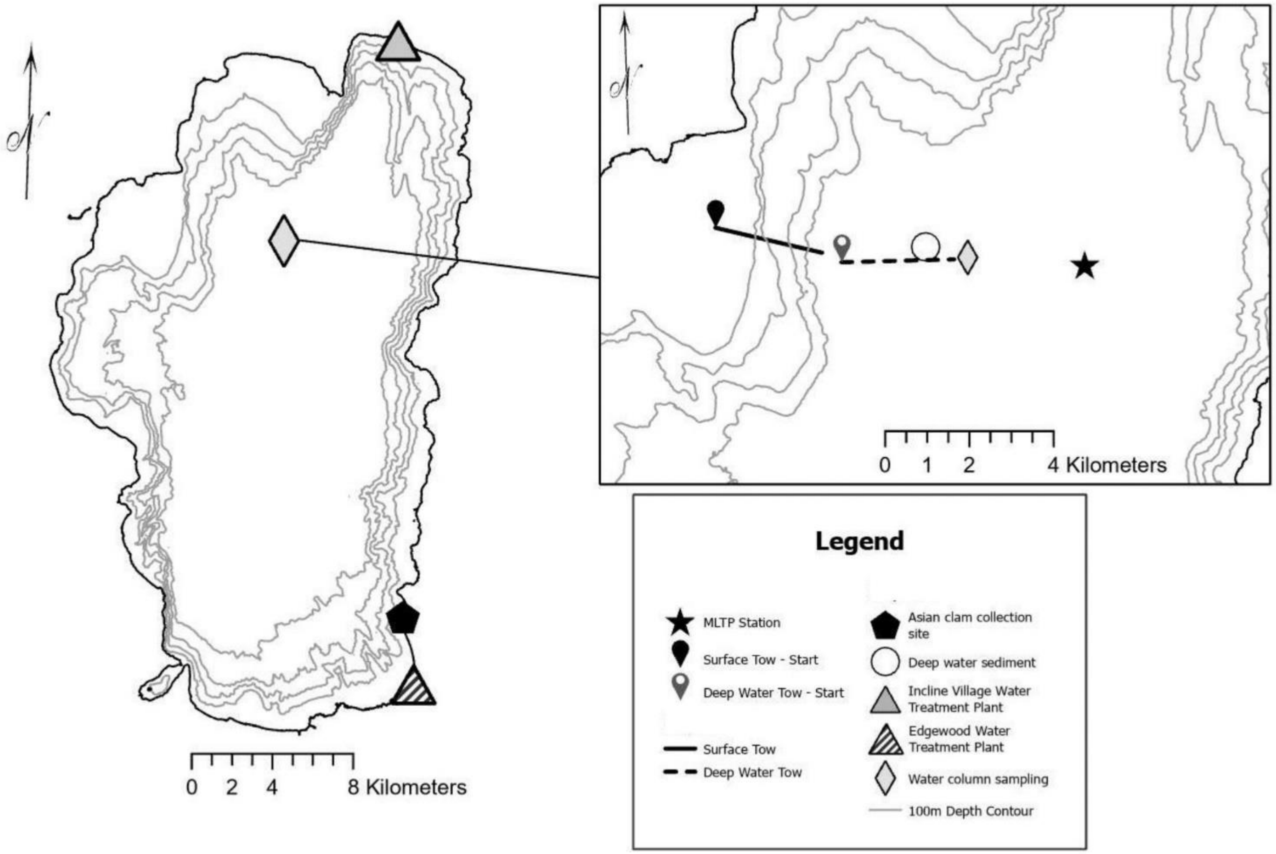


FIGURE 1. MAP OF LAKE TAHOE INDICATING PROJECT SAMPLING LOCATIONS. THE MAP INDICATES THE LOCATIONS WHERE EACH TYPE OF SAMPLE WAS COLLECTED. THESE LOCATIONS ARE CONSIDERED TO BE REPRESENTATIVE OF EACH TYPE OF ENVIRONMENT AT LAKE TAHOE, ALTHOUGH NO SPATIAL RECORD OF MICROPLASTIC POLLUTION EXISTS TO CONFIRM THIS ASSUMPTION. HOWEVER, BASED ON MANY DECADES OF LAKE MONITORING OF A BROAD RANGE OF ENVIRONMENTAL VARIABLES WE BELIEVE THAT THIS ASSUMPTION TO BE CORRECT

MATERIALS AND METHODS
HORIZONTAL TOWS OF SURFACE AND SUBSURFACE WATERS

Sample Collection

A horizontal oceanic sampling trawl net (Hydro Bios®; Am Jägersberg 5-7, 24161 Altenholz, Germany) with mechanical flow meter was used to collect 12 monthly samples from surface and subsurface waters of Lake Tahoe during the period of August 27th, 2020 to August 4th, 2021. The net had a rectangular opening of 40cm x 70cm, length of 260cm, and mesh size of 335µm. The trawl net was towed by boat for 30 minutes at 3 knots along a fixed heading transect between the Tahoe City Marina and Mid Lake Tahoe Profile (MLTP) monitoring site (see figure 1). The sampling net was towed alongside the vessel to prevent contamination from the boat or interference from the propeller. In order to collect the subsurface water samples, floats were removed from the sides of the net and a 3.6kg metal weight was affixed to the bridle in order to submerge the trawl to a proper depth. The net was deployed over the side of the vessel and 85m of cable was paid out to reach the sampling depth. Cable length and target boat speed were fixed during subsurface trawls. A water level data logger (HOBO® U20L-02; Onset Computer Corporation, 136 Richmond Rd, Marleston SA 5033, AU) was attached to the net to measure actual tow depth during subsurface sample collection. Actual sampling depth varied with a transect length weighted average of 14.9m (Table 4B). GPS location, heading, speed and duration were recorded during each tow.

Table 4A. Tow depth, average vessel speed and total transect length sampled for each unique sampling event of Lake Tahoe's surface waters with the manta trawl.

Horizontal Tow Field Sampling Data Surface Water			
Sampling Date	**Tow Depth**	**Avg Speed**	**Total transect length**
	m	**knots**	**km**
8/27/2020	0	1.9	1.8
10/2/2020	0	3.5	3.2
10/29/2020	0	3.1	2.9
11/20/2020	0	2.9	2.7
12/9/2020	0	3.3	NR
1/13/2021	0	3.2	3
2/10/2021	0	3.3	3.1
3/16/2021	0	3.2	2.9
4/12/2021	0	2.8	2.5
5/5/2021	0	3	2.9
6/30/2021	0	3.1	2.6
8/4/2021	0	3	2.8

NP = No pressure sensor data from sampling event
NR = Not recorded

Table 4B. Tow depth, average vessel speed and total transect length sampled for each unique sampling event of Lake Tahoe's subsurface waters with the manta trawl.

Horizontal Tow Field Sampling Data Subsurface Water			
Sampling Date	**Tow Depth**	**Avg Speed**	**Total transect length**
	m	**knots**	**km**
8/27/2020	12.6	1.7	1.7
10/2/2020	NP	3.2	3
10/29/2020	8.6	2.8	2.6
11/20/2020	18.1	2.8	2.8
12/9/2020	15.5	3	NR
1/13/2021	19.2	3	2.8
2/10/2021	15.5	2.9	2.8
3/16/2021	17.5	2.8	2.7
4/12/2021	17.7	2.9	2.7
5/5/2021	14.0	3.1	2.9
6/30/2021	22.8	3.1	2.9
8/4/2021	17.7	3	2.8

NP = No pressure sensor data from sampling event
NR = Not recorded

Following completion of a tow, the net was carefully brought onboard ensuring it did not come into contact with the vessel, and a high-pressure deck hose was used externally on the net to flush all collected material into the cod-end of the net. The cod-end was detached and all collected material was flushed into a pre-cleaned glass jar using de-ionized water. Large organic debris was manually removed and rinsed over the sample jar to remove any attached particles before being discarded. Field blank samples were collected prior to tows by rinsing two liters of pre-filtered de-ionized water through the suspended trawl net. Sample jars were stored in coolers until they were transferred to the lab. At the lab, 70% ethanol (Fisher Scientific, Reagent Alcohol, Cat. No. A995-4) was added to each sample jar until full and then stored in a dark refrigerator at 4°C to prevent bacterial and algal growth. Processing of samples were completed on average 13 days after sample collection.

MATERIALS AND METHODS

HORIZONTAL TOWS OF SURFACE AND SUBSURFACE WATERS

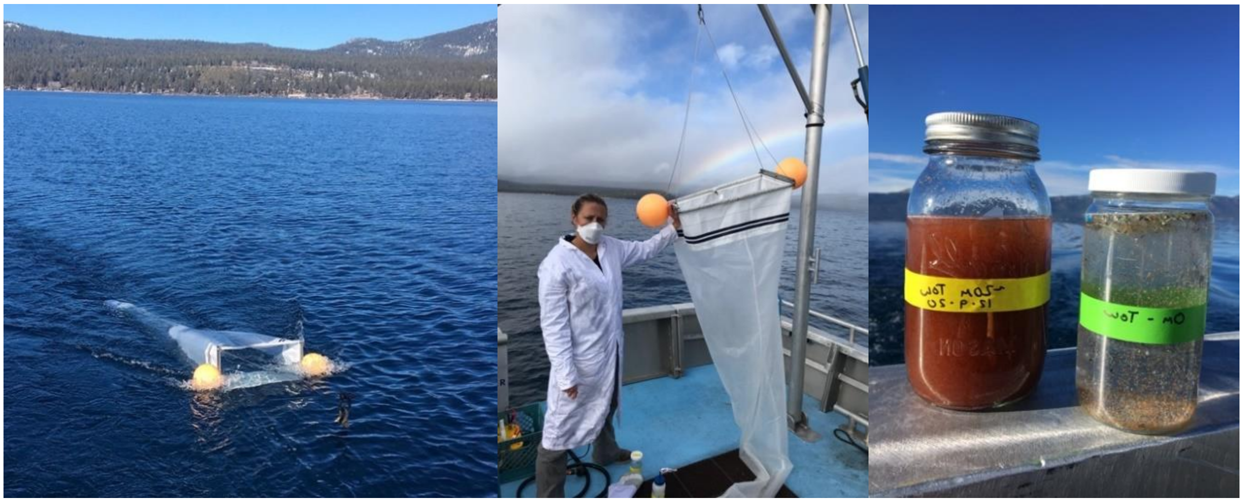


FIGURE 1. SAMPLING OF SUSPECTED PLASTIC PARTICLES FROM LAKE TAHOE SURFACE WATERS. A TOW NET WAS USED TO COLLECT SAMPLES FROM SURFACE AND SUBSURFACE WATERS (LEFT IMAGE). FOLLOWING A TOW, ALL MATERIAL FROM THE NET WAS TRANSFERRED INTO A SAMPLING JAR (MIDDLE IMAGE). SAMPLES FROM EACH JAR WERE PROCESSED AT THE LAB TO REMOVE ORGANIC MATERIAL AND ISOLATE ANY POTENTIALLY PLASTIC PARTICLES FOR FURTHER ANALYSIS.

Sample Preparation

Each sample was then processed to isolate particles suspected to be plastic from other natural materials. The sample was first filtered through 500µm and 300µm mesh-sized sieves. If the sample contained high organic content (i.e. algae, grass, twigs, etc.), each large piece of organic material was removed with pre-cleaned forceps and thoroughly rinsed with de-ionized water over the 500 µm sieve. When high organic content was observed for a sample after use of sieves, a digestion step was used to remove this organic material and isolate suspected plastic particles for analysis. Digestion was accomplished using 10% KOH (w/v) with a minimum of a 3:1 digestion solution to sample volume ratio. The samples were incubated at 60°C with reciprocal shaking at 70 rpm overnight. These samples were then transferred into a 1 L separatory funnel and density separation was performed by filling the separatory funnel to maximum volume with 5M NaCl solution. This step allows dense material, like sand to settle to the bottom while less dense materials (like plastic particles) float to the surface. The solution was allowed to separate overnight. Material suspended at the surface of each sample was collected and vacuum filtered through a 0.8 µm filter (Millipore Sigma, Isopore™ PC Membrane Filter, Cat. No. ATTP04700). A dissection microscope and forceps were used to transfer and mount any particles from the filter onto double-sided tape attached to a pre-cleaned transparent plastic disc (see figure 3). The digestion, filtration, and density separation steps are designed to eliminate non-plastic materials from each sample and retain only particles with characteristics consistent with plastic to remain. Although these remaining particles are suspected to be plastic, particles composed of other materials with qualities similar to plastic may also remain in the sample. These particles that are likely to be plastic but that have not yet been definitively identified as composed of plastic are referred to in this report as 'suspect' or 'suspected plastic' particles. The chemical identity of each particle must be confirmed using analytical tools before it can be considered a 'microplastic'. Each suspect particle attached to the disc was catalogued and labeled with a number for reference. The discs were stored inside pre-cleaned petri dishes until Raman microspectroscopic analysis for particle identification and characterization could be performed.

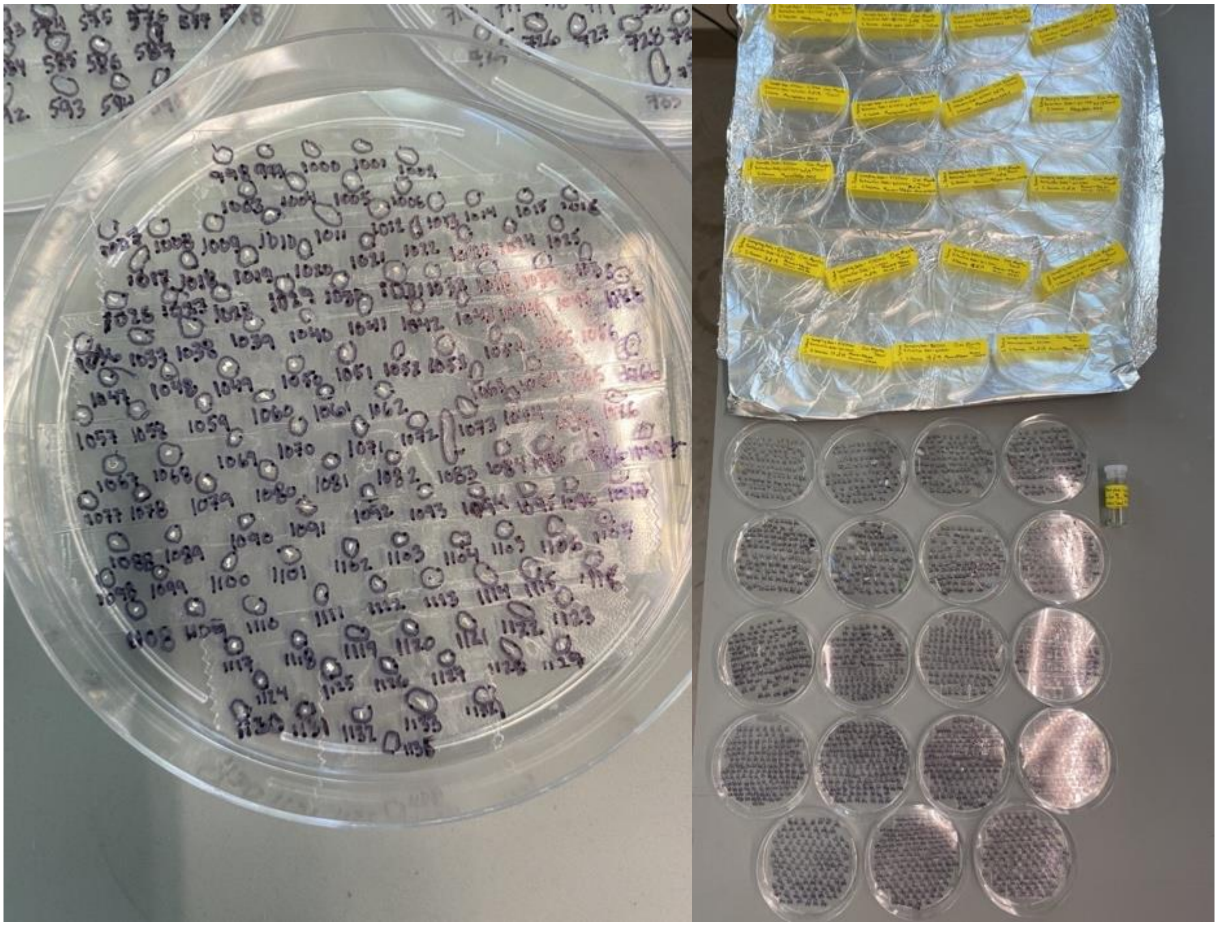


FIGURE 3. PREPARATION OF TRAWL NET SAMPLES FROM LAKE TAHOE SURFACE WATERS. FOLLOWING DIGESTION OF ORGANIC MATERIAL, EACH SUSPECT PARTICLE WAS TRANSFERRED AND MOUNTED FOR CHARACTERIZATION AND ANALYSIS.

VERTICAL WATER SAMPLES

Sample Collection

Using a 5 L Van Dorn sampler, water was collected from various depths of Lake Tahoe. The same location (39.155483N, 120.0324W) was visited for each quarterly sampling event and represented the approximate end of the subsurface horizontal tow transect. To collect subsurface water along a vertical depth profile, the van Dorn bottle was hung vertically on a metal cable and sent to the desired depth. When being deployed on the cable, both end plugs of the Van Dorn sampler were secured to the side so water can flow through the tube until it reaches the correct depth. Once at the correct sampling depth, a metal “messenger” was sent down the cable, striking a trigger and allowing the end plugs to snap into place at the ends of the tube. A taut elastic cable connecting the two end plugs ensured that the water sample remained intact while being brought back to the surface. Once onboard, the sample was placed into a pre-cleaned jar using the side hose of the Van Dorn. Samples were collected at 0 m, 15 m, 30 m, 50 m, 250 m and 450 m as well as a field duplicate from 15 m. Sample jars were stored in coolers on the boat following collection. Pre-filtered 70 % ethanol was added to each sample and they were stored in the dark at 4° C to prevent bacterial and algal growth until further processing and analysis.

Sample Preparation

Samples were vacuum filtered onto a polycarbonate filter (10 µm pore size). All detectable particles on the filter surface were identified with the aid of a dissecting microscope. Each was mounted onto double-sided tape attached to a pre-cleaned transparent disc and labeled as previously described. The discs were stored inside pre-cleaned petri dishes until Raman microspectroscopic analysis and characterization could be performed.

Table 5. Sampling dates for vertical water column sampling at Lake Tahoe

Vertical Water Sample Collection Dates	
Collection Date	**Quarter**
11-20-2020	Fall
1-21-2021	Winter
6-10-2021	Spring
8-25-2021	Summer

LAKE SEDIMENT SAMPLES

Sample Collection

A large metal box core sampler (Figure 4) was deployed from the research vessel John LeConte to collect deep water sediment samples from Lake Tahoe on September 25, 2020. The sampling site (39.155483N, 120.0324W) was located approximately 8 km offshore of the Tahoe City Marina (Figure 1) with a water depth of approximately 470 m. The box core sampler was lowered off the A – frame on the rear of the vessel, taking special care to avoid having it touch other surfaces on the boat during both deployment and retrieval.

Once the box corer had been retrieved, sediment was removed on the rear deck of the sampling vessel. Two pre-cleaned 500mL glass jars were filled with sediment collected from the center of the grab (at least 5cm away from the sides) and down to a depth of 5cm using a pre-cleaned stainless-steel spoon which was rinsed with DI water in-between samples. Collecting samples from the top 5 cm of sediment is consistent with recommendations (European Commission Joint Research Center, 2013) and work recently completed by the San Francisco Estuary Institute (Sutton et al., 2019). Both samples were placed in the dark and on ice until they could be stored in the freezer later that day. At the lab, the samples were frozen and stored in the dark to prevent bacterial and algal growth until analysis.

FIGURE 4. BOX CORE SAMPLER PREPARING TO BE DEPLOYED DURING SEDIMENT SAMPLING ON LAKE TAHOE

MATERIALS AND METHODS - LAKE SEDIMENT SAMPLES

Sample Preparation

The sediment was allowed to thaw, homogenized, sub-sample, freeze dried, and weighed (dry weight of 1.53 g). It was digested in 10% pre-filtered KOH at approximately three times the volume of the sample. The samples were covered with fresh aluminum foil and allowed to incubate for 3-6 weeks at room temperature. Target particles were then isolated from the sediment using a separatory funnel for 7 days. The supernatant was vacuum-filtered onto a polycarbonate filter (10 µm pore size). All detectable particles on the filter surface were identified with the aid of a dissecting microscope and were mounted onto double-sided tape attached to a pre-cleaned transparent plastic disc as previously described. Each suspect particle attached to the disc was catalogued and labeled with a number for reference. The discs were stored inside pre-cleaned petri dishes until Raman microspectroscopic analysis for particle identification and characterization could be performed.

LAKE BIOTA

Sample Collection

Kokanee Salmon Gastrointestinal Tracts

Kokanee (*Oncorhynchus nerka*) stomachs were collected in the summer and fall of 2021 with the help of a local fishing guide. Fish were caught with line and lures along the southern shores of Lake Tahoe and were kept in a cooler on ice until they were cleaned. To minimize contamination of stomach contents, the entire digestive tracts were removed from fish intact and frozen at -20° C in sealable plastic bags until further processing could take place in a clean lab environment.

Asian Clams

Asian clams (*Corbicula fluminea*) for this project were collected from the southern portion of Lake Tahoe. The south shore is an ideal sampling location since it has the highest densities of clams in the lake and also has the greatest human population density. Thus, it is potentially experiencing the largest impacts from human influences (i.e. litter, microplastics in stormwater runoff, etc.).

Clams were collected during the late summer period when water temperatures were still above 15° C, meaning they had higher water filtration rates to their support higher metabolic demands. Clams were collected on October 19, 2021 from a 15 m depth in Marla Bay, NV using a petite Ponar grab sampler. Once onboard, the clams were scrubbed with a natural fiber coconut brush and rinsed thoroughly with DI water in order to remove any plastic particles which may be adhered on their shells. Clams ≥12 mm were selected as they filter a greater volume of water per day and may be more likely to have ingested plastics. Size class data were collected and clams were immediately placed into prewashed sample jars with no lake water or preservative to prevent the intake or excretion of any particles while in transport. Six replicate samples were collected with 10 individual clams pooled together for each replicate. Sample jars were stored on ice in coolers during transport and immediately placed in the freezer at -20° C upon arrival back at the TERC labs.

Sample Preparation

Bags containing kokanee digestive tracts were slowly thawed in a cool water bath until they were malleable. The thawed digestive tracts were carefully rinsed with DI water prior to handling to remove any plastic particles which might be adhered to the outside. The outside of the stomach was carefully examined for any damage which may have occurred during handling in the field (i.e. accidently cut by fishing guide while they were processing on the boat). After the stomach was rinsed and inspected for damage, it was removed from the rest of the digestive tract using pre-cleaned glass petri dishes and razor blades. Whole stomachs were placed individually in pre-cleaned glass jars and frozen again at -20° C until additional processing and analysis were performed at the Gjeltema Lab.

MATERIALS AND METHODS - LAKE BIOTA

Whole salmon stomachs and composites of 10 clams removed from their shells were digested in 10% pre-filtered KOH at approximately three times the volume of each sample. Samples were covered with fresh aluminum foil and allowed to incubate for 3-6 weeks. Samples were heated to 40° C for 1 hour prior to vacuum filtration onto a polycarbonate filter (10 µm pore size). All detectable particles on the filter surface were identified with the aid of a dissecting microscope. Each was mounted onto double-sided tape attached to a pre-cleaned transparent disc and labeled as previously described. The discs were stored inside pre-cleaned petri dishes until Raman microspectroscopic analysis and characterization could be performed.

MUNICIPAL WATER

Sample Collection

Working with the Tahoe Water Suppliers Association (TWSA), quarterly samples were collected at two municipal drinking water intakes within the Lake Tahoe Basin. The Incline Village pump station, operated by the Incline Village General Improvement District (IVGID), served as the north shore sampling location. While the Edgewood pump station, operated by the Edgewood Water Company, served as the south shore sampling location. Municipal water samples for this project were collected from the same sample collection spigots used by the water operator to collect their water quality samples. At the Edgewood pump house, the sampling point was located off a large metal pipe which is estimated to have consistent high-water flows moving through it. All samples at the Edgewood pump house were collected from this point except for the summer quarterly sample due to repairs taking place on the spigot. An alternative sampling spigot was identified by the pump house manager and used for this one event. The sampling point for the Incline Village pump house was not off a main water pipe but a small PVC pipe that ran approximately 15m off the main line before the water could be collected from the sampling spigot. It is suspected that water inside the small PVC pipe was often stagnant since it was not a part of the main line constantly pumping water.

At the sampling spigots, water was flushed for 10 minutes prior to collecting the sample in order to move any stagnant water through the system. Once the water line had been purged, municipal water was collected directly into pre-cleaned 3.75 L glass jars. A duplicate sample was also collected at the Edgewood pump house. Field blanks were collected at each pump house by placing a pre-cleaned 3.75 L glass jar filled with DI water next to the sampling spigot with the lid off for the same amount of time it took to collect the municipal water. This was done in order to account for any airborne contamination which may have occurred during sample collection. Municipal water samples, duplicates, and blanks were stored in a dark 4° C cooler or cold room until samples could be filtered.

Municipal samples collected for the summer quarter were collected on different dates due to staffing changes at one of the pump houses. During the summer collection at the Edgewood pump house in August 2021, the Tahoe Basin was experiencing heavy smoke effects from the Caldor wildfire activity. Wildfire smoke had dissipated from the basin for approximately 2 weeks prior to the September 2021 water collection at the IVGID pump house.

Table 6. Sampling dates and locations for municipal water sampling at Lake Tahoe.

Municipal Water Sample Collection Dates		
Edgewood Water Company	**Incline Village GID**	**Quarter**
June 10, 2021	June 10, 2021	Spring
August 24, 2021	September 19, 2021	Summer
December 2, 2021	December 2, 2021	Fall
February 8, 2022	February 8, 2022	Winter

Table 7: TWSA partner agencies' intake length, depth, and distance from the lake bottom. Intake water depth is reported based on measurements from the lake's rim since water depth varies depending on water level.

Municipal Water Intake Pipe Systems			
Municipal Water Source	**Length (m)**	**Depth (m)**	**Bottom (m)**
Incline Village GID	204.2	9.1	1.2
Edgewood Water Company	1676.4	182.9	1.2

Sample Preparation

Samples were vacuum filtered onto a polycarbonate filter (10 µm pore size). All detectable particles on the filter surface were identified with the aid of a dissecting microscope. Each was mounted onto double-sided tape attached to a pre-cleaned transparent disc and labeled as previously described. The discs were stored inside pre-cleaned petri dishes until Raman microspectroscopic analysis and characterization could be performed.

MATERIALS AND METHODS
PARTICLE IDENTIFICATION AND CHARACTERIZATION

Particle Classification and Identification

Following collection, processing, and isolation of particles suspected to be plastic, the particle composition must be determined. Raman spectroscopy is considered one of the current gold standards for confirming whether a particle is composed of plastic or another type of material. A Horiba XploRA™ Plus confocal Raman microspectroscopic unit operated using LabSpec6 spectroscopy suite software (Horiba Instruments Inc., 2890 John R Road, Troy, MI 48083, USA) was used for analysis. This unit was equipped with a motorized stage, three objectives (10X/0.25BD/FN22, 50X/0.75BD/FN22, and 100X/0.9BD/FN22), two lasers (532nm and 785nm), and a cooled charge-coupled device detector (1024 x 256 pixels). Confocal hole width was 300nm and slit width was 150nm. Laser spot diameter ranged from 0.72 to 3.83µm depending on the objective and laser wavelength used for analysis. Spectral acquisition parameters including laser wavelength, laser power, acquisition time, grating (600 - 2400gr/mm), number of accumulations, and objective used varied based on individual particle characteristics to obtain spectra optimized for efficient identification within the spectral range of 150 - 3500cm^{-1}. Calibration of the unit was performed by zero-order correction of each grating on the 520.7cm^{-1} peak of a reference silicon wafer and observation of laser alignment prior to sample analysis.

Post-acquisition spectral processing including polynomial baseline correction, noise correction, and range extraction were used as needed to adjust for fluorescence and to facilitate spectral identification. Raman spectra from each particle were identified by peak matching comparisons to Raman spectral libraries using KnowItAll™ software (Wiley) in conjunction with KnowItAll™, SLOPP, SLOPP-E, and in-house Raman spectral libraries. Spectral library matches were then screened individually for appropriate particle identification.

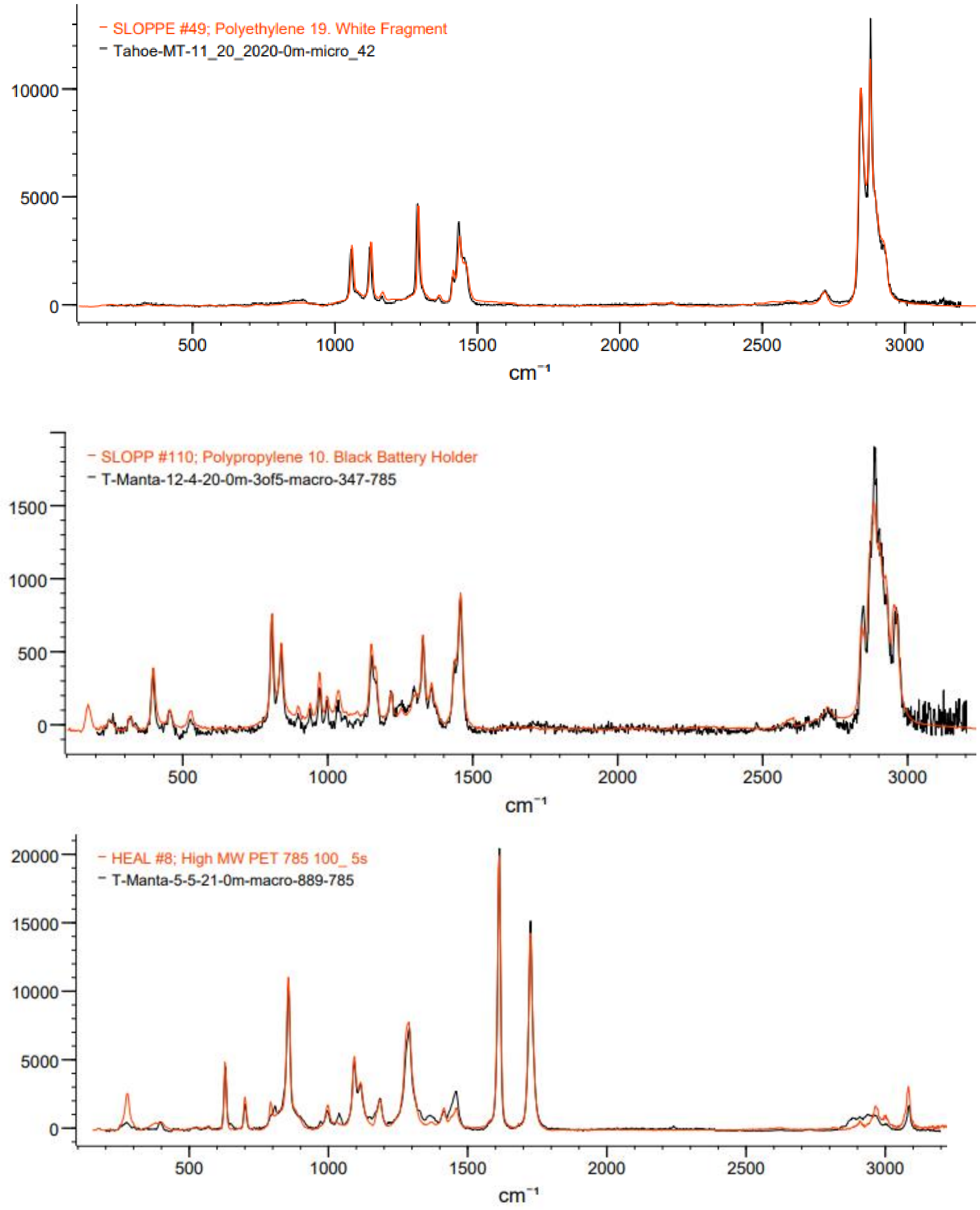


Figure 5. Examples of synthetic polymer (plastic) types identified using Raman microspectroscopy for microplastic particles collected from surface and subsurface waters of Lake Tahoe. The black line is the spectra obtained from a suspected plastic particle that is compared to a reference library spectra indicated by the red line to identify the particle.

MATERIALS AND METHODS
PARTICLE IDENTIFICATION AND CHARACTERIZATION

Based on chemical identity determined by Raman spectroscopy, particles were classified into one of 6 different categories: (1) Synthetic polymers, (2) Synthetic/natural polymer blends, (3) Natural polymers, (4) Dye prominent, (5) Other, or (6) Unknown. For all particles considered to be at least partially synthetic polymers, the primary plastic type(s) were identified and reported.

Table 8. Classifications used to categorize suspected particles found in Lake Tahoe water, sediment and biota samples after Raman analysis.

PARTICLE CLASSIFICATION	
Category	**Description**
Synthetic polymer	Particles composed of man-made plastic materials
Synthetic/natural polymer blend	Particles composed of both natural and man-made plastic materials
Natural polymer	Particles composed of polymers derived from biological materials
Dye prominent	Particles with undetermined chemical identity due to the presence of dye/pigment within the material that masks underlying chemical composition
Other	Particles with known chemical identity other than polymers or dyes
Unknown/other	Particles with undetermined chemical identity due to poor inherent spectral quality or lack of a discernable match to known library spectra

Particle Characterization

Key characteristics including size, morphology, and color of each suspect particle were individually measured and recorded. Measurements were made using the LabSpec6 software measurement tool and microscopic digital mosaic imaging. Straight length (longest dimension) and width (shortest dimension) were recorded for each particle as depicted in the table below. Each particle was categorized by form and shape. Color of each particle was also recorded and classified as Red, Orange, Brown, Yellow, Green, Blue, Violet, Grey, White, Black, or No color.

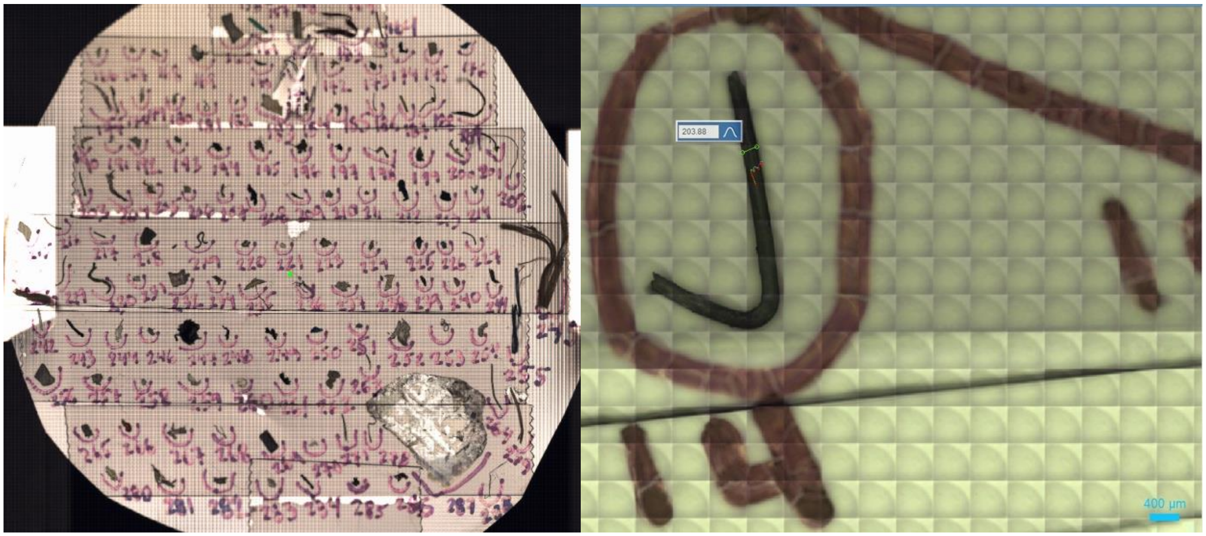
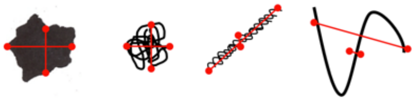
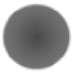
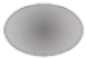


FIGURE 6. EXAMPLE OF DIGITAL MICROSCOPIC MOSAIC IMAGES OF LABELED AND MOUNTED SUSPECTED PLASTIC PARTICLES USED FOR PRECISE PARTICLE SIZE MEASUREMENT AND CHARACTERIZATION. EACH SMALL SQUARE MAKING UP THE LARGER MOSAIC REPRESENTS A SINGLE HIGH-RESOLUTION IMAGE TAKEN THROUGH THE MICROSCOPE OBJECTIVE.

Table 9. Size measurements and particle morphology were collected for each particle analyzed using Raman spectroscopy.

Method of Particle Measurement		
Length	(longest dimension)	
Width	(shortest dimension)	

MATERIALS AND METHODS
PARTICLE IDENTIFICATION AND CHARACTERIZATION

PARTICLE MORPHOLOGY		
Form	**Shape**	**Depiction/description**
Beads and Pellets	Spherical	
	Ovoid	
	Cylindrical	
	Cuboidal	
Fragments	Angular-shard	
	Angular	
	Subangular	
	Subrounded	
	Rounded	
	Granular	
	Amorphous/Irregular	Any other shape
Fibers and Filaments	Monofilament/ Fiber	Single, individual strand 
	Multifilament	Two or more strands or fibers twisted or braided together 
	Filament aggregate/ Fiber bundle	Tangled fibers with varied organization and orientation 
Other	Foam	Contains numerous air bubbles or pockets; compressible
	Film	Thin with relatively long and wide dimensions compared to thickness; often foldable

QUALITY CONTROL MEASURES

General Quality Control Measures

Due to potential for plastic contamination of samples, strict laboratory protocols and hygiene were used to preserve sample integrity and avoid background contamination. To reduce the likelihood of contamination from clothing, field personnel were instructed to avoid synthetic clothing (i.e. fleece) and wear natural materials (cotton and wool) when possible. Clean 100% cotton lab coats were worn onboard the vessel over clothing and at all times in the lab to minimize potential contamination. Nitrile gloves were worn during sample processing as well. All sampling equipment was pre-cleaned in the TERC labs prior to field work. Glass jars and other sampling equipment were be washed with Liquinox® soap and rinsed 8 times with pre-filtered DI water and a final rinse with pre-filtered 70% ethanol. Once clean, the jars remained sealed until the sample was added on the boat to avoid airborne sources of contamination.

Additional quality control measures unique to specific sample collections are described below.

Quality Control Measures - horizontal

De-ionized water passed through a 0.22 µm filter (Millipore Sigma, Millipak 0.22 µm, Cat. No. MPGP02001) and ethanol were pre-filtered with 0.8 µm filters (Millipore Sigma, Isopore™ PC Membrane Filter, Cat. No. ATTP04700) prior to lab use. Work surfaces were cleaned with 70% ethanol and paper towels prior to use. All sample jars for field collection and laboratory supplies were pre-cleaned with Liqui-Nox® soap (Alconox, Mfr. #1232-1), rinsed 8 times with tap water, 6 times with pre-filtered de-ionized water (for sample jars) or tap water (for laboratory supplies and equipment), and then rinsed once with pre-filtered 70% ethanol. Once clean, the jars remained sealed until sample collection and equipment was stored in clean containers or covered with fresh aluminum foil to prevent airborne sources of contamination between uses. The trawl net was cleaned with a high-power hose prior to each tow to remove any particles. The cod-end of the trawl net was removed and cleaned thoroughly in the lab to remove any particles, rinsed with pre-filtered de-ionized water, and stored in a clean container between uses. Samples, glassware, and supplies were also covered with aluminum foil during sample processing and preparation. When appropriate (where loss of sample particles due to air currents was not a concern), lab work was performed in a clean fume hood (Labconco, Protector Laboratory Hood, Cat. No. 72801001697).

Quality Control Measures - vertical tow

The Van Dorn samplers were pre-cleaned by running them through at least 10 m of the water column to “rinse” them immediately prior to each sampling event.

One field blank was collected during each quarterly sampling event to account for background contamination. The field blank was collected just prior to the start of water collections for the day and immediately after rinsing them by lowering and raising them through 10 m of the water column. After the initial rinse with lake water, 1 L of DI water was poured into the bottle and then shaken vigorously for 30 seconds with both ends closed in order to thoroughly rinse the inside of the bottle. The rinse water was disposed of and an additional one liter of pre-filtered DI water was added to the van Dorn to serve as the field blank. The blank was then poured out the side tubing and into a prepared sample jar. Field duplicates were collected at 15 m during each sampling event by collecting a second sample using identical methods. Individual van Dorn bottles were assigned to specific depths during the first sampling event and remained the same throughout the duration of the project. Staff recorded various physical parameters (e.g. surface water temperature, wind speed/direction, recent precipitation events, etc.) and notes on a field datasheet.

Quality Control Measures - sediment

All sample jars for field collection were pre-cleaned in the TERC labs prior to field work. Glass jars were washed with Liquinox® soap and rinsed 8 times with pre-filtered DI water and a final rinse with 70% ethanol. Once clean, the jars remained sealed until the sample was added.

No field blank was collected during the deep-water sediment sampling. One field replicate was collected using the same methods detailed above. The replicate sample was handled, stored and processed in the lab in the same manner as the sample.

Quality Control Measures - lake biota

To reduce the likelihood of contamination from clothing, fishing guides were instructed to avoid synthetic clothing (i.e. fleece) and wear natural materials (cotton and wool). All sampling equipment was pre-cleaned as described above and stored in sealed containers until use to avoid airborne or other sources of contamination.

Glass jars were washed with Liquinox® soap and rinsed 8 times with pre-filtered DI water and a final rinse with 70% isopropyl alcohol. Once clean, the jars remained sealed until the clams were added.

Quality Control Measures - municipal

All sample jars for field collection were pre-cleaned in the TERC labs prior to field work. Glass jars were washed with Liquinox® soap and rinsed 8 times with pre-filtered DI water and a final rinse with 70% ethanol. Once clean, the jars remained sealed until the sampling.

One field blank was collected at each pump house during every quarterly sampling event to account for background contamination. The field blanks were collected by filling a randomly selected pre-cleaned glass jar with 3.75 L DI water and leaving it open to exposure to ambient pump house conditions. One lab blank was also collected at the TERC labs by filling a pre-cleaned glass jar with 3.75 L of DI water. One field duplicate was also collected during each quarterly sampling event by taking an additional 3.75 L sample at one of the water treatment plants.

Quality Control Measures - Raman

Contamination was evaluated with field/lab blank samples that were concurrently processed with their corresponding field samples. Comparison of sample spectra to those from field blank particles was used to identify potential contaminant particles. Sample spectra were also compared to spectra from mounting tape to account for any interference from the sample substrate during Raman analysis. Both spectral quality (based on observed signal to noise ratio of obtained spectra) and confidence of identification based on the library match and presence of characteristic peaks for common plastics during identification were subjectively scored for each particle during analysis to ensure the quality of results.

RESULTS AND DISCUSSION - HORIZONTAL TOW SAMPLES

RESULTS OF HORIZONTAL TOW SAMPLES

Quantity of Suspected Plastic Particles and Confirmation of Plastic Composition

A total number of 8178 (monthly average of 682, SD 856) suspected plastic particles were collected by horizontal tow from surface and subsurface waters during the 12 monthly sampling dates during the period of 8/27/2020 - 8/4/2021. Of the particles collected, 7620 (93%) were collected from the lake surface and 558 (7%) were from subsurface water samples.

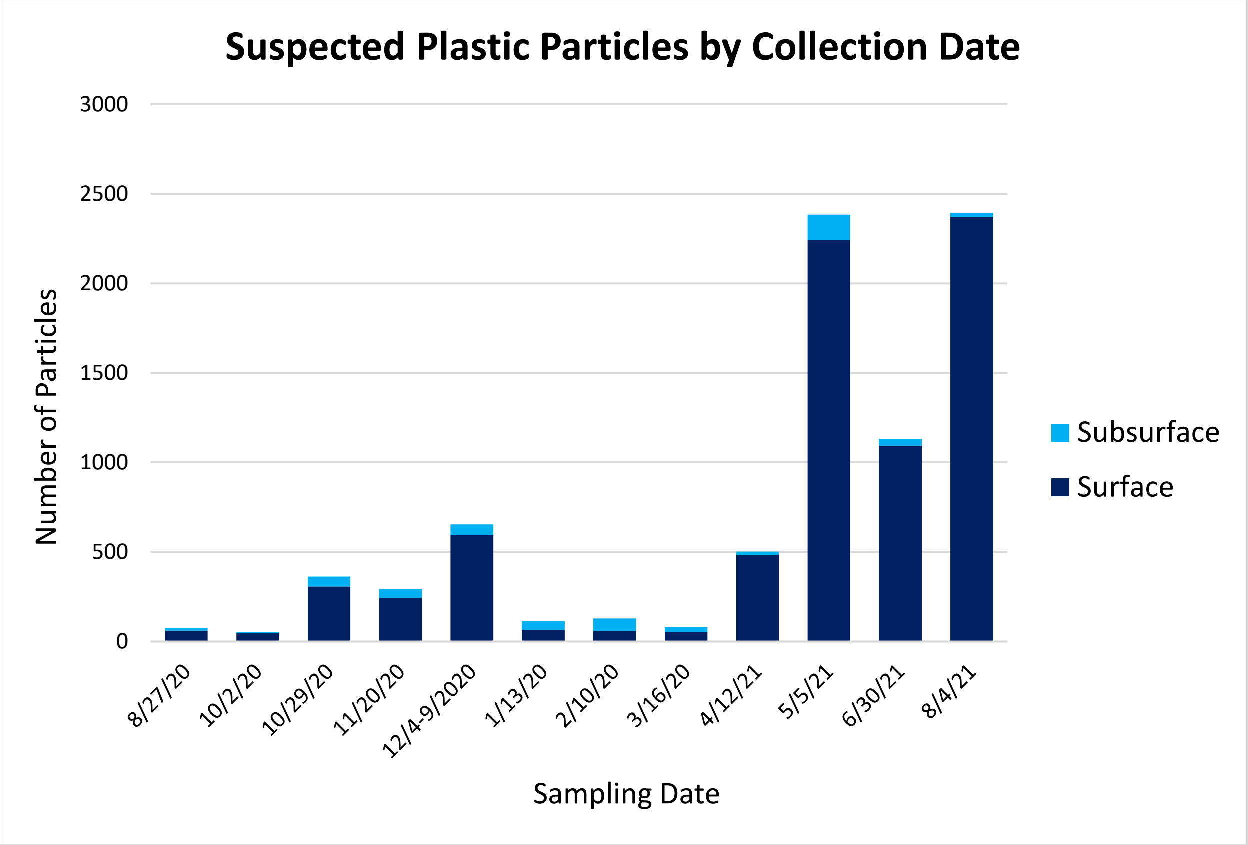


FIGURE 7. SUSPECTED MICROPLASTIC PARTICLES WERE COLLECTED FROM SURFACE AND SUBSURFACE WATERS OF LAKE TAHOE VIA TOW NET ON A MONTHLY BASIS DURING THE PERIOD OF 8/27/2020 TO 8/4/2021.

A randomly selected subset of 1682 suspected plastic particles (16% of the total number with a minimum of 10% from each sampling date) were analyzed via Raman spectroscopy to confirm the particle's chemical composition. Out of the analyzed particles, 81% (n=1356) were positively confirmed to be synthetic or partially synthetic polymers (composed of plastic).

RESULTS AND DISCUSSION - HORIZONTAL TOW SAMPLES

Table 9A. Results from Raman analysis of suspected plastic particles in Lake Tahoe's surface waters.

Microplastic Particles in Lake Tahoe Horizontal Tow of Surface Waters (0 m depth)				
Sampling Date	Suspected Plastic Particles Collected	Suspected Plastic Particles Analyzed	Particles Confirmed to be Microplastic	Microplastic Particles Matching Control Samples
	number	number (%)	number (%)	number (%)
8/27/2020	61	61 (100)	48 (79)	12 (25)
10/2/2020	46	44 (96)	32 (72)	2 (6)
10/29/2020	308	59 (19)	57 (97)	8 (14)
11/20/2020	244	62 (25)	58 (94)	2 (3)
12/4/2020	593	144 (24)	133 (92)	15 (11)
1/13/2021	64	64 (100)	39 (63)	1 (2)
2/10/2021	59	59 (100)	41 (69)	6 (15)
3/16/2021	53	53 (100)	25 (47)	1 (4)
4/12/2021	484	123 (25)	110 (89)	3 (3)
5/5/2021	2243	219 (10)	201 (92)	4 (2)
6/30/2021	1093	114 (10)	96 (84)	6 (6)
8/4/2021	2372	232 (10)	207 (89)	8 (4)
Total	7620	1234 (16)	1047 (89)	68 (6)

Table 9B. Results from Raman analysis of suspected plastic particles in Lake Tahoe's subsurface waters.

Microplastic Particles in Lake Tahoe Horizontal Tow of Subsurface Waters				
Sampling Date	Suspected Plastic Particles Collected	Suspected Plastic Particles Analyzed	Particles Confirmed to be Microplastic	Microplastic Particles Matching Control Samples
	number	number (%)	number (%)	number (%)
8/27/2020	16	16 (100)	1 (6)	1 (100)
10/2/2020	8	8 (100)	4 (50)	0 (0)
10/29/2020	55	53 (96)	49 (92)	4 (8)
11/20/2020	50	49 (98)	37 (76)	5 (14)
12/9/2020	61	59 (97)	47 (80)	7 (15)
1/13/2021	51	51 (100)	32 (63)	1 (3)
2/10/2021	69	67 (97)	53 (79)	1 (2)
3/16/2021	28	28 (100)	13 (46)	0 (2)
4/12/2021	18	18 (100)	11 (61)	1 (9)
5/5/2021	140	37 (26)	32 (86)	5 (16)
6/30/2021	39	39 (100)	21 (54)	3 (14)
8/4/2021	23	23 (100)	9 (39)	0 (0)
Total	558	448 (80)	309 (69)	28 (9)

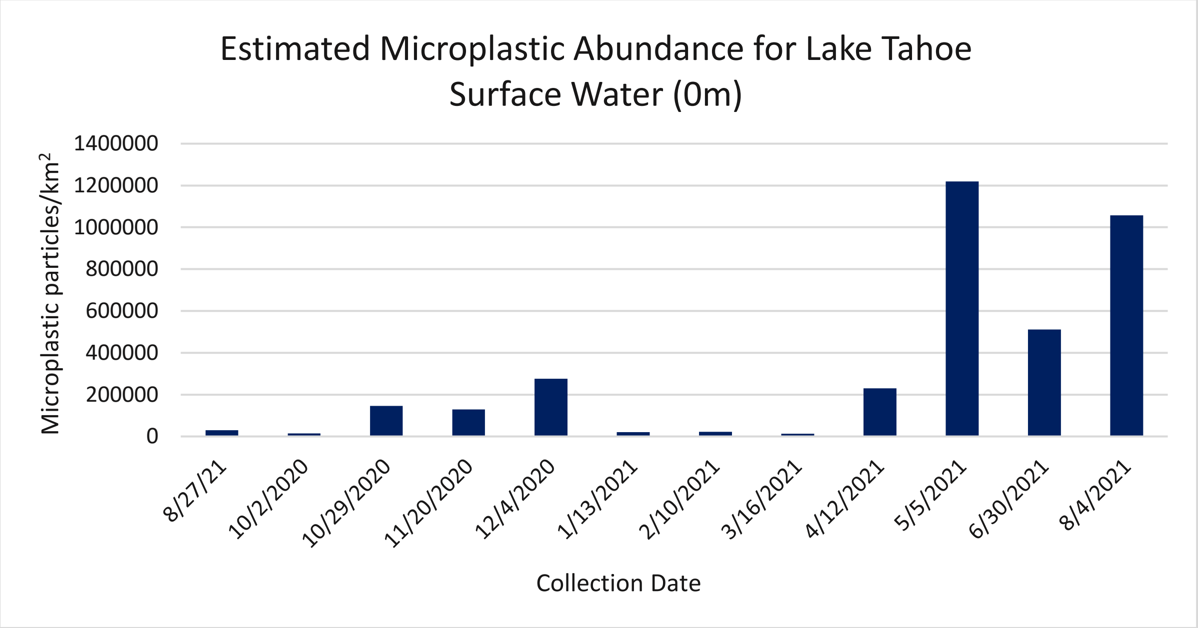
Microplastic Abundance

Estimated microplastic abundances in surface and subsurface waters of Lake Tahoe were calculated for each sampling date using the following formulas:

Microplastics/km^2 = (Total # Suspected Plastic Particles x % Confirmed Microplastics) / (Towing Distance x Trawl Net Width)

Microplastics/km^3 = (Total # Suspected Plastic Particles x % Confirmed Microplastics) / (Towing Distance x Trawl Net Area)

Assuming particles are evenly distributed throughout each water column plane, the average estimated abundance of microplastics at the lake surface (0m) was 306,044 (SD 417,012) microplastic particles/km^2 and 0.043 (SD 0.04) microplastic particles/km^3 in the lake's subsurface waters during the sampling period.



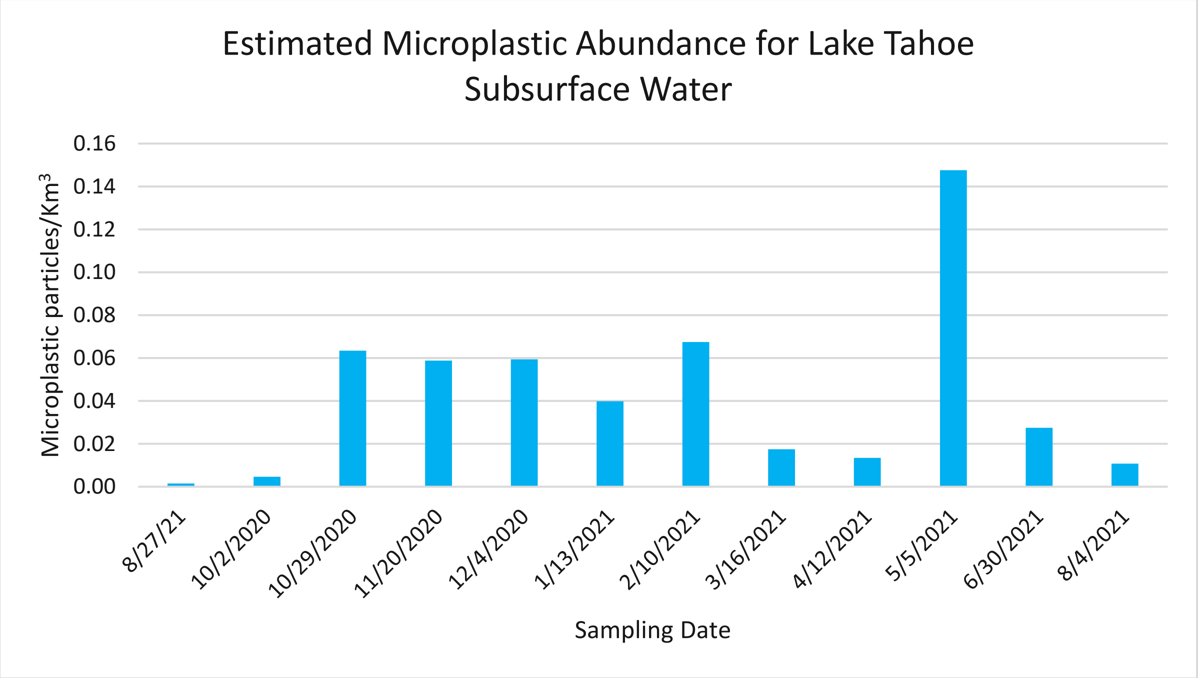


FIGURE 8. ESTIMATED MICROPLASTIC PARTICLE ABUNDANCE IN SURFACE AND SUBSURFACE WATERS OF LAKE TAHOE DURING THE PERIOD OF 8/27/2020 TO 8/4/2021.

Suspected Plastic Particle Classification

The randomly selected subset of 1682 suspect particles were classified into groups based on results from Raman spectroscopic analysis. Out of the analyzed particles, 81% (n=1356) were confirmed to be synthetic or partially synthetic polymers (composed of plastic), 11% (n= 191) were considered dye-prominent, 2% (n=34) were determined to be natural polymers (like cotton or plant material), and 6% (n=100) could not be classified/identified.

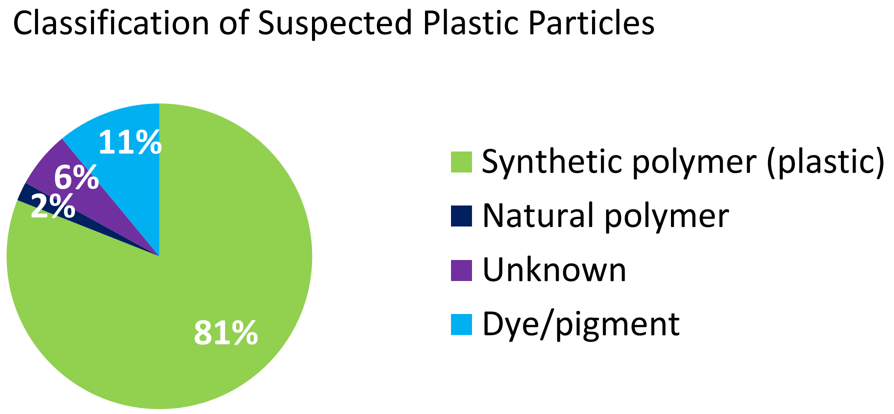


FIGURE 9. A SUBSET OF SUSPECTED PLASTIC PARTICLES COLLECTED FROM SURFACE WATERS OF LAKE TAHOE WERE CLASSIFIED BY PARTICLE COMPOSITION BASED ON RESULTS FROM RAMAN MICROSPECTROSCOPIC ANALYSIS.

Types of Microplastics

In addition to classifying particles as microplastics, the type of plastic composition for each microplastic particle was also determined using Raman microspectroscopy (Figure 10). The majority of analyzed plastic particles from surface waters were identified as polypropylene (41%) and polyethylene (39%) with a smaller proportion of particles identified as polyesters (15%). Additional synthetic polymers including polystyrene, nylon, acrylics, and co-polymer mixtures were also identified but made up less than 5% of all analyzed particles.

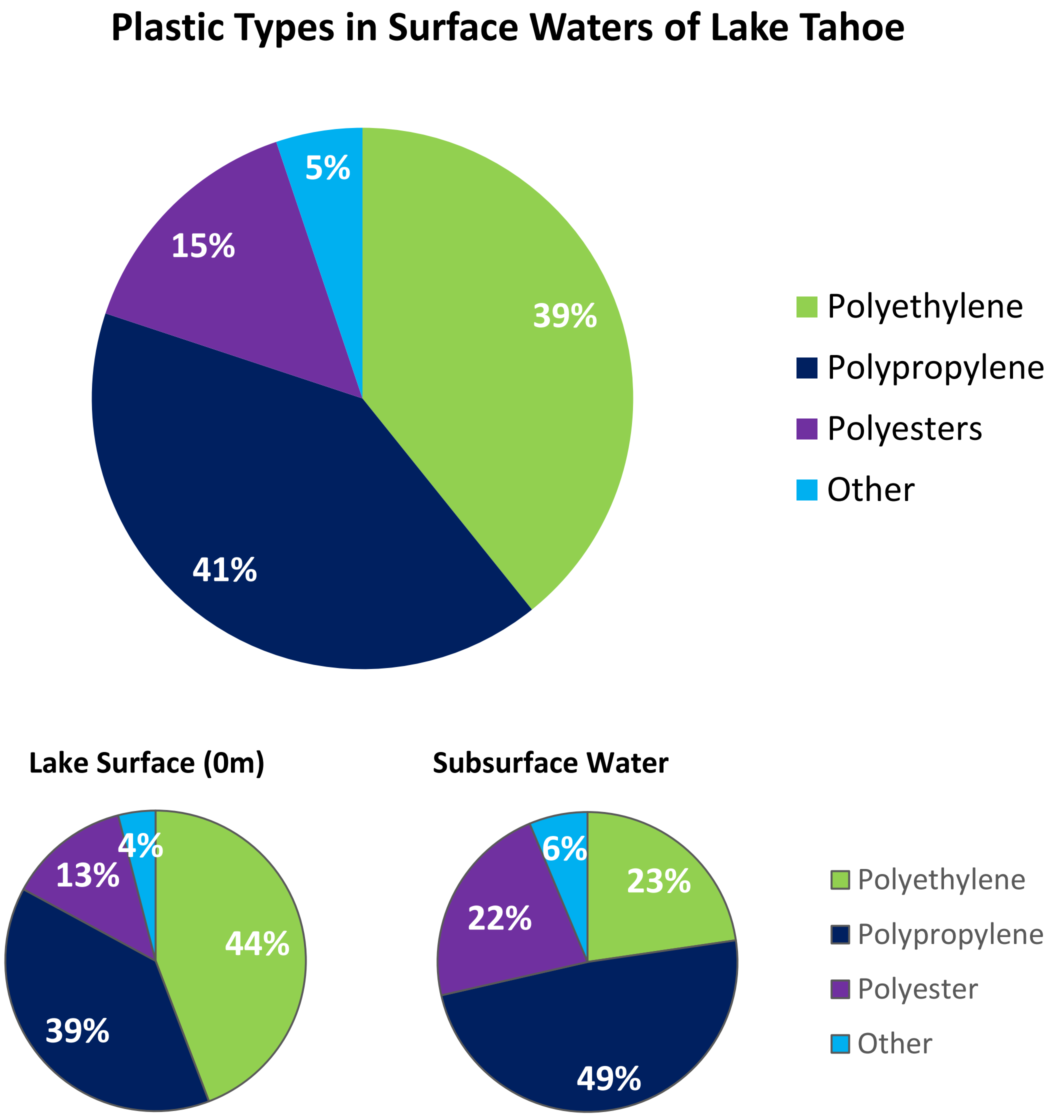


Figure 10. Synthetic Polymer (plastic) types of confirmed microplastic particles collected from surface and subsurface waters of Lake Tahoe. The majority of microplastics were identified as polyethylene and polypropylene. A larger proportion of polyethylene particles were collected from the lake's surface.

Plastic particle types from the water surface were compared to those collected from 20 m below the surface at the location of the lake's thermocline. A larger proportion of particles were identified as polyethylene (47%) at the lake's surface (0 m water depth) compared to those collected at 20 m below the surface. Particles collected at a water depth of 20 m below the surface was comprised of larger proportions of polypropylene and polyester particles compared to the surface of the lake.

The proportion of microplastic polymer types identified showed some variation between collection dates, although polyethylene, polypropylene, and polyester were consistently found to comprise the majority of all samples.

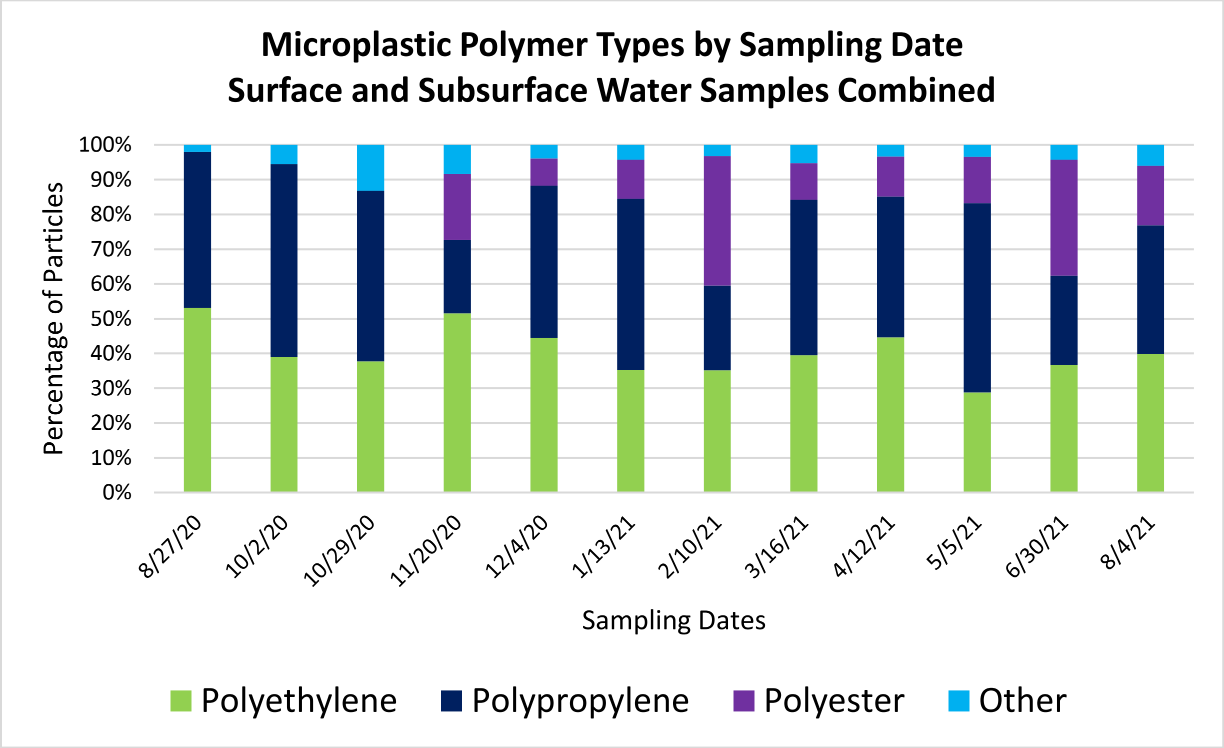


FIGURE 11. POLYMER TYPES WERE DETERMINED BY RAMAN MICROSPECTROSCOPY FOR A SUBSET OF MICROPLASTIC PARTICLES COLLECTED FROM HORIZONTAL NET TOWS OF LAKE TAHOE'S SURFACE AND SUBSURFACE WATERS.

Particle Characterization

A total of 1325 particles that had been confirmed to be microplastics were also characterized and categorized by shape, color, and size. Of these, 808 (61%) were fragments, 475 (36%) were filaments, 25 (2%) were films, 11 (1%) were beads or pellets, and 6 (<1%) were foam. The average particle length was 2.33mm (3.47 SD) and ranged from 159 µm to 5.9 cm. The average particle width was 591 µm (1258 SD) and ranged from 15 µm to 4 cm.

RESULTS AND DISCUSSION - HORIZONTAL TOW SAMPLES

Table 10. Particle morphology and size class of Raman validated microplastics from Lake Tahoe's surface and subsurface waters.

Shape and Size of Microplastics in Lake Tahoe					
Particle Shape	**All**	**0 m**	**Subsurface**	**Avg Length**	**Avg Width**
	number (%)	number (%)	number (%)	um (SD)	um (SD)
Fragment	808 (61)	669 (65)	139 (46)	1440 (1238)	727 (573)
Fiber/Filament	475 (36)	318 (31)	157 (53)	3868 (5204)	311 (1938)
Film	25 (2)	23 (3)	2 (1)	2966 (1749)	1333 (802)
Bead/Pellet	11 (1)	7 (1)	1 (0)	604 (168)	445 (111)
Foam	6 (<1)	6 (1)	0 (0)	1296 (849)	813 (632)
Total	1325	1023	1047 (89)	2330 (3470)	591 (1258)

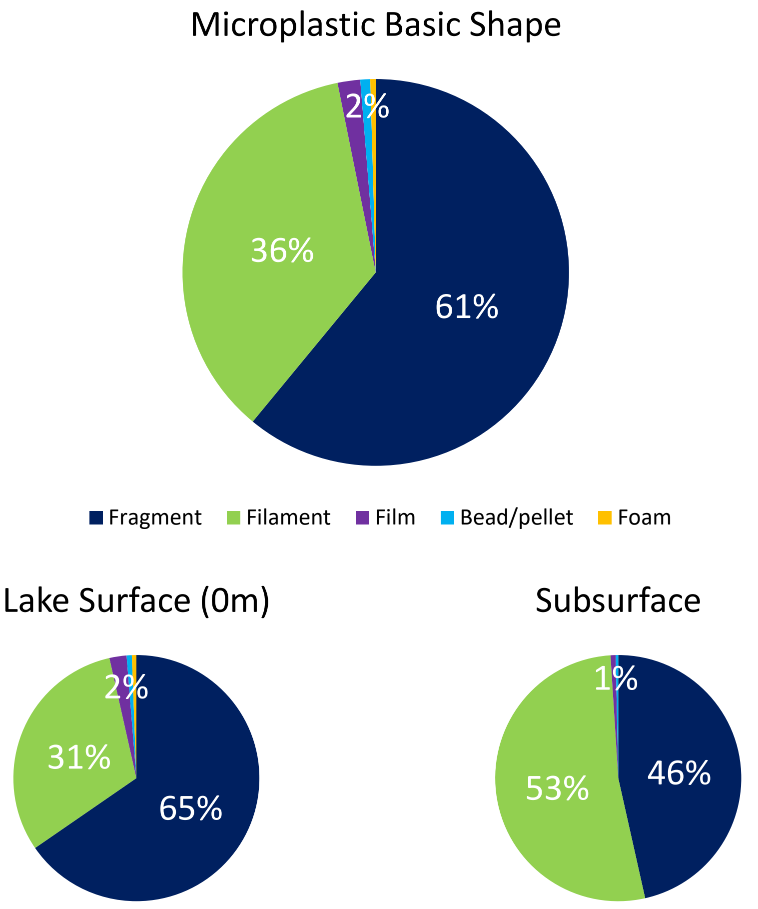


FIGURE 12: MORPHOLOGY OF MICROPLASTICS ISOLATED FROM THE SURFACE AND SUBSURFACE WATERS OF LAKE TAHOE. FRAGMENTS FOLLOWED BY FILAMENTS ARE THE DOMINANT MORPHOLOGIES OVERALL WITH FILMS, BEADS/PELLETS AND FOAMS ACCOUNTING FOR A SMALL FRACTION OF MICROPLASTICS.

RESULTS AND DISCUSSION - HORIZONTAL TOW SAMPLES

DISCUSSION OF HORIZONTAL TOW SAMPLE RESULTS

The microplastic abundance on the surface waters of Lake Tahoe are some of the highest reported amongst North American lakes (range: 13,000 – 1,220,000 particles/km^2, mean: 306,000 particles/km^2) although higher values have been report in other systems such as Lake Taihu, China (range: 10,000 – 6,800,000 particles/km^2) and the San Francisco Bay (range: 34,000 – 1,800,000 particles/km^2, mean: 390,000 particles/km^2) (Su et al., 2016; Sutton et al., 2019). A comparison of surface water microplastic abundance in Lake Tahoe and other large North American lakes is provided (Table 11). There are a number of factors which may contribute to this high abundance compared to other systems.

Table 11. Comparison of microplastic abundance in the surface waters of North American lakes.

Microplastic abundance in the surface waters of North American lakes						
	Lake Winnipeg	**Lake Superior**	**Lake Michigan**	**Lake Erie**	**Flathead Lake**	**Lake Tahoe**
Surface area (km^2)	25,514	82,100	58,030	25,744	510	490
Mean depth (m)	12	147	85	19	50	300
Residence time (years)	4	191	99	2.6	2.2	650
Population in watershed	7,000,000	600,000	12,000,000	12,000,000	121,000	40,000
Watershed area (km^2)	982,900	127,700	118,000	78,000	7,615	1,298
Wastewater treatment	30% combined, 70% sanitary with separate system for stormwater treatment	Combined	Combined	Combined	~70% of residents and business' on spectic systems	All wastewater removed from basin. No treatment of stormwater
Mean microplastic abundance (# per km^2)	193,420	30,000	17,276	105,503	189,000	306,044
Standard deviation	± 115,567			± 173,587		± 417,012
Dominate particle type	Fiber (90%)	Fiber (67%)	Fragment (79%)	Pellets (48%)	Fiber (79%)	Fragment (61%)
Dominate polymer	n/a	Polyethylene (51%)	Polyethylene (46%)	n/a	Polyethylene	Polyethylene (44%)
Sample collection	Manta trawl, 333 µm	Paired nueston net, 500 µm	Manta trawl, 333 µm	Manta trawl, 333 µm	Paneled trawling net, 330 µm	Manta trawl, 335 µm
Sample analysis	WPO digestion, SEM/EMS validation	WPO digestion, FTIR validation	WPO digestion, FTIR and SEM/EMS validation	Density separation, SEM/EMS validation	WPO digestion, Raman validation	Density separation, KOH digestion, Raman validation
Sample size (n=)	36	187	59	8	12	12
Citation	Anderson et al., 2017	Cox et al., 2021	Mason et al., 2016	Eriksen et al., 2013	Xiong et al., 2022	Present study

Sampling Methodology

The field of microplastics has long struggled with inconsistent sampling methods making it difficult to compare results across multiple studies (Tamminga et al., 2019). The studies in Table 11 were chosen for comparison because the methodology was most similar, although not identical, to our own. Larger mesh sizes (Cox et al., 2021) and the lack of sample digestion (Eriksen et al., 2013) may have caused an underestimation of microplastics in lakes Superior and Erie compared to what would have been detected using methods described in this study.

Treatment of Stormwater Effluent

Combined sewer systems are common in the Great Lakes watershed potentially preventing the release of microplastics collected from the landscape, into local waters. Combined sewer systems collect both household wastewater and stormwater runoff from rain and snowmelt for processing at a wastewater treatment plant (WWTP) prior to release back into the environment. While combined sewer systems can have a number of drawbacks, the most critical being the system can be overwhelmed by copious volumes of wastewater during large precipitation events causing untreated stormwater and wastewater to discharge directly into nearby waterbodies, they may still prevent many microplastics found in stormwater, from entering local waterways. Grbić et al. (2020) found anthropogenic particle concentrations in untreated stormwater runoff from the Lake Ontario watershed averaged 15.4 particles L^{-1}. There is currently no treatment system for stormwater in the Tahoe Basin prior to it flowing into the lake potentially contributing to a large microplastic load from a range of sources such as trash, rubber tire wear and road paint. Microplastics deposited by atmospheric deposition may also be a contributor.

As an initial step towards understanding factors that may influence the presence of microplastics in surface water of Lake Tahoe, data for environmental factors and human activities were obtained for the months during the study period (Figure 13). As a proxy measurement for snow melt, average monthly water discharge data for Ward Creek was obtained (located 7 km southwest of the horizontal tow sampling transect). Monthly average hotel room nights for South Lake Tahoe obtained from the Lake Tahoe Visitors Authority was used as an indicator of tourism activity in the vicinity of the lake. Monthly precipitation data for Tahoe City was obtained from the National Oceanic and Atmospheric Administration. Out of these factors, it appears there could be relationships related to runoff from precipitation and snow melt that warrant further investigation. Due to the unusual circumstances related to the COVID-19 Pandemic and its effect on the tourism industry during the period of this study, it is unclear whether there is any relationship between tourism activity in the region and the abundance of microplastics in Lake Tahoe.

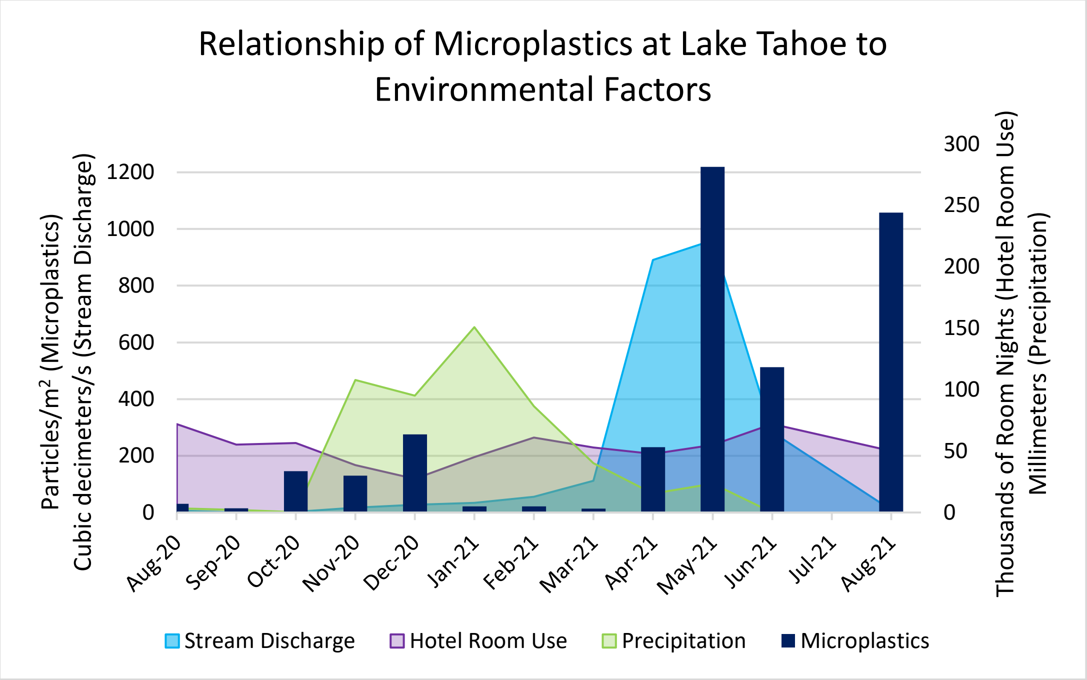


FIGURE 23. ESTIMATED MICROPLASTIC ABUNDANCE AT THE SURFACE OF LAKE TAHOE IS SHOWN OVERLAYED WITH DATA FOR STREAM DISCHARGE, HOTEL ROOM USE, AND PRECIPITATION FOR THE SAMPLING PERIOD AS A PRELIMINARY EXPLORATION INTO POTENTIAL RELATIONSHIPS BETWEEN MICROPLASTIC ABUNDANCE, SNOW MELT, TOURISM ACTIVITY, AND PRECIPITATION.

Tourism and Litter in the Tahoe Basin

The Tahoe Basin sees an enormous fluctuation in population throughout the year. 40,000 – 60,000 people reside year-round in the basin with 15 million visitors estimated as coming to the lake each year. During peak days, the Tahoe Basin can see a total population of 300,000 people putting immense pressure on local resources and intensifying anthropogenic impacts. The majority of microplastics removed from surface tow samples in the present study were identified as fragments (61%). Fragments are likely secondary microplastics created by the weathering and subsequent breakdown of anthropogenic trash which has been improperly disposed of. From 2014 to 2020, community beach clean ups conducted after popular summer holidays (e.g. Fourth of July, Labor Day, etc.) have removed over 48,500 lbs of trash from the shoreline of Lake Tahoe (The League to Save Lake Tahoe, 2022). Additionally, over 25,000 lbs of submerged anthropogenic waste was removed from Tahoe’s nearshore areas in 2020 – 2021 (Clean Up The Lake, 2022).

RESULTS - VERTICAL WATER SAMPLES

Quantity and types of plastic particles

A total number of 159 suspect particles were collected during vertical sample collection at depths of 0 m, 15 m, 30 m, 50 m, 250 m, and 450 m during 4 quarterly sampling dates between the period of 11/1/2020 - 8/31/2021. Of these particles, a total of 16% (n=25) were determined to be composed of plastic and 42% (n=12) particles were considered a match to particle spectra obtained from control samples (field and procedural blanks). These particles were excluded from further analysis and the particle abundance and plastic types present for each depth are detailed in the table below. Polyvinyl Chloride (PVC) was not frequently identified in surface water samples, but made up a larger proportion of the plastic particles in vertical water samples, particularly at deeper sampling depths.

Table 11. Abundance and polymer composition of microplastics found throughout the water column depth gradient in Lake Tahoe.

Microplastic particles at Different Water Depths of Lake Tahoe						
	Particles/L	**PE**	**PES**	**PP**	**PVC**	**Total**
0m	**0.87**	**2**	**1**	**0**	**0**	**3**
15m	**0.57**	**0**	**2**	**0**	**0**	**2**
30m	**0.00**	**0**	**0**	**0**	**0**	**0**
50m	**0.02**	**0**	**0**	**1**	**1**	**2**
250m	**1.40**	**1**	**2**	**2**	**0**	**5**
450m	**0.27**	**0**	**0**	**0**	**1**	**1**
All depths	**0.12**	**3**	**5**	**3**	**2**	**13**

RESULTS - VERTICAL WATER SAMPLES

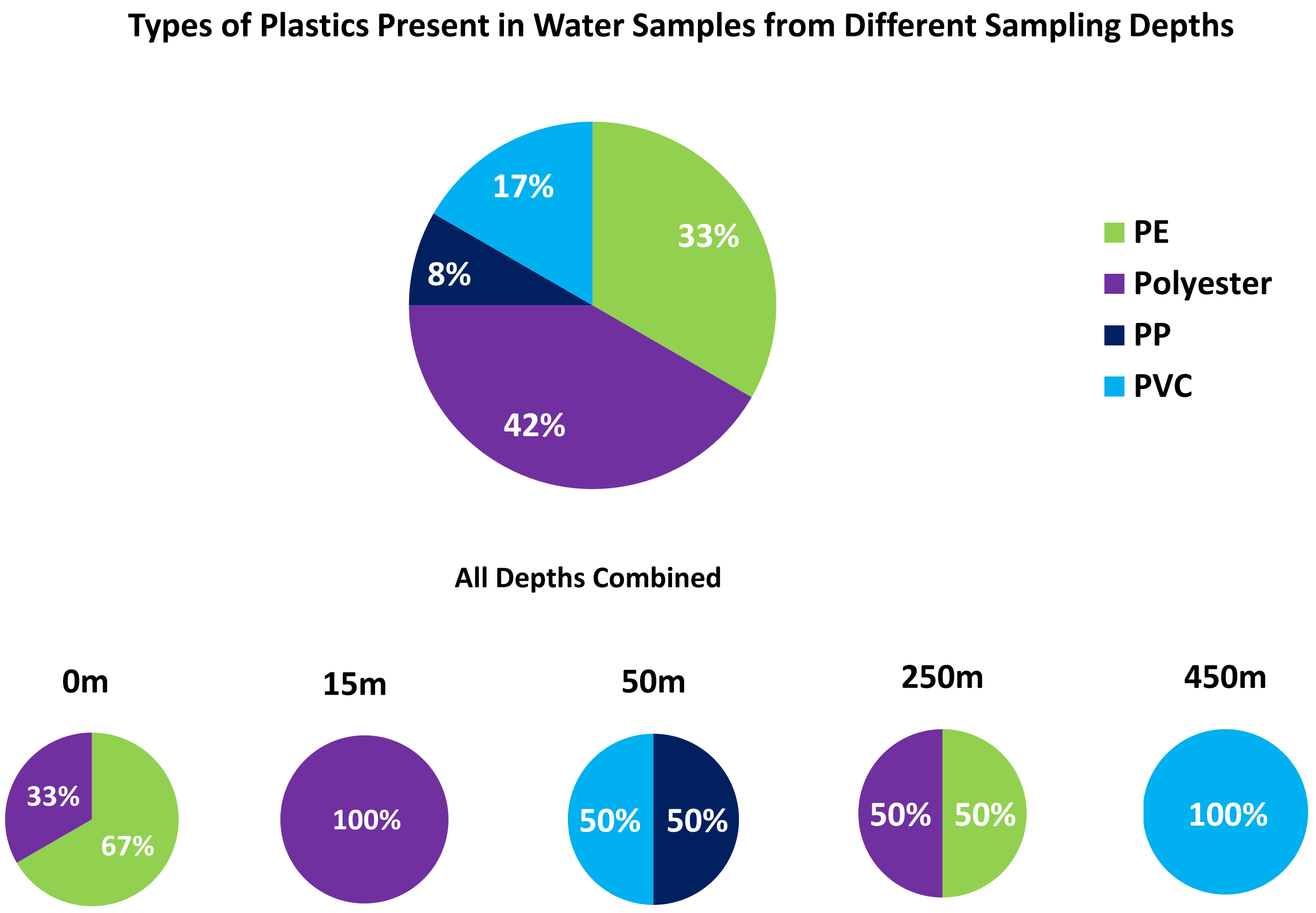


FIGURE 14. SYNTHETIC POLYMER (PLASTIC) TYPES OF CONFIRMED MICROPLASTIC PARTICLES COLLECTED FROM A VERTICAL DEPTH GRADIENT OF THE SUBSURFACE WATERS OF LAKE TAHOE. THE MAJORITY OF MICROPLASTICS WERE IDENTIFIED AS POLYETHYLENE AND POLYESTER. PVC AND POLYESTER REPRESENTED A LARGER PERCENTAGE OF PARTICLES AT DEPTH COMPARED WITH PARTICLES COLLECTED FROM SURFACE WATERS.

Discussion

Microplastics found in the vertical water profiles of Lake Tahoe showed denser polymers make up a larger proportion of microplastics found at depth. When considering only particles collected below the surface (15 m – 450 m), 60% of particles were composed of denser polymers (PES, PVC) while 40% were less dense (PP, PE). Lenaker et al. (2019) found similar results with water column (max depth: 13.7 m) and sediment sampling along the Milwaukee River Estuary to Lake Michigan. Polymer presence varied with depth as low density particle presence decreased with depth while high density polymers came more common with depth. It is likely denser particles would settle more quickly and the buoyancy of lighter particles would eventually change as they accumulate biofilm or algal growth that would increase overall density.

RESULTS - VERTICAL WATER SAMPLES

As with surface waters, many different methods have been utilized to quantify microplastics abundance in the vertical water column making it difficult to compare results from this study to others. This is made even more challenging by relatively few of these studies occurring in lakes. Two studies were identified with similar methodologies on Gehu Lake in China (average depth: 1.2 m, max sampling depth: 2.0 m) and Lake Tollense in northern Germany (max depth: 31 m, max sampling depth: 7 m). The present study found mean vertical water column abundance of microplastics at Lake Tahoe to be 0.12 particles/L or 120 particles/m^3. These results place Tahoe in line with Lake Tollense with mean vertical water column abundance of 41 to 71 particles/m^3 and sustainably lower than Gehu Lake in China with a mean microplastic abundance of 2.15 particles/L (Tamminga and Fischer, 2020; Yang et al., 2022).

RESULTS - SEDIMENT, BIOTA, MUNICIPAL SAMPLES

LAKE SEDIMENT

Quantity and types of plastic particles

A total number of 20 suspect particles were collected from a homogenized lake sediment sample (1.53 g dry weight). Three of the particles were determined to be composed of plastic and two particles were matched to particle spectra obtained from control samples (procedural blanks). These particles were excluded from further analysis. A single particle consisting of polypropylene was identified, suggesting an estimated 0.65 particles/g abundance of microplastics in sediment. A more robust sampling and evaluation of lake sediment would be required before any conclusions can be drawn about the amount and types of microplastics present in lake sediment.

LAKE TAHOE BIOTA

Quantity and types of plastic particles in Asian Clams

A total of 116 suspect particles were collected from 30 Asian clams (3 sets of 10 pooled clams). 12 particles were determined to be composed of plastic and 6 (50%) of these particles were matched to control sample spectra. All 6 of the microplastic particles recovered from the clam samples were composed of polypropylene.

Discussion

Su et al. (2018) collected Asian clams from lakes, rivers and estuarine waters of the Middle-Lower Yangtze River Basin and found an average abundance of 0.4 – 5.0 microplastics/clam. These values are higher than the 0.2 microplastics/clam found in Lake Tahoe's clams during the present study but not surprising given the microplastic abundance in the studies sample sites ranged from 0.5 – 3.1 items/L in water.

Due to their ability to concentrate and accumulate pollutants substantially above background levels, bivalves are valuable organisms for indicating levels of many pollutants in the environment (Boening, 1999). As Asian clams have invaded waterways worldwide Su et al. (2018) believes they have potential as a bioindicator for freshwater microplastic pollution. Additional work would need to be done to establish the correlation between microplastic abundance in surface waters, sediments and clams but based on the limited results from Su et al. and the present study, it may merit further investigation.

RESULTS - SEDIMENT, BIOTA, MUNICIPAL SAMPLES

Quantity and types of plastic particles in Kokanee Salmon

A total of 46 suspect particles were collected from 3 individually digested Kokanee Salmon gastrointestinal tracts. A total of 12 particles were composed of plastic, however, all of these particles were matched to control sample spectra indicating that all particles found were either non-plastic or could not be eliminated as a potential contaminant particle. A more robust sampling and evaluation would be required before any conclusions can be drawn about the amount and types of microplastics ingested by filter feeding fish such as Kokanee in Lake Tahoe.

MUNICIPAL WATER

A total of 155 suspected plastic particles were collected from municipal waters obtained via samples collected quarterly from two separate sites. A total of 19 particles were composed of plastic based on Raman microspectroscopic analysis. Out of these 19 particles, 84% (n=16) matched to control spectra obtained from blanks and background spectra. Three microplastic particles were composed of plastics not found in control samples. Two particles were composed of polypropylene and one particle was composed of polyester.

Microplastic Particles From Municipal Water Samples

Date	Site (# replicates)	Total Sample Volume (L)	Suspected plastic particles	Confirmed plastic particles	Microplastic abundance	Plastic type
		L	number	number	particles/L	
6/13/2021	Edgewood (3)	10.49	10	1	0.100	PP
	IVGID (2)	6.91	18	1	0.055	PP
8/24/2021	Edgewood (2)	7.45	8	0	0.000	
	IVGID (2)	7.38	10	0	0.000	
11/15/2021	Edgewood (3)	10.67	21	1	0.094	PES
	IVGID (2)	7.21	39	0	0.000	
2/9/2022	Edgewood (3)	10.99	38	0	0.000	
	IVGID (2)	7.34	11	0	0.000	
All dates	Edgewood (11)	39.6	77	2	0.050	
	IVGID (8)	28.84	78	1	0.035	
All dates	**All sites**	**68.44**	**155**	**3**	**0.044**	**PP, PES**

Discussion

Pivoknosky et al. (2018) monitored three water treatment plants in the Czech Republic for microplastic presence in treated drinking water using methods similar to those in the present study. A microplastic abundance of 338 ± 76 to 628 ± 28 particles L^{-1} was found in the treated water from those plants which is orders of magnitude greater than the results of this study. Additionally, Oßmann et al. (2018) found the amount of microplastics in bottled mineral water varied from 2649 ± 2857 per liter in single use PET bottles and up to 6292 ± 10521 per liter in glass bottles illustrating that packaging water has the potential to contribute a significant amount of microplastics to drinking water. One notable difference between the present study and the others discussed, is the lower size detection limit. Both studies are of the very few to determine microplastics down to the size of 1µm, while the lower size detection limit of the Lake Tahoe study is 10µm. Pivoknosky found microplastics smaller than 10µm were the most plentiful treated water samples, accounting for up to 95% while Oßmann concluded 90% of microplastics detected in bottled water were smaller than 5µm.

Additional research on microplastic abundance in drinking water sources is needed but monitoring microplastics in drinking water has struggled with lack of standardized methods as seen in other branches of the field. In May 2022, California's State Water Resources Control Board issued the world's first standard protocols for monitoring microplastics in drinking water (SWB-MP2-rev1) establishing a critically important standard for future research and monitoring programs to adhere to.

Beginning in 2023, water suppliers within the TWSA will begin mandatory water sample collections in compliance with the Fifth Unregulated Contaminant Monitoring Rule (URCM 5) established by the U.S. Environmental Protection Agency (EPA). URCM 5 requires nationwide monitoring for 29 per- and polyfluoroalkyl substances (PFAS) and lithium in public drinking water systems from 2023 – 2025. Microplastics, such as polytetrafluorethylene used as nonstick coating on cooking pans, can be composed of PFAS meaning they will be monitored in the municipal waters of Lake Tahoe under URCM 5. This monitoring is a critical first step but additional monitoring is recommended using protocols set forth by the California State Water Resources Control Board to understand microplastic presence in municipal water supplies for polymers that are not included under URCM 5.

RECOMMENDATIONS

This work has established the presence of microplastics throughout the water column of Lake Tahoe as well as in biota and municipal waters. It is not possible to say whether microplastics are increasing or decreasing. However, the data collected have established a baseline, one in which Tahoe is surprisingly high in microplastics relative to other water bodies. Additional data may indicate how large the year-to-year variability is in the short term.

Additional work with biota could be considered in the future as the amount of sampling in this project was not sufficient to come to strong conclusions. In particular zooplankton sampling for microplastics could be undertaken in the future. Because of the prey size of many Tahoe zooplankton, they could be an important pathway for removing accumulated microplastics.

Sediment results from this study were inconclusive so additional sampling may be warranted to understand polymer abundance and type potentially accumulating in Lake Tahoe. Given the variation in polymer density and ability to settle out of the water column, sampling sediment centered on stormwater inflows and urbanized tributaries in addition to mid lake sites may improve our understanding of microplastic abundance in lake sediments.

For any future microplastic research in Lake Tahoe, it is imperative to include analysis of smaller size classes, specifically the 1 – 10µm range. This work is critical as plastics continue to accumulate in our natural environment breaking into ever smaller pieces but not fully degrading. These smaller particles will impact lake clarity (the degree to which they do so is unknown until further research has been conducted). These small particles may also pose the greatest risk for accidental ingestion by humans and wildlife. The long-term health impacts of plastic consumption is an area of current research worldwide.

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