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Quantifying temporal and geographic variation in sunscreen and mineralogic titanium-containing nanoparticles in three recreational rivers

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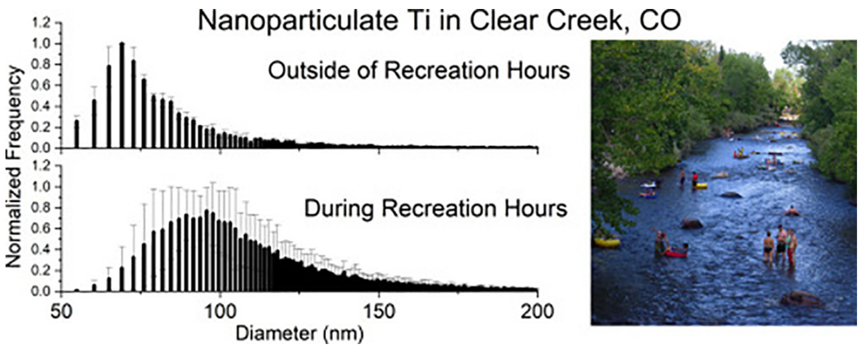
HIGHLIGHTS

- Nanomaterials in products, such as sunscreens, are released to the environment.
- Engineered nanoparticle detection is complicated by natural nanoparticle background.
- Recreational stream waters were sampled to examine the range and variability of Ti.
- Several instances of elevated Ti during recreation were statistically significant.
- Natural variation in suspended sediments affects engineered Ti detectability.

GRAPHICAL ABSTRACT



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ABSTRACT

Detection of metal nanoparticles (NPs) in the environment is an analytical challenge of interest due to increasing use of nanomaterials in consumer and industrial products. Detecting NPs associated with human activities is affected by both the magnitude and variation in background concentrations of natural NPs. In this work, we investigated the potential release of titanium dioxide (TiO_2) NPs from sunscreen in three recreational rivers, with a time-intensive sampling regime on one river, in order to determine the range and variability of natural, background titania (Ti). Conventional ICP analysis for total metal concentrations, single particle ICP-MS for NP concentrations, and electron microscopy aided in assessing mineralogical morphology and composition. Oxybenzone, a widely-used organic sunscreen, was measured and used as a surrogate for the intensity of recreational activity in the water. Statistically significant increases in Ti concentrations were observed in Clear Creek, CO during one recreation period, but the significance of other instances of recreation-associated Ti increases was unclear, in part due to storm impacts on the natural suspended sediment load of the stream. A comparison of three recreational rivers showed increases in both Ti mass concentrations and NP sizes occur during recreation in both Clear Creek, CO and the Salt River, AZ, but no detectable changes in the Truckee River, NV. However, size distributions were variable in background samples, which make the significance of differences observed during recreation unclear. These results underline that the release of engineered nanoparticles to a natural system cannot be detected without a well-defined background, including measures of its variability during the study period.

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1. Introduction and previous studies

Engineered TiO_2 NPs are effective UV filters(Popov et al., 2005; Serpone et al., 2007; Petrazzuoli, 2000) and widely used in commercial sunscreen products.(Botta et al., 2011; Johnson et al., 2011; Labille et al., 2010; Adams et al., 2006; Hanigan et al., 2018) The surfaces of TiO_2 ENPs in sunscreen are typically coated with Al or Si oxides, organic polymers, or some combination to mitigate TiO_2 toxicity(Adams et al., 2006; Dransfield et al., 2000; Brezová et al., 2005; Johnston et al., 2009) and to improve dispersion.(Dransfield et al., 2000) However, the coatings have been shown to readily break down upon release to surface waters and expose TiO_2 cores that may have intrinsic ecotoxicity.(Labille et al., 2010; Auffan et al., 2010) Concerns over the negative environmental implications of sunscreen ENPs motivate research on the fate and transport of sunscreen upon environmental release.

Various studies have attempted to examine release of sunscreen ENPs to natural water bodies as a result of recreational activities. (Holbrook et al., 2013; Gondikas et al., 2014; Reed et al., 2017; Venkatesan et al., 2018) In these scenarios it has proven challenging to differentiate Ti as having natural or engineered sources.(Gondikas et al., 2014; Reed et al., 2017; Venkatesan et al., 2018) This is a consequence of the presence of natural nanoparticles (NNPs) containing Ti and the expected low amounts of ENPs released. A prior study on Clear Creek in Golden, CO found elevated Ti and oxybenzone, a common active ingredient in non-ENP containing sunscreens, that coincide with human water recreation.(Reed et al., 2017) However, sediment resuspension was implicated by the both simultaneous elevation of Fe and Al, and by the much greater Ti concentration increase over the amount expected if ENPs were the only Ti source.

Studies of sunscreen ENP release have been limited by the reliance on total mass concentration (mg L^{-1}) measurements of the ENP element of interest in water (e.g. Ti) and generally lack particle-specific analyses.(Holbrook et al., 2013; Gondikas et al., 2014; Reed et al., 2017; Venkatesan et al., 2018) Most studies compare the mass concentration of the primary ENP element during stream recreation to a reference stream sample using standard ICP-MS analysis.(Reed et al., 2017) Unlike the organic active ingredients used in sunscreens, such as avobenzone and oxybenzone, Ti is present in the stream naturally and will thus be present in samples both upstream and downstream of recreation. Differentiating an input of anthropogenic sunscreen-derived Ti from Ti-containing NNPs is therefore challenging.

Other research areas in environmental nanoparticle analysis have taken different approaches to differentiate ENPs from NNPs. One study examined the quantifiability of CeO_2 ENPs in soil by comparing a soil spiked with CeO_2 to a non-spiked soil.(Praetorius et al., 2017) This allowed quantification of the minimum detectable CeO_2 above the natural Ce level in that specific soil. Another approach is to compare the elemental ratios of mass concentrations.(Gondikas et al., 2014; Viers et al., 2009; von der Kammer et al., 2012) The addition of an ENP containing a specific ratio of elements would be expected to change the naturally occurring elemental ratios of the sample, as long as the NNPs present are different from the ENPs added. However, natural variation in elemental ratios, a known problem for using Ti:Al ratios, made this approach unsuccessful in the prior study on sunscreen in Clear Creek.(Reed et al., 2017)

Alternatively, ENPs may be observed directly with a particle-specific analysis technique, rather than inferred from total mass concentrations. (Hassellöv et al., 2008; Zänker and Schierz, 2012; Montaño et al., 2016) This could be the detection of uniquely-sized particles by ICP-MS in single particle mode or the observation by electron microscopy of a unique morphology characteristic of the ENP. Single particle ICP-MS (spICP-MS) is especially useful because it can rapidly determine element-specific nanoparticle (NP) number concentrations and size distributions in addition to the total mass concentration at very low (parts-per-trillion) levels.(Montaño et al., 2016; Degueldre and Favarger, 2003; Pace et al., 2011) An ENP input to a natural water could be detectable using this method based on changes to the number concentration or size distribution of NNPs in the reference sample. However, spICP-MS is challenging to apply to samples with high dissolved concentrations or complex matrices that result in a high background signal. Assumptions regarding elemental composition can result in experimental artifacts when the actual composition of the sample NPs is unknown. (Reed et al., 2012; Schwertfeger et al., 2016) This limitation may be overcome with the development of multi-element detection capabilities (time-of-flight and dual element quadrupole mass analyzers), but such instrumentation is not yet widely available.(Loosli et al., 2019)

Sampling in similar published work is often infrequent and lacking replicates.(Holbrook et al., 2013; Gondikas et al., 2014; Reed et al., 2017; Venkatesan et al., 2018) However, observing an elevated level of the NP-associated element in a single sample as compared to a single reference or control is often insufficient. Concentrations in the sample of interest must significantly exceed the range of background values to support the argument the ENPs are present. Correspondingly, when lacking distinguishing ENP characteristics (specific size, elemental composition, and morphology),(Montano et al., 2014) consideration of the variability (temporal and/or spatial) in the NP population may represent a pathway forward in order to detect a significant perturbation from background. Understanding uncertainties in temporal variations is key to determining what amount of ENP input to natural systems would be detectable.

These concerns motivated this study of sunscreen ENPs which more heavily emphasized sampling variability and particle-specific methodology. Collaboration provided an opportunity for sampling to be extended to two additional waterways with recreational activity: the Truckee River in Reno, NV and the Salt River near Phoenix, AZ. Temporal and spatial variability, and reproducibility were examined. We hypothesized that with greater sampling frequency and replication, Ti and oxybenzone concentrations during periods of recreational activity over the Labor Day holiday weekend would be found to be statistically different from naturally occurring background concentrations. An additional hypothesis was that elevated Ti observed during recreation in Clear Creek would occur in other streams with similar recreational activity. Total mass concentrations, particle-specific analyses (spICP-MS and TEM-EDS), and sampling schemes resulted in more certainty as to the differentiation of anthropogenic and natural NP signals.

2. Materials and methods

2.1. Temporal study sampling protocol (2016)

Sampling of Clear Creek in Golden, CO was conducted from Friday September 2 to Friday September 9, 2016. Automated ISCO™ samplers were placed 1.5 km upstream (bridge site) from the main recreational areas, and at a site immediately downstream of the recreation (downstream site). The downstream site was located immediately upstream of the beach where people using flotation tubes leave the creek (see map in SI Fig. S.1). In the intermediate reaches of the stream the water is very shallow (<1 m) and wading is a popular activity. The ISCO™ samplers were programmed to withdraw samples at variable intervals, described subsequently. The sampler intake was positioned ~10 cm below the water surface. Water samples were stored in HDPE plastic bottles within the ISCO™ sampler until being transferred into sample splits for refrigerated (4 °C) storage and laboratory analyses.

Both ISCO™ samplers were programmed to sample at high, medium, and low frequency depending on the amount of recreation use in stream, with the goal of achieving better temporal resolution of chemical peaks associated with bathing. High frequency sampling: during peak bathing periods (2–5 pm Saturday, Sunday, and Labor Day Monday), a 1 L sample was taken every 10 min. Medium frequency sampling: during daytime hours with no/low bathing on Saturday, Sunday, and Monday, a 1 L sample was taken from the creek every hour. Low frequency sampling: during Friday 9/2, at nighttime Saturday, Sunday, and Monday, and all day each of the following weekdays, a 250 mL sample was withdrawn every hour and combined with the next three 250 mL samples to make 4-hour composite samples of 1 L.

The bridge site was intended to serve as a reference and had been used in the previous study by Reed et al. (2017). However, in the 2016 study described here, many bathers were observed entering the creek directly above the bridge site during recreation times. This prompted the collection of additional grab samples on Saturday, Sunday, and Monday from a site 2 km upstream from the Bridge site. These additional samples help to establish the natural background during recreation but provide limited temporal information. These samples are denoted as upstream (as opposed to bridge) samples.

2.2. Geographic study sampling protocol (2017)

To explore reproducibility and geographic variability, three locations were sampled on Sunday September 3 through Wednesday September 6, 2017: Clear Creek, CO, Salt River, AZ, and Truckee River, NV (site descriptions in SI). Water pH, conductivity (mS cm^{-1}), and alkalinity (mg $CaCO_3$ L^{-1}) of the river water were measured on site during sampling (details given in SI). Reported values for depth and discharge rates (m^3 s^{-1}) were obtained from the USGS National Water Information System.

Grab samples for metals (mg L^{-1}) and NPs analysis were collected in triplicate in 250-mL HDPE bottles. Sample collection times were 8 am and 4 pm, at both upstream and downstream locations. Grab samples for oxybenzone analysis were collected in triplicate using 500-mL amber glass bottles. Since prior results for Clear Creek have shown no oxybenzone present at non-recreational times, samples were collected at 4 pm at only the downstream location. At the 4 pm sampling time each day, the number of people currently in the water were counted at each downstream river site.

An estimation of the number of bathers was determined at each site in order to compare relative recreation use between the rivers. At Truckee River and Clear Creek, the number of bathers in the stream between the upstream and downstream locations were counted with a clicker counter at 4 pm. The length between the upstream and downstream locations was much greater and not walkable at the Salt River, so instead the number of bathers visible at the downstream location during afternoon sampling were counted.

2.3. Sample processing

In 2016 after each sampling day, the 1 L samples collected by the ISCO samplers were transferred into the following sample splits: 500 mL into amber glass bottles for oxybenzone analysis, 10 mL into HDPE Falcon™ tubes for total metals analysis, and 250 mL HDPE bottles for NPs analysis and archiving.

In 2017, all 250 mL grab samples for metals and NPs analysis were shipped to Colorado School of Mines for analysis as soon as possible, generally within 24 h of the final sample collection. Sample bottles were shaken, then 10 mL was poured into 15-mL Falcon™ tubes. 30 mL was withdrawn via polypropylene syringe and filtered through a 0.02 μm (Anotop) filter, discarding the first 20 mL as a rinse and collecting the remaining 10 mL filtrate into a 15-mL Falcon™ tube. All samples were stored refrigerated until shipment and/or analysis.

2.4. Laboratory analyses

The 10 mL unfiltered and filtered sample splits were acidified with several drops of concentrated nitric acid prior to shipment at room temperature to the U.S. Army Corps of Engineers Environmental Laboratory for total metals analysis. Metals concentrations were determined using ICP-AES (PerkinElmer Optima 8300DV ICP-AES) or ICP-MS (PerkinElmer NexION 300D ICP-MS), as appropriate for expected concentration ranges, following EPA methods 6010C and 6020A (*Test Methods for Evaluating Solid Waste: Physical/Chemical Methods (SW-846), 2007*). Calibration was with NIST-traceable standards (SPEX CertiPrep and CPI International). Quality control utilized Sc, Ge, Y, Rh, Tb, and Ho internal standards, which varied less than 15% during the analysis. Continuing calibration verification and independent second-source verification standards were also analyzed regularly during each batch and each analyte was within 10% of the certified value.

The 500-mL samples for oxybenzone analysis were bubble-wrapped and shipped on ice overnight to the U.S. Army Corps of Engineers Environmental Laboratory. Samples were first pre-concentrated by solid phase extraction, then analyzed for oxybenzone via high performance liquid chromatography (HPLC) with UV absorbance detection at 315 and 365 nm as described by Peck (2006). Separation of the analyte was carried out on an Agilent 1200 HPLC equipped with a Phenomonex Synergi 4u Hydro-RP 80A column and using an isocratic mobile phase of 90:10 methanol:water at a flow of 1 mL/min. Calibration was achieved with standards purchased from U.S. Pharmacopeia. Continuing calibration verification and independent second-source verification standards were also analyzed regularly during each batch with analyte recovery within 10% of the certified value.

Selected samples were shaken and analyzed at Colorado School of Mines for ^{49}Ti NPs using a PerkinElmer NexION 300D ICP-MS operated in single particle mode with 100-μs dwell times. The nano application module in the PerkinElmer Syngistix software differentiated NP pulses from background signal via a 5σ statistical algorithm, computed NP sizes using the bulk density and Ti mass fraction of TiO_2, and generated NP number concentrations and size histograms.

The 2017 samples from the Salt River, the Truckee River, and Clear Creek downstream Monday afternoon (9/4/2017) were characterized for particle morphology, size, and elemental composition by TEM-EDS. After bath sonication for 30 min, 50 mL of collected river samples were directly centrifuged for 2 h at ~3000*g* onto 200 mesh carbon type B (Ted Pella) TEM grid as described previously.(Kaegi et al., 2010) TEM grids were placed on the flat bottom of 50 mL centrifuge tubes and a swinging bucket rotor was used (Eppendorf Centrifuge 5810). The applied centrifugation conditions should result in total removal from the water column height (5 cm) for >20 nm TiO_2 NPs. After the supernatant was decanted, the grids were collected and dried under ambient conditions. The TEM grids were washed with a drop of distilled water to remove the dissolved components. The TEM samples were then transported to Arizona State University for analysis on a JEOL 2010F TEM operated at an acceleration voltage of 200 kV coupled with energy dispersive X-ray spectroscopy (EDS).

All statistical calculations of concentrations of total mass (mg L^{-1}) and NPs (# L^{-1}) were carried out in OriginPro 2018, with the add-in app “Post-hoc Analysis for Non-parametric Tests”.

3. Results and discussion

3.1. Temporal variability of metal and oxybenzone concentrations

In Clear Creek, CO, summer thunderstorms can more than double the daily stream flow and impact the suspended sediment loads accordingly. The 2016 sampling campaign was affected by a storm that occurred immediately before the study. This resulted in elevated streamflow (4 m^3 s^{-1} on Thursday August 31) that gradually returned to baseflow (2.5 m^3 s^{-1}) over the following week, and is the likely cause of a noticeably higher background of Ti and Al in the first few days of the study (Fig. 1a). In spite of this higher background, samples collected downstream during recreation (2–5 pm on Saturday, Sunday, and Labor Day Monday – a national holiday when many people recreate outdoors) contained Ti and Al up to three times their morning concentrations. Simultaneous increases in oxybenzone concentrations were observed during recreation, whereas most oxybenzone measurements outside of recreational periods were below the detection limit (over half of the oxybenzone samples were lost due to bottle breakage during

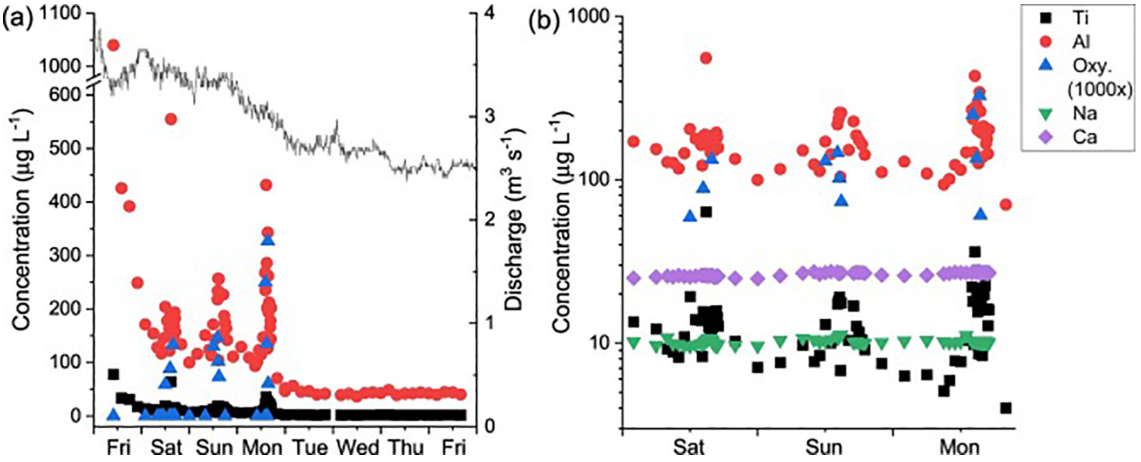

Fig. 1. Clear Creek downstream unfiltered samples 9/2–9/9/2016. Oxybenzone data are shown at 1000 times the measured concentrations for clarity. (a) Concentrations (colored markers) and USGS flow rates measured at 15-min intervals (solid line). (b) Weekend-only concentrations, including Na and Ca, excluding oxybenzone samples that were below detection (0.026 μg L^{-1}). The weekend metal concentrations were affected by a storm early during sampling and diurnal cycling during recreation is only visible for metals that are particle-associated, rather than dissolved.

shipment, resulting in fewer observations of this analyte). These recreation-associated increases in oxybenzone as well as Ti and Al concur with diurnal cycling previously reported by Reed, et al. (Reed et al., 2017) In contrast, there is no evidence of variation of dissolved elements, with Na and Ca being key examples (Fig. 1b).

The Ti and oxybenzone data were grouped by site, day, and recreation use (Fig. 2) to investigate the impact of background variability on the statistical significance of elevated downstream concentrations. The recreational periods were defined as 2–5 pm Saturday, Sunday, and Monday, based on the times that most people were observed entering the stream. The high frequency of sampling (every 10 min) provided data to investigate variations occurring on time scales much shorter than the hourly/daily resolution obtained in prior studies (Botta et al., 2011; Johnson et al., 2011; Holbrook et al., 2013; Gondikas et al., 2014; Reed et al., 2017; Venkatesan et al., 2018). The bridge site was intended to serve as a control but was just downstream of where some bathers were observed to be entering the stream, and thus was also impacted by recreational bathing. To separate bridge samples that could serve as a background control, the dataset was treated with two grouping methods (Fig. 2): "All bridge" includes all samples collected at this site. "Bridge without Friday and recreation" includes all samples except those collected on Friday 9/2 (to avoid the immediate effects of the storm) and samples from recreational periods (2–5 pm). This allows many of the bridge data to still serve as a control group. The small sample group collected upstream of all recreation activity during recreational periods are shown as a single group. Nearly all samples collected outside of recreating periods had oxybenzone levels below the detection limit, supporting that sunscreen release is minimal when bathers are not present. The occasional elevated oxybenzone in the Saturday, Sunday, and Monday periods outside of high recreational activity are likely attributable to a few bathers that were observed entering the creek in the early afternoon.

The Friday Ti mass concentrations are elevated compared to other samples and concentrations were more variable across the samples (Fig. 2a), resulting from the large concentration decrease over Friday from the storm on Thursday (Fig. 1a). Friday was relatively cool and overcast (see SI); no bathers were observed. The Friday median was 32 μg Ti L^{-1} and decreased steadily to below 10 μg Ti L^{-1} over the Saturday, Sunday, and Monday non-recreation periods. The decreasing discharge shown in Fig. 1a indicates that the stream did not return to normal flow conditions until the middle of the following week. The gradual decrease in naturally perturbed Ti over the weekend tracks with this decrease in discharge, supporting that it is associated with re-suspension by increased flow.

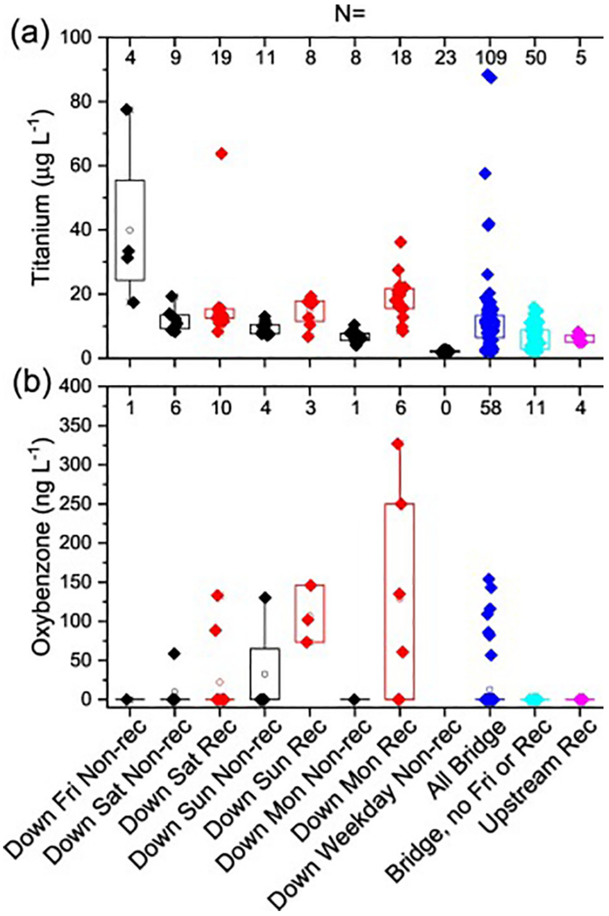

Fig. 2. Ti (a) and oxybenzone (b) concentrations from Labor Day week (Sept 2–9), 2016. Downstream data are grouped by day and recreational activity at the time of sampling (black, low or no recreational activity; red, high activity). Bridge data are grouped together (blue) and for times that exclude the Friday 9/2 rainfall event and the recreational periods (cyan). Upstream data (magenta) are for four samples collected Saturday, Sunday, and Monday only. Top x-axis indicates the number of observations in each group. "Downstream Monday recreation" is significantly different from "downstream Monday non-recreation" and "upstream recreation".

Table 1
Summarized findings from statistical pairwise comparisons between grouped data, analyzed by Dunn's Test at the 0.05 significance level. "NR" designates samples that were collected at non-recreational (background) periods while "R" designates samples collected while recreation was occurring in the stream.

Downstream Sample Group	Differs From Control Group	Explanation Discussed
Mon R	Upstream R Downstream Weekday NR Bridge-no Fri or R	Recreational impact to Ti levels
Sat R	Downstream Weekday NR Bridge-no Fri or R	
Sun R		
Sat NR	Downstream Weekday NR	Rain event on Thurs preceding study elevated background Ti
Sun NR		

Statistical analyses ($\alpha = 0.05$) were conducted to evaluate the differences between recreational and non-recreational periods at the downstream site as well as bridge and upstream site data shown in Fig. 2. After initial descriptive statistics (see SI), Dunn's pairwise test for equality of means was conducted to determine which groups in Fig. 2 differ significantly from one another. Loads computed from concentrations multiplied by discharge were examined in the same manner, and the results supported conclusions drawn from the concentration data and statistical analysis. The oxybenzone data were not examined with pairwise comparisons due to the low number of observations. All *p*-values from these pairwise comparisons are shown in the SI. Significant results are summarized in Fig. 2 and Table 1.

Monday downstream samples differed significantly from all grouped background data. This strongly supports that human activity perturbed the natural levels of suspended Ti on that day. However, there is less evidence for a recreational effect on Ti concentrations downstream on Saturday and Sunday afternoons. Downstream Saturday and Sunday recreation samples differ significantly only from some of the background controls (Table S.3). This is likely because Saturday and Sunday Ti levels were naturally higher before any human activity took place, obscuring recreation impacts.

Downstream non-recreation samples collected Saturday and Sunday differ significantly from samples collected at the same site on weekdays. This is clear evidence that the downstream background levels of Ti changed substantially over the study period. After the Thursday rain event increased the natural sediment loading in the creek, this elevated background took multiple days to return to base concentrations and complicates the differentiation of any recreation impacts on those days.

There was high variability among the 10-min samples during recreation hours for both Ti and oxybenzone (Fig. 2, red series). While many recreation concentrations were elevated, many were similar to non-recreational periods. Saturday, Sunday, and Monday non-recreation groups mostly had less variability in concentrations than the recreation groups (Table S.2). The distributions of the "downstream weekday non-recreation" data from after the storm impact are particularly narrow, having a coefficient of variation of 0.157, reduced from 0.291 for Monday non-recreation, implying minimal natural variation occurred after the effects of the storm passed. Apart from Friday 9/2, the effects of human activity on Ti, Al, and oxybenzone concentrations appear to cause far more variation than is naturally present in the stream. Additionally, this variability between samples collected only 10 min apart from each other was only observed in concentrations of metals expected to be particle associated, while dissolved metals, such as Na and Ca, were nearly constant across the study and within recreation hours (Fig. 1b).

3.2. Geographic variability of metal and oxybenzone concentrations

The 2017 sampling campaign examining Ti in three recreational rivers showed that the recreation-associated elevation of Ti and oxybenzone observed in Clear Creek was also visible in the Salt River and, to a lesser extent, the Truckee River (Fig. 3). Of the three streams in this sampling year, Clear Creek exhibited the highest peaks in oxybenzone and Ti. Clear Creek oxybenzone and Ti concentrations were higher on Sunday than Monday afternoon while Salt River's maximum oxybenzone concentrations occurred on Monday afternoon and maximum Ti concentrations occurred on Sunday afternoon. Elevated concentrations were not observed on Tuesday or Wednesday, consistent with the low counts of people in the streams. Two people were

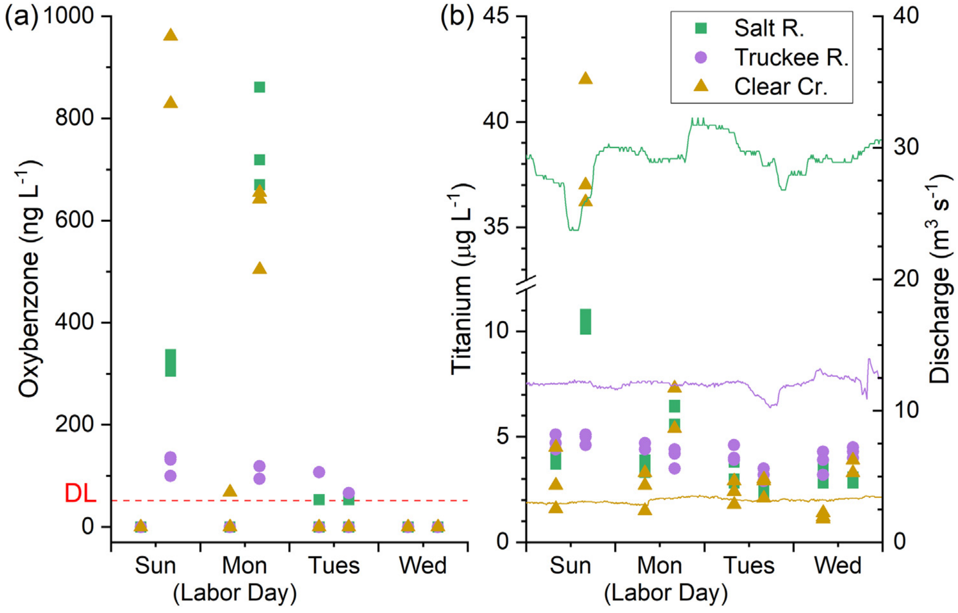

Fig. 3. Oxybenzone (a) and Ti (b) concentrations in each triplicate sample from the Salt River, Truckee River, and Clear Creek during the week of Labor Day, 2017. Samples were collected downstream of the recreational sections of the streams. Discharge (green, purple, and gold solid lines for Salt River, Truckee River, and Clear Cree, respectively) of all three streams is shown in (b). Individual triplicates are shown at morning and evening sampling times. Oxybenzone was elevated during recreation (Sunday and Monday afternoons) in all three streams, but only just above the detection limit in the Truckee River. Only Salt River and Clear Creek exhibited elevated Ti during recreation.

observed in both Salt and Truckee Rivers at the time of sampling and no people were observed in Clear Creek on those days.

Due to the small sample sizes, it was not possible to compare data grouped into separate days as was done in the temporal variability campaign (Section 3.1). Instead, all data for each river were simply grouped into a recreation versus non-recreation category and compared using a two-sample Kolmogorov-Smirnov test ($\alpha = 0.05$). The oxybenzone in all rivers was significantly different during recreation versus non-recreation. The Ti, however, only differed significantly during recreation in the Salt River and Clear Creek, not the Truckee River.

The Truckee River's Ti and oxybenzone concentrations on Sunday and Monday were low relative to the other study sites, with oxybenzone only increasing just above the detection limit during recreation and no elevated Ti. However, at the Truckee site, the stream bed has been engineered as a kayaking whitewater park. Some of the streambed has been cemented to create hydraulics and the stream bed has less loose sediment than a natural streambed. The lack of change in Ti in the Truckee River is therefore unsurprising, given the evidence that the majority of the Ti measured in these studies is believed to be from sediment resuspension.

The Salt and Truckee Rivers' Ti relative standard deviations of 4–15% and 8–50% in Clear Creek were observed. The standard deviation of Ti triplicates is greater during recreation than non-recreation times in all streams. This finding, like the temporal observations of recreation versus non-recreation measurements in Section 3.1, suggests that Ti concentrations during human activity are more variable than the natural stream background.

In all systems, the afternoon oxybenzone loads correlate to the amount of river use, as estimated by the bather counts (Fig. 4). However, the magnitude of oxybenzone release as a function of bathers is different for each river. Based on the linear regression slopes, the Salt River, the Truckee River, and Clear Creek each respectively had 89, 9.3, and 10 μg oxybenzone released per bather per second. The Truckee River and Clear Creek slopes are substantially lower than the Salt River, but still statistically different from a slope of zero ($\alpha = 0.05$). A plausible explanation is that bathers in the Salt River apply much more sunscreen than in the Truckee River and Clear Creek, which would also affect the detectability of released sunscreen ENPs.

Based on these results, statistically significant perturbation to background Ti and oxybenzone concentrations was detectable in at least two of the three rivers sampled. Important factors for detecting released sunscreen ENP detection limits in a variety of systems include the amount of NNP content making up the stream's natural Ti background, hydrological variables such as flow rate, depth, and mixing, and the rate of sunscreen use among bathers in that location.

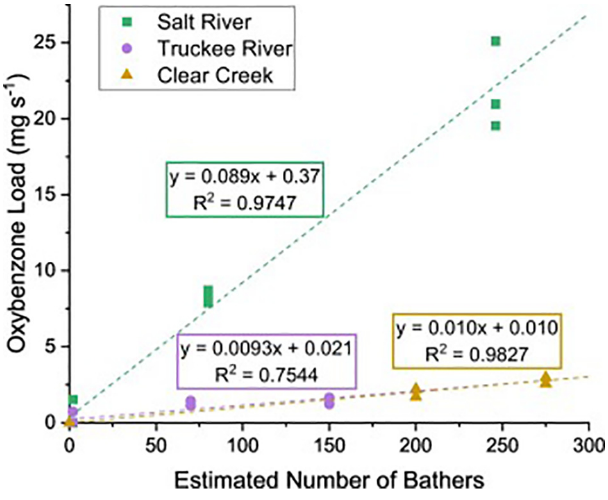

Fig. 4. Downstream oxybenzone loads from each triplicate sample as a function of the estimated number of bathers. The greatest amount of oxybenzone release was observed in the Salt River.

3.3. NP size distributions from spICP-MS

As a particle-specific alternative to examining mass concentrations, spICP-MS size distributions were examined for changes to the Ti NP size distribution during recreational activities. While this method is unable to differentiate between NPs containing only Ti versus NPs with a more complex elemental composition, it provides a useful estimation of the relative NP sizes based on the magnitude of Ti pulses detected during analysis. The size detection limit of this analysis in equivalent TiO_2 diameter was 48 nm. Samples selected for spICP-MS analysis in the multi-river study included the triplicate samples collected downstream Sunday morning and afternoon, upstream Sunday morning and afternoon, and downstream Wednesday afternoon. Only the downstream Sunday afternoon samples were collected during recreation, so all others were expected to serve as controls and generate identical distributions of background-only NPs.

The size distributions (shown in SI) of each stream were tested for equivalency using one-way Kruskal-Wallis ANOVA, followed by Dunn's test for non-parametric pairwise comparison of means ($\alpha = 0.05$). The mean and most frequent NP sizes, number concentrations, and interquartile ranges of these distributions are summarized in Table 2. The mode of the size of downstream Salt River Ti NPs increased from 73 nm on Sunday morning to 92 ± 3 nm during recreation (Table 2). Downstream Sunday NP concentrations also doubled, from 1.6 ± 0.3 to $3.1 \pm 0.2 \times 10^5$ particles mL^{-1}. These NP distributions are significantly different from each other, suggesting that an increase in Ti NP size and concentration is statistically supported. However, all other samples expected to be background-only (and therefore identical) also differed from each other significantly (Fig. S.6). This suggests the natural Ti NP population in the river is highly variable, making it difficult to attribute the differences in the Sunday afternoon samples to recreation activity. This variability in NP sizes seems counter to the relatively consistent trends in Ti mass concentrations during and outside of recreation (Fig. 1), but those measurements with conventional ICP-MS do not account for the number or size of particles making up the overall mass concentration.

The mode of the Truckee River's Ti NPs was 69 nm downstream on Sunday morning and 72 ± 6 nm Sunday evening, with number concentrations of $2.5 \pm 0.4 \times 10^5$ particles mL^{-1} at both times (Table 2). These samples are not statistically different, as were the other samples from upstream Sunday morning and afternoon and downstream Wednesday afternoon. Consistent with the Ti mass concentrations observed (Fig. 3b), recreation did not appear to significantly affect particulate Ti in Truckee River.

Downstream Clear Creek Ti NPs increased from a mode size of 68 ± 2 nm Sunday morning to 92 ± 13 nm during afternoon recreation and the NP number concentration increased by an order of magnitude (Table 2). The difference between these two is statistically significant and in fact, the downstream Sunday afternoon sample was statistically different from all four background-only samples. This shows a change in the Ti NPs that only occurs during recreation, supporting the hypothesis of an anthropogenic effect in this stream.

3.4. TEM characterization of NPs

The multi-river sampling campaign utilized TEM-EDS as another particle-specific analysis tool complimentary to spICP-MS. Due to the time requirements needed to generate quantitative (concentration and size distribution) data for the NP population of one sample, a comprehensive comparison between NPs during and outside of recreational activity using the TEM was not feasible. However, the in-depth characterization of individual NPs within a sample using this method can potentially allow identification of NNPs and ENPs.

Table 2
The most frequent and mean ^{49}Ti NP sizes, interquartile range of sizes, and number concentration determined by spICP-MS. Changes to NP size distributions during afternoon recreation were only significant ($\alpha = 0.05$) in Salt River and Clear Creek.

		Size (nm)			Concentration (10^5 particles mL^{-1})
		Mode	Mean	Interquartile Range	
Salt River, Arizona	Down Sun AM	73	102 ± 2	36.6	1.6 ± 0.3
	Down Sun PM	92 ± 3	128 ± 4	45.2	3.1 ± 0.2
	Up Sun AM	66 ± 2	79 ± 4	16.9	0.22 ± 0.02
	Up Sun PM	68 ± 2	81 ± 4	22.0	0.3 ± 0.1
	Down Wed PM	68 ± 2	86 ± 2	28.7	0.44 ± 0.03
Truckee River, Nevada	Down Sun AM	69.1	91 ± 5	35.8	2.5 ± 0.4
	Down Sun PM	72 ± 6	100 ± 7	45.3	2.5 ± 0.4
	Up Sun AM	68 ± 2	90 ± 8	33.8	2.5 ± 0.6
	Up Sun PM	68 ± 2	90 ± 7	34.1	2.4 ± 0.2
	Down Wed PM	69.1	91 ± 1	34.1	1.7 ± 0.2
Clear Creek, Colorado	Down Sun AM	68 ± 2	94 ± 5	30.5	0.6 ± 0.2
	Down Sun PM	90 ± 12	120 ± 13	33.6	3.2 ± 0.6
	Up Sun AM	68 ± 2	96 ± 3	30.5	0.8 ± 0.3
	Up Sun PM	69	94 ± 2	30.5	0.6 ± 0.1
	Down Wed PM	69	90 ± 2	30.5	0.46 ± 0.02

A representative particle visualized with TEM from each site is shown below in Fig. 5. In the downstream Sunday samples from all three sites, the morphology of the inorganic particles were diverse and highly branched aggregates were often visible. Amorphous, sheet-like particles were commonly found in the mixture of particles of all three rivers. EDS showed the common presence of Si, Al, K, Fe, and Mn elements, suggesting that phyllosilicate clay minerals were the dominant mineral components of the collected particles. Several instances of amorphous SiO_2 were observed. The results agree with previous studies showing that most aquatic NNPs are composed of mixture of inorganic materials, mainly Fe and Mn oxides, and clays.(Banfield and Zhang, 2001) From their size, chemical composition, and morphology, these clay-rich nanophases are interpreted to most likely be (hydr)oxides, but further analysis with high-resolution TEM and selected-area electron diffraction would be needed for structure confirmation. No clear differences in mineralogy between three locations were found.

In most cases, Ti was found coexisting with Fe oxides and clay NPs (Fig. 5c). However, one instance of TiO_2 was found in Clear Creek (Fig. S.9). At 300 nm in one dimension and nearly 1 μm in another, it is much larger than the average Ti-containing NP size reported by spICP-MS. In comparison with spICP-MS, spICP-MS sizes are likely underestimates of observed TEM particles because the equivalent TiO_2 size is calculated from Ti mass measured in individual NPs, which may often be present as a trace element of clay NP. It should be noted that this TEM analysis is challenging to interpret given the large NP sampling size and inclusion of large μm-sized particles on the grids. While clay particles predominate natural waters, as was represented in these TEM-EDS analyses, the comparatively few TiO_2 ENPs may not have been captured on the TEM grids. It is also possible that TiO_2 ENPs deposited on the grids were not identified during the analysis due to the complexity of the particle mixture. Heteroaggregation between TiO_2 released from sunscreen and other suspended material is likely to occur in these rivers and could further obscure these ENPs from visualization. This TEM work clearly showed the presence of nanoscale particles, which can contain Ti. However, obtaining sufficient quantitative data for statistical testing of pure TiO_2 samples is difficult in natural samples, because of the large number of heterogeneous Ti-bearing particles. Future research should continue to differentiate discrete from heteroaggregated nanoparticles over broad size ranges (<100 nm to >1 μm), using emerging counting and classification methods (Verleysen et al., 2019).

4. Conclusions

Statistical testing in the 2016 Clear Creek sampling campaign, emphasizing temporal variability, supports that Ti levels were elevated from background during Labor Day Monday's recreation. While there is strong statistical evidence of recreational effects on particulate metals during Monday afternoon, there is more uncertainty interpreting the Saturday and Sunday results due to the difficulty in establishing an appropriate control for those days. Clearly, the definition of a background signal is important for gauging environmental contamination. When the background source is altered, either naturally (i.e. the storm preceding Friday) or unnaturally (i.e. bathers and tubers in the water near the bridge sampling location), it is more difficult to establish certainty in an anthropogenic effect.

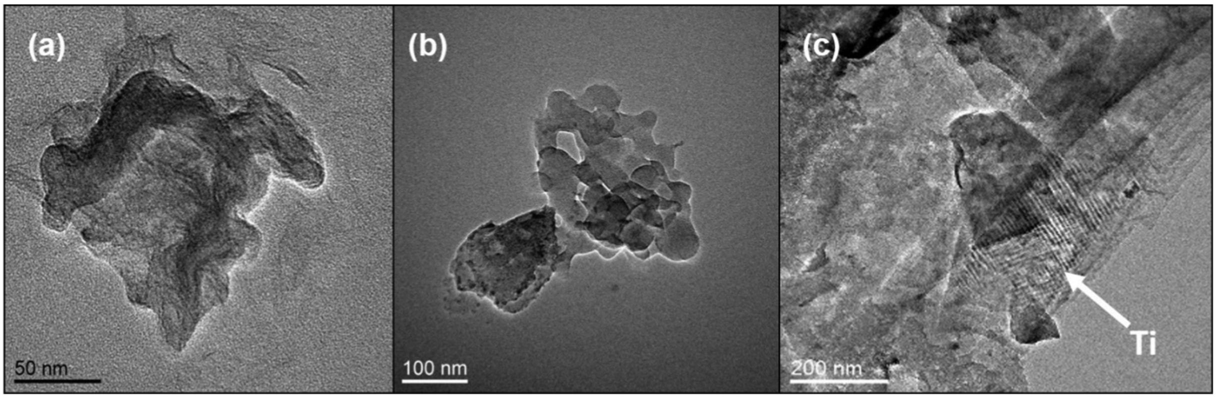

Fig. 5. TEM images of NPs collected from (a) the Salt River, Arizona, (b) the Truckee River, Nevada, and (c) Clear Creek, Colorado. The NPs observed in all three rivers ranged from amorphous to crystalline and included both spheroids and sheets. Most Ti detected by EDS was present in trace amounts with clay and Fe oxide NPs.

The results of the 2017 multi-river sampling campaign support that recreational effects on oxybenzone and particle-associated metal concentrations occur in multiple recreational stream systems. The data collected during recreation showed significantly elevated concentrations of oxybenzone in all three rivers, and significantly elevated Ti in two of the three rivers. This further substantiates an anthropogenic effect on stream concentrations of oxybenzone and particle-associated metals, and that this could be generalizable to other recreational streams.

Although small-scale spatial variability was not investigated in either of the sampling campaigns, a separate experiment suggested only small differences in concentrations across the stream width and that fluctuations in the number of bathers may be more important to concentration variability (see SI Section S.5 and Fig. S.8). However, small changes to flow rates and mixing may impact fate and transport in some systems. Therefore, we recommend further consideration of this in future studies of stream NPs. Using discharge-weighted volume and integrated sampling across multiple lengths and depths of the stream cross section could provide a better assessment of the conditions at any given time. Collection of time-integrated samples (hourly or daily) could eliminate the effects of short-term variation.

Finally, spICP-MS and TEM allowed for particle-specific characterization of the water samples, which supported the hypothesis that bed sediment resuspension causes elevated Ti from natural particles during recreation in Clear Creek and possibly the Salt River. The variation observed in the four background samples from the Salt River demonstrates that high background variability can be problematic for detecting anthropogenic changes in spICP-MS data as well and that establishing background needs to be emphasized with all methodology. Although TEM is invaluable for a full NP characterization of individual particles, obtaining sufficient quantitative data for statistical testing would be difficult for natural unknown samples. SpICP-MS arguably combines the best of both analytical worlds by providing particle-specific quantitative data that lends itself well to statistical hypothesis testing.

CRediT authorship contribution statement

Logan N. Rand: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Yuqiang Bi:** Methodology, Investigation, Data curation, Writing - review & editing, Visualization. **Andrew Poustie:** Investigation. **Anthony J. Bednar:** Methodology, Validation, Investigation, Data curation, Writing - review & editing, Supervision. **David J. Hanigan:** Conceptualization, Investigation, Writing - review & editing, Supervision. **Paul Westerhoff:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **James F. Ranville:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140845.

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