Investigating Impacts of Ultraviolet Filters on the Cowichan River Ecosystem

Year 2

Prepared by

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February 2022

Prepared with financial support from:
Habitat Conservation Trust Foundation, Mitacs, and the RBC Foundation

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EXECUTIVE SUMMARY

Ultraviolet filters (UVFs) are compounds added to chemical sunscreens, personal care products, and plastics to block or absorb ultraviolet radiation from the sun, protecting human skin or extending the usable life of a product. However, several different UVFs have been found to disrupt normal hormonal and genetic function in aquatic organisms at high doses. Scientific evidence also suggests that several UVFs bioaccumulate and can biomagnify within aquatic and terrestrial ecosystems at higher concentrations than found in ambient water.

The British Columbia Conservation Foundation (BCCF) oversaw collection of river and lake water, sediment, juvenile fish and adult fish from the Cowichan watershed in 2021 with the valuable assistance of community streamkeepers and angling guides. This adds to the baseline of data collected over the past two years. Literature review (Evans, 2019; Rodgers, 2020) and preliminary water sampling in the upper 4 km of river were developed from "Seed" funding in 2019 where, across 4 water samples, oxybenzone was found to range from 68 – 570 ng/L (Traynor, 2019). An intensive and socially-distanced sampling protocol began in 2020, intended as Year 1 of a 5-year monitoring program to investigate and mitigate UVF contamination in the Cowichan River ecosystem. Sampling occurred at a range of sites along the length of the Cowichan River and at beaches around Cowichan Lake. In 2020 (Year 1 of 5), across 64 water samples, oxybenzone ranged from 0 – 211 ng/L (Rodgers *et al.,* 2021); juvenile fish, benthic macroinvertebrates, and freshwater mussels were collected and stored for future analysis.

In 2021 (Year 2 of 5), water samples were analyzed by Vancouver Island University's Applied Environmental Research Lab (VIU-AERL) using condensed phase membrane introduction mass spectrometry-liquid electron ionization with *in situ* liquid reagent chemical ionization (CP-MIMS-LEI/CI), adapted to determine oxybenzone concentrations in water. Additional work was undertaken to adapt the method for other UVFs of concern in water (enzacamene, octinoxate, octocrylene, and octisalate), alongside efforts to further develop methods for analyzing sediment and fish tissue using CP-MIMS-LEI/CI. BCCF and community volunteers continued with the sampling protocol in Year 2 where, across 105 water samples, oxybenzone ranged from 0 – 1,761 ng/L. Juvenile and adult fish were also collected and stored for future analysis.

Differences in river flow, precipitation, tourism and recreation patterns between years are likely factors affecting the year-to-year differences in oxybenzone (Rodgers *et al.,* 2021). Analysis of Year 2 results showed a relatively similar average oxybenzone concentration between Lake and River sites, although Lake sites experienced much higher point-in-time concentrations than River sites. Similar to what was found in Year 1, sites sampled in Year 2 with high recreational use had relatively higher concentrations of oxybenzone than sites where wastewater inputs were present. However, oxybenzone downstream of the Town of Lake Cowichan wastewater outfall persists; further investigations are needed to understand potential UVF contributions from the outfall to the river. From limited time-delay samples collected in Year 2, persistence in the water column of both Lake and River sites appears to be short-lived; more samples are needed to assess timeliness of oxybenzone degradation/dispersion at Lake sites in future sampling years. Natural molecule degradation coupled with repeated "flushing" downstream appears to prevent significant accumulation of oxybenzone within the river (Rodgers *et al.,* 2021); however, the question remains whether repeated seasonal exposure to UVFs (also known as chronic exposure) is resulting in bioaccumulation in aquatic organisms. The upper 10 km of the Cowichan River contains the highest densities of resident rainbow trout, as well as brown trout fry and parr, which increases the likelihood these species will be exposed to UVFs during the summer season due to overlap of habitat with recreation areas.

Recommendations for continued monitoring in 2022 include a focus on quantifying the presence and concentrations of enzacamene, octinoxate, octisalate and oxybenzone in the upper Cowichan River and at high-use beaches surrounding Cowichan Lake. Additionally, quantifying oxybenzone in tissues of resident trout, freshwater mussels and invertebrates is a continued priority goal (pending success with the CP-MIMS-LEI/CI method development to overcome challenges caused by fatty acids passing the probe membrane). Data collected since 2019 will be compiled into a summary report to analyze trends, and this should be used to inform a contamination mitigation plan and an education/outreach campaign related to lake and river stewardship.

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1.0 INTRODUCTION

"Ultraviolet filter" (UVF) is a term used to describe compounds added to chemical sunscreens that block or absorb ultraviolet radiation emitted from the sun. The presence and impact of UVFs in aquatic ecosystems is a rapidly emerging global issue, but information about UVF contamination in Canadian freshwater systems and potential impacts from chronic environmental exposure is currently limited.

UVFs have been demonstrated as dose-dependent endocrine disruptors, affecting reproduction and hormonal activity in several different aquatic species (Coronado *et al.*, 2008; Fent, Kunz and Gomez, 2008; Gago-Ferrero *et al.*, 2013; Molins-Delgado *et al.*, 2017). Some UVFs are relatively stable against degradation in the aquatic environment, and certain UVFs may be prone to bioaccumulation and biomagnification within the aquatic food web (Fent, Zenker and Rapp, 2010; Gago-Ferrero *et al.*, 2015). Both wastewater effluent and recreational inputs have been identified as major sources of UVF contamination worldwide (Semones *et al.*, 2017; Tsui *et al.*, 2014).

Located on southern Vancouver Island, the Cowichan watershed encompasses the traditional territory of the historic Cowichan Nation (present-day Cowichan Tribes, among others) (Cowichan Tribes, 2022). Significant cultural history is interwoven with the landscape. Today, listed as one of three Canadian Heritage Rivers in British Columbia (Madrone, 2013), the Cowichan River is an international destination known for drifting, angling, and fly fishing.

Besides supporting the lower Georgia Strait Chinook salmon (*Oncorhynchus tshawytscha*) indicator stock for the Pacific Salmon Treaty (Pacific Salmon Commission, 2020), the Cowichan watershed also provides critical habitat for hundreds of species of birds, fish, mammals, insects, and amphibians (BC Parks, n.d.), notably several populations of salmonid fishes including three species of trout: rainbow/steelhead (*Oncorhynchus mykiss*), coastal cutthroat (*O. clarkii clarkii*), and the introduced European brown trout (*Salmo trutta)*; along with four species of Pacific salmon: Chinook (*O. tshawytscha*), coho (*O. kisutch*), chum (*O. keta*), and kokanee salmon (*O. nerka*). Many of these fish species have declined relative to historical abundances (LGL Ltd., 2005).

The Cowichan watershed currently faces extensive anthropogenic impacts such as urbanization, deforestation, and climate change (LGL Ltd., 2005). Additionally, each summer thousands of visitors seek out water-based recreational activities in the lake and river, which makes the water highly susceptible to UVF inputs from sunscreen products (Evans, 2019; Traynor, 2019). Considered most at-risk for UVF impacts in the Cowichan River ecosystem are those species spending a significant portion of their life in the freshwater environment, near areas of UVF contamination.

The British Columbia Conservation Foundation (BCCF) began investigating the issue of UVF contamination in the Cowichan River ecosystem in 2019 with Seed funding from the Habitat Conservation Trust Foundation, and partnership support from the Cowichan Lake and River Stewardship Society and BC Ministry of Environment. Oxybenzone, a UVF added to many sunscreens and widely studied due to its estrogen-mimicking properties and propensity for bioaccumulation (Fent *et al.*, 2008; Kim and Choi, 2014), was chosen as an indicator contaminant.

Additional financial support from the Habitat Conservation Trust Foundation, RBC Foundation, BC Ministry of Environment, Regional District of Nanaimo, and Mitacs has resulted in an annual sampling protocol undertaken in Year 1 (2020) and Year 2 (2021), and continuous method development at Vancouver Island University's Applied Environmental Research Lab (AERL). The following report summarizes Year 2 activities and results.

2.0 GOALS AND OBJECTIVES

This project's overarching goals are to: 1) describe the nature and extent of UVF contamination within the Cowichan River ecosystem, 2) understand the potential impacts of UVF contamination on resident aquatic organisms, and 3) encourage public education, outreach and regulatory measures to help mitigate UVF inputs to the ecosystem. This study also aims to provide a project model for stewardship groups to undertake UVF investigations in other freshwater bodies of concern throughout BC and Canada.

The specific objectives for Year 2 were to:

- Continue describing the spatiotemporal distribution and interannual variability of oxybenzone at select sites in the Cowichan watershed.
- Investigate the presence of other UVFs of concern in water, including octocrylene, benzophenone-2, octinoxate, enzacamene, and 3-benzylidene camphor.
- Continue to assist Vancouver Island University (VIU) with sample collection and support student research to refine analysis methods for oxybenzone in sediment and tissue.
- Engage community stakeholders and volunteers in the project and its results.

3.0 METHODS

3.1 Study Area

The Cowichan watershed drains the Cowichan Valley basin, an area of approximately 940 km^2 on the southeast coast of Vancouver Island (LGL Ltd., 2005); the river flows east out of Cowichan Lake (elev. 180 m) for approximately 47 km before emptying into the Cowichan estuary and bay (Fig. 1).

The Cowichan Valley experiences a coastal Mediterranean climate, with warm-to-hot, dry summers and mild, wet winters (LGL Ltd., 2005). Since 2008, maximum summer temperatures in the region have reached between 30-36°C (Environment Canada 2020); increasingly warm mean annual temperatures, which have risen approximately 1.5°C since the 1980s, leave the valley prone to drought in the summer when water demand is highest (Smith *et al.*, 2019; Westland Resource Group, 2007).

Several communities are located along the shores of Cowichan Lake, mainly concentrated towards its eastern end; the largest of these is the Town of Lake Cowichan (pop. ~3,000) (Statistics Canada, 2017a). Near its terminus, the Cowichan River flows through the City of Duncan (pop. ~23,000) and Cowichan Tribes First Nation (local pop. ~2,200) (Statistics Canada, 2017b and 2018). Two wastewater treatment facilities currently discharge treated effluent into the Cowichan River: the Town of Lake Cowichan outfall (located approx. 3.8 km downstream from the weir in Lake Cowichan) and the Joint Utility Board (JUB) sewage treatment plant outfall (located east of Duncan on Cowichan Tribes land, approx. 44 km downstream of the weir; currently slated for relocation to Cowichan Bay (North Cowichan, 2020).

Several sites along the Cowichan River are used as year-round recreation areas. The most common summer recreation areas along the river include the Cowichan River Provincial Park and campground facilities (including Stoltz Pool, Skutz Falls); Sandy Pool Regional Park; and the privately-owned Little Beach lot, a popular exit location for river tubers coming from the Town of Lake Cowichan. An additional site serving as a tubing take-out location in 2022 was Spring Pool. On the lake, beaches at Arbutus Park in Youbou and Gordon Bay Provincial Park are heavily used (Fig. 1).

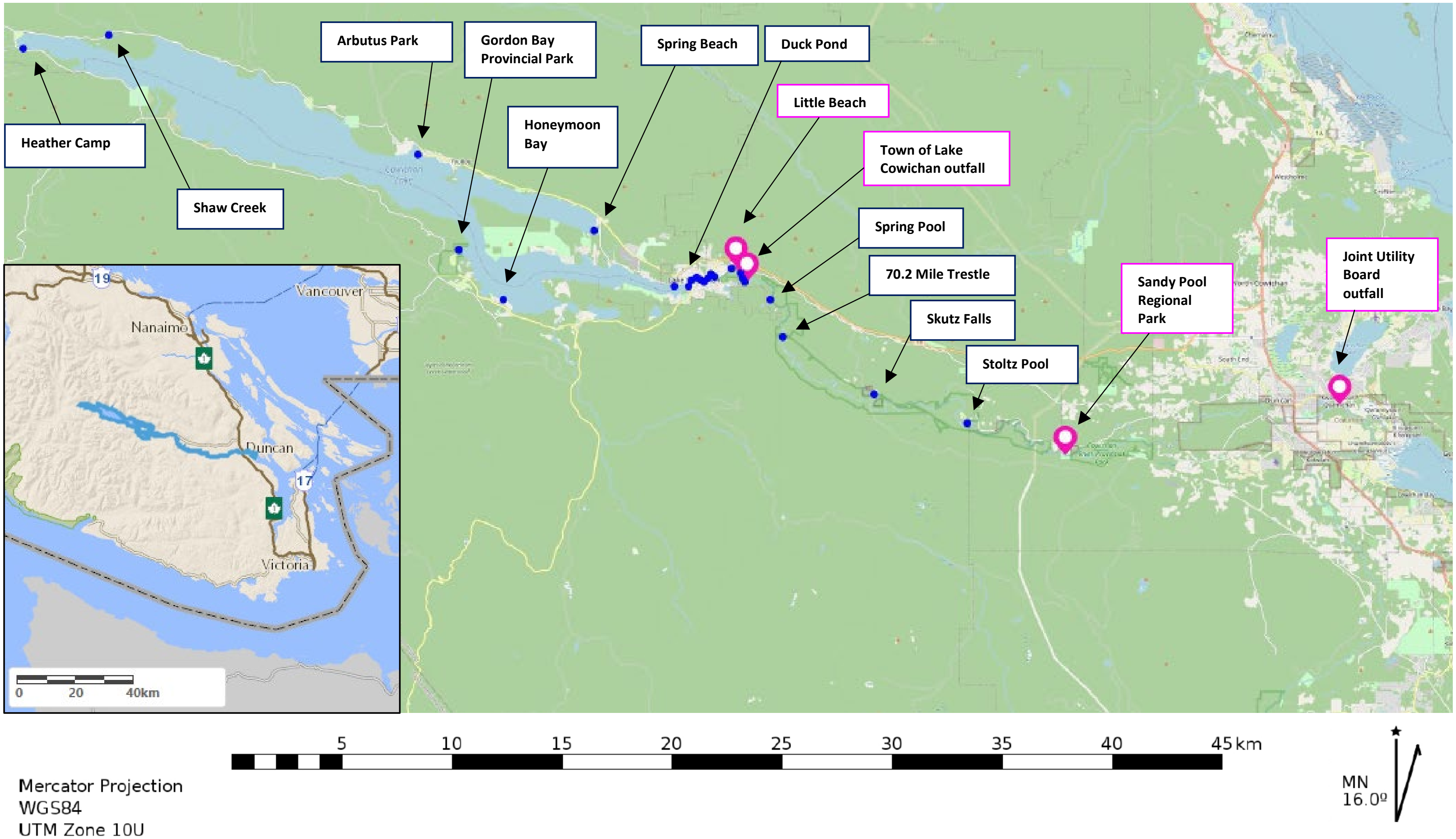

Figure 1. Cowichan Lake and River water sampling locations in Year 2, showing long-term Recreation vs. Wastewater monitoring sites (pink) and Year 2 sampling sites (blue) (Image source: CalTopo; Inset source: iMapBC).

3.2 Data Collection

3.2.1 River Discharge & Precipitation

Hourly water temperature and discharge data were downloaded from the Water Survey of Canada real-time hydrometric station database for Station ID "Cowichan River at Lake Cowichan" (08HA002), situated at 48° 49' 33" N, 124° 03' 10" W (ECCC 2021a). This hydrometric station monitors a total drainage area of 594 km^2 (ECCC 2021). Historical station data were downloaded using Cygwin DLL (3.3.4) terminal.

Daily air temperature and precipitation data were downloaded from Environment and Climate Change Canada's historical database for Station ID "North Cowichan" (1015630), situated at 48° 49' 27" N, 123° 43' 08" W (ECCC 2021b). Data were downloaded using cygwin terminal. Note that data were used for the North Cowichan station due to significant data gaps in the Lake Cowichan weather station.

3.2.2 Water Sampling

River water samples were collected at four long-term monitoring sites established in Year 1 (2020) (Fig. 1; in pink), on four weekends between July–August 2021. These sites were chosen for monitoring in Year 1 based on site accessibility and surrounding uses; two of the sites have consistent recreational use in summer (Little Beach, ~ 2.8 km downstream from the Cowichan Lake weir, and Sandy Pool Regional Park, ~ 28 km downstream of the weir) while two sites have wastewater treatment facility outfalls upstream (~500 m below the Town of Lake Cowichan wastewater outfall (TLCO), and ~100 m below the Joint Utility Board wastewater outfall (JUBO) on Cowichan Tribes land).

Additional river water samples spanning the length of the upper 25 km of the Cowichan River were collected on four weekends between July–August 2021. These sites spanned from the weir to Stoltz Pool, with a focus on the upper 4 km of river above the TLCO (Fig. 1). The upper 4 km of river was selected for close assessment in Year 2 (2021) due to the high recreational pressures in this area.

Lake water samples were collected from five popular swim beaches (Fig. 1) on seven weekends between July–August 2021 (Table 2). As a negative control, an additional sample was collected from Shaw Creek near the lake headwaters, approximately 900 m upstream of the creek's outlet to the lake.

Sample collection & equipment

To reduce the risk of cross-contamination, samplers did not apply sunscreen to their skin on the morning of sample collection. The date, time, weather conditions, and river discharge as listed on the Water Survey of Canada website for "Cowichan River at Lake Cowichan" and "Cowichan River at Duncan" stations were noted. Water temperature was collected using one of several handheld thermometers calibrated to a known reference thermometer (YSI ProPlus).

Samplers waded to knee-depth and faced upstream (in the case of river samples) or out toward the swimming area (in the case of lake samples), as close to the center of the river channel or swimming area as possible. In cases where wading was not possible, a dock or sloping bank was used to access the water.

Samplers wore nitrile gloves before handling vials. Pre-cleaned, 50 mL amber glass vials with PTFE-lined polypropylene caps were uncapped, inverted, and submerged ~ 30 cm below the water surface, then righted to allow water to fill the vial. This water was discarded downstream behind the sampler, and vials were rinsed this way three times with river water before a sample was collected, then capped and immediately placed in a 9-quart field cooler with ice to keep cool and dark. All samples were kept in the cold (~ 4°C) and dark after sampling (e.g. volunteer's refrigerator) until they could be packaged and transferred to a larger cooler (Coleman Xtreme® 5 Marine Cooler, 26.4-L) for delivery to VIU-AERL.

The rationale for sampling at depth (~ 30 cm) was to capture the ambient concentrations to which fish would be exposed to oxybenzone and other UVFs during rearing (Coronado *et al.,* 2008; Ziarrusta *et al.,* 2018; Labille *et al.,* 2020).

Quality Assurance & Control

Sampling QA/QC involved collecting field blank and duplicate samples. Field blank bottles were filled with deionized water in a sterile lab, then received, uncapped, handled, stored, and shipped in the same manner as standard sample vials. Duplicates were sampled simultaneously in both gloved hands of the sampler; samples were stored and handled under identical conditions until they underwent independent analysis.

3.2.3 Recreation Monitoring

Recreation monitoring was conducted for each site at the same time as water sampling. Samplers counted in-water users (*i.e.,* the number of people in the water upstream, and/or adjacent to the sampling location within the beach swimming area) at time of sample collection. This differs slightly from Year 1, when samplers counted in-water users for 10 minutes prior to sample collection. The reason for this change was due to logistics associated with daily sampling schedules in Year 2. In-water user counts were not directly compared for Year 1 to Year 2 for this reason.

At beach areas, public surveys were conducted with willing participants using a questionnaire based on Labille *et al.* (2020). A copy of the questionnaire is found in Appendix A. Respondents were asked if they would like to participate in a survey, and all answers were recorded (Yes or No). Positive respondents were read all questions aloud, and asked to provide a response verbally; this response was marked on the questionnaire sheet. Answers were recorded by the interviewer using pencil and paper. All respondents had the option to pass or skip a question. Negative respondents were given the final option to answer just the question “Are you wearing sunscreen?”, and to have their sunscreen bottle photographed for active ingredient analysis. Photos were taken of sunscreen products and linked to survey results, when available.

The Cowichan Lake District Chamber of Commerce (CLDCC) and Tube Shack businesses were again contacted to request public engagement information for Year 2 (2021). Data from both the CLDSS and Tube Shack were provided via email. Methods of data collection stayed consistent across both sources, thus recreation totals (by month) were able to be compared between project years.

The CLDCC recently transitioned operation of the Cowichan Lake Visitor Center, which impacted visitation numbers from January–March 2021. The Visitor Center, located beside Saywell park in the heart of downtown Lake Cowichan, is now operated by the Town of Lake Cowichan. The Visitor Center was operational from May 14–September 27, 2021, with closures every Wednesday and on Statutory holidays (K. Worsley, pers. comm., Feb 2022). The Chamber of Commerce continued to operate its mobile outreach wagon, which was in use from April–November 2021 (K. Worsley, pers. comm.).

3.2.4 Sediment Sampling

Preliminary sediment monitoring sites were chosen based on swim beaches with higher oxybenzone concentrations recorded during Year 1 (2020) sampling (Spring Beach Recreation Site and Gordon Bay Provincial Park; Fig. 1). Swim beaches were chosen for preliminary analysis instead of river sites, as available field equipment restricted sampling capabilities in-river. Samples were collected on July 18, 2021 and express couriered to ALS Global (Kelso, WA) for analysis. Duplicates were provided to VIU-AERL for method development.

Sample collection & equipment

To reduce the risk of cross-contamination, samplers did not apply sunscreen to their skin on the morning of sample collection. The date, time, weather, in-water users and water temperature were noted upon arrival at a sampling location. The general sampling location, depth, and distance from shore were marked on a diagram of the beach, along with distinguishing features and site access notes.

An inflatable dinghy (NRS Tributary Strike II) and Ekman sampler were used to access the beach to collect sediments. The Ekman sampler was lowered to the sediment surface and, while resting open on the bottom, triggered to close using a messenger weight. A successful sample was raised out of the water, allowed to drain, then scooped into pre-labelled 200 mL amber glass sample jars with PTFE-lined lids. Samplers wore nitrile gloves and processed successful samples as quickly as possible to avoid exposure to heat and light. After processing, jars were capped and immediately transferred to a dark cooler for holding. Supplementary water quality information was recorded on the field sheet using a YSI ProPlus.

Two samples were collected at Spring Beach and Gordon Bay and analyzed for Solids, Moisture, and Oxybenzone. One duplicate lab sample for Spring Beach was also analyzed (Table 3). A comparison sample was collected from Westwood Lake in Nanaimo, BC, on the morning of July 14, 2021.

Quality Assurance & Control

The Spring Beach sample was processed at ALS Global as a lab duplicate, alongside a method blank and lab control. Due to the presence of debris on the lake bottom (leaf litter, sticks, small cobbles), the jaws of the Ekman sampler were prevented from closing on several occasions, and sample collection had to be repeated in a slightly different area of the beach to avoid re-sampling any disturbed surface sediment.

3.2.5 Fish Sampling

Juvenile

Juvenile trout (brown, rainbow and/or steelhead) were collected opportunistically by BCCF staff working with MFLNRORD staff during annual electrofishing surveys (Scientific Fish Collection Permit #NA21-620245). Collection occurred during early September 2021, which coincided with low summer flows. Electrofishing-related mortalities were bagged, labelled, and placed in a cooler until they could be frozen.

Due to the small size, juvenile trout samples were grouped according to fork length (smallest fish ≥ 75% length of largest fish). Five composite samples were produced for Year 1 (2020) fish, while four composite samples were produced for Year 2 (2021). Composite samples were stored in a labelled freezer bag and are kept frozen until processing.

Adult

Adult trout (n=3 brown and n=1 cutthroat) were caught and retained while drift fishing on two dates in February 2022 (Scientific Fish Collection Permit #NA21-620245). Samples meeting permit criteria were immediately stunned and euthanized, measured (fork length, weight, sex, maturity), and photographed. Samples were wrapped in aluminum foil, bagged and placed in a cooler until vacuum sealing. Samplers noted the capture site's location, date, time, weather, and water temperature.

Processed samples were labelled with identifying information prior to being frozen until processing. Samples were processed by dissecting and removing otoliths; analyzing stomach contents; and separating out major organs, which were weighed, re-wrapped in aluminum foil and plastic, then re-frozen for individual analysis in future.

3.3 Analysis

3.3.1 Laboratory Analysis

Water samples were transported to VIU-AERL on the first weekday morning after sampling, with an average transit time of 2 hours (total holding time <48 hours). Analysis was conducted using condensed phase membrane induction mass spectrometry with direct liquid electron/chemical ionization (CP-MIMS-LEI/CI). CP-MIMS LEI/CI is a novel direct mass spectrometry technique developed by VIU-AERL researchers that eliminates sample preparation steps, requires less than 10 mL of sample for analysis, and provides parts-per-trillion detection limits for oxybenzone and related contaminants within minutes (Vandergrift *et al.*, 2022).

Sediment samples were split into duplicates. One set of samples was couriered to ALS Global (Kelso, WA) with a transit time of 4 days, while the duplicate set was transported to VIU-AERL within 48 hours and held under similar conditions as the samples couriered to ALS (*i.e.*, within the same brand of cooler and with same amount of ice packs and bubble wrap) until extraction occurred at ALS. The goal was to conduct concurrent analysis in order to compare method results between ALS and VIU-AERL. At ALS, samples were extracted and analyzed within one week of sample receipt using EPA Method 1694 adapted for Dry Sediment (Table 3). At VIU-AERL, samples were attempted run using CP-MIMS-LEI/CI adapted for sediment slurry.

3.3.2 Method Development

Delays caused by instrument repair at VIU-AERL in spring and early summer of Year 2 prevented significant method development for additional UVFs of concern prior to the start of the summer sampling window. Therefore, all Year 2 water samples were analyzed for oxybenzone using the confirmed method (Vandergrift *et al.,* 2022), although the number of samples was increased relative to Year 1.

Through the winter of Year 2, analysis methods were tested for four additional UVFs of concern (enzacamene, octocrylene, octinoxate, and octisalate) at VIU-AERL using a modified version of CP-MIMS-LEI/CI (Appendix C). Extraction recovery experiments were also performed for oxybenzone in sediments. Both a marine clay sediment and lab standard sediment were loaded with varied concentrations of oxybenzone, then extracted and directly measured using a modified CP-MIMS-LEI/CI (Appendix C).

Extraction recovery experiments for oxybenzone in fish tissue are still in progress as of spring 2022. Technical challenges must be overcome to allow for analysis of fatty tissues, without compromising the function of the instruments.

3.3.3 Data Analysis

Environmental data were processed and analyzed using Microsoft Excel (2016) and R (R Core Team, 2021) under R *Studio* (1.4.1717 "Juliet Rose"). Data wrangling was conducted using *tidyverse* (Wickham *et al.,* 2019) package. All values below laboratory detection limits (<20 ng/L) were assumed to be 0 ng/L for data analysis purposes.

All data were presented as values using descriptive and inferential analysis. Water sample results were summarized by site, type, and compared with river discharge between years; QA/QC results were presented for discussion. Recreation monitoring results were summarized by site, type, and compared between years. Public questionnaire results were tallied and presented as values. Standard regression analysis was performed to determine the relationships between air temperature and oxybenzone, or air temperature and number of in-water users. Sediment and fish sample results were presented as values.

General Linear Regression models (glm), developed using the *lme4* package and plotted using *ggplot2* (Wickham H., 2016; Bates *et al.*, 2015), were applied with a Pearson's correlation coefficient for six sample sites, to determine the relationship between recreational users and concentration of oxybenzone in water.

Spatiotemporal variation in oxybenzone concentrations at Recreation (Rec) vs. Wastewater (WWTP) monitoring sample sites (Fig.1, in pink) were described between sites and sampling dates in Year 2 (2021). These results were compared using analysis of variance (ANOVA). A Levene's test was conducted to test for equality of variances prior to a one-way ANOVA; where the assumption of equal variances was not met, a Welch's F test (Welch's ANOVA) was used. A Shapiro-Wilk test was conducted to determine the normality of distribution, followed by a non-parametric Mann-Whitney test to assess differences in oxybenzone concentrations between Lake and River samples.

4.0 RESULTS

4.1 River Discharge & Precipitation

Mean daily river discharge (MDD) for the Cowichan River at Lake Cowichan (Station ID 08HA002) was consistent between Year 1 (2020) and Year 2 (2021) for the month of July. However, MDD was 30% higher in Year 1 than in Year 2 for the month of August (Table 1). The MDD for the summer of 2019 (project "Seed" year) remains extremely low relative to both Year 1 and Year 2.

Table 1. Comparison of mean daily river discharge, water temperature (Cowichan River at Lake Cowichan) and air temperature (North Cowichan Climate Station) for the months of July and August.

		Seed 2019	Year 1 2020	Year 2 2021
Mean daily air temperature (°C)	July	16.7*	18.05	20.25
	August	19.0*	18.09	18.81
Mean daily water temperature (°C)	July	Not available	20.12	23.02
	August	Not available	21.74	22.35
Mean daily river discharge (m^3/s)	July	4.46	7.11	7.11
	August	4.45	7.08	4.92

*Data from nearby Station ID 1012055, Lake Cowichan (data missing for North Cowichan)

Hourly water temperature and discharge data were graphed for Cowichan River at Lake Cowichan (Station ID 08HA002) for the period May 1–October 31, 2021 (Fig. 2). The maximum discharge within this period was recorded on October 31 at 60 m^3/s.

Within the Year 2 sampling period only (July 3–August 29, 2021), indicated by red points on the x-axis, the maximum and minimum river discharges were 7.76 and 4.03 m^3/s on July 11 and August 19, respectively; the MDD was 5.91 m^3/s (Fig. 2). The maximum and minimum river temperatures within the Year 2 sampling period were 25.1 and 20.4°C on July 13 and August 28, respectively; the mean river temperature was 22.8°C (Fig. 2).

Daily air temperature and precipitation data were graphed for North Cowichan climate station (Station ID 1015630) for the period June 15–September 15, 2021 (Fig. 3). The maximum air temperature was recorded on June 28 at 41.9 °C. Precipitation events >5 mm occurred only once on September 4, with a rainfall amount of 6.5 mm.

Within the Year 2 sampling period only (July 3–August 29, 2021), indicated by red points on the x-axis, maximum and minimum daily air temperatures were 33.1 and 7.1°C, on August 11 and 24, respectively; the mean air temperature was 19.7 °C (Fig. 3). Light precipitation was only recorded on August 26 and 27, 2021, at 0.2 mm/day (Fig. 3).

Precipitation was negligible during the Year 2 sampling period (July 3–August 29, 2021; n=58 days) with an average rainfall amount of 0.01 mm/day. The Year 1 sampling period had a longer duration (June 20–September 7, 2020; n=80 days) and had a higher average daily precipitation (0.71 mm/day).

Data gaps exist in the daily meteorologic data available for North Cowichan station, with data missing between July 22–30, August 3–13, and August 25, 2021.

4.2 Water Quality

4.2.1 Year 2 Results

108 water samples were collected within the Year 2 sampling period (July 3–August 29, 2021) at a range of sites throughout the Cowichan watershed (Fig. 1). All samples were submitted to VIU-AERL for analysis of oxybenzone (Table 2, Table 3). For all water samples collected from the Cowichan watershed, 45% (n=49) were below the CP-MIMS-LEI/CI method limit of detection (LoD) of 20 ng/L, while 52% (n=56) were detectable for oxybenzone. 3% (n=3) were status unknown due to loss of sample.

Cowichan River

Of the water samples collected from the Cowichan River, point-in-time oxybenzone concentrations were highest approximately 0.6 km downstream of the weir, at Kinsmen Duck Pond beach on July 10, 2021 (421 ng/L). The Duck Pond also had the highest mean oxybenzone concentrations for all river sites sampled in Year 2 (*M*=175 *SD*=165, n=6).

Results were compared between the four long-term Recreation vs. Wastewater monitoring sites (Fig. 1, in pink) for Year 2. For the Recreation group, oxybenzone concentrations were highest in the at Sandy Pool (M=54.4, SD=70.8, n=4), closely followed by Little Beach (M=53.9 *SD*=61.7, n=8). For the Wastewater group, oxybenzone concentrations were highest downstream of the Town of Lake Cowichan Outfall (TLCO) (*M*=80.4, *SD*=110.8, n=4), whereas values did not rise above the detection limit downstream of the Joint Utility Board outfall (JUBO) (*M*=0, *SD*=0, n=3) (Fig. 4).

Cowichan Lake

Of the water samples collected from Cowichan Lake, point-in-time oxybenzone concentrations were highest at Gordon Bay Provincial Park Beach on August 1, 2021 (1,761 ng/L). However, the beach with the highest mean oxybenzone concentrations in Year 2 was Arbutus Park (*M*=514, *SD*=769, n=4), whereas the lowest average oxybenzone concentrations were seen at Honeymoon Bay (*M*=13, *SD*=22, n=6).

All water samples from Year 2 were grouped by location (Lake vs. River) and plotted (Fig. 5) to compare oxybenzone concentrations between site type.

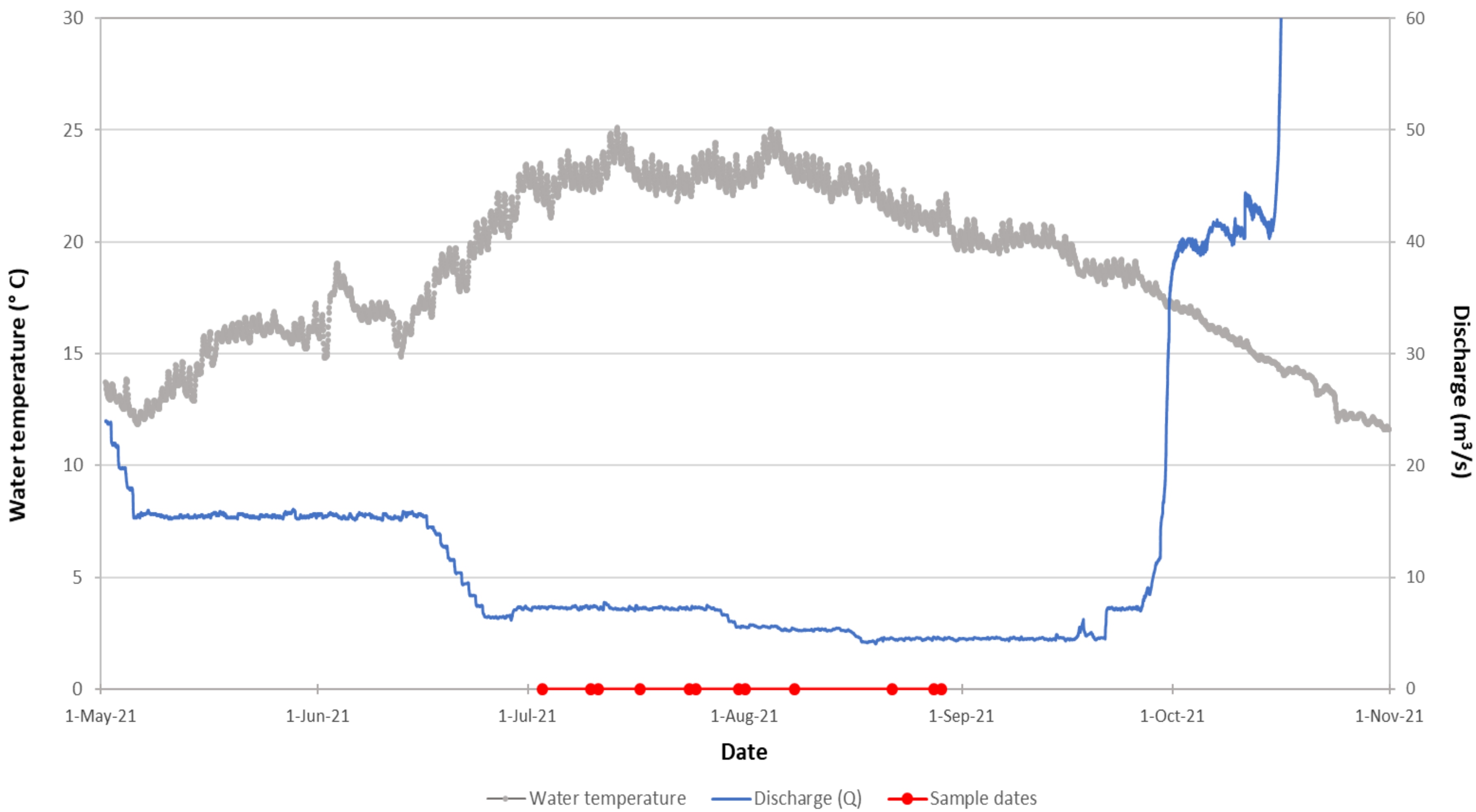

Figure 2. Water temperature (°C) and river discharge (m^3/s) for Cowichan River at Lake Cowichan, May 1–Oct 31, 2021.

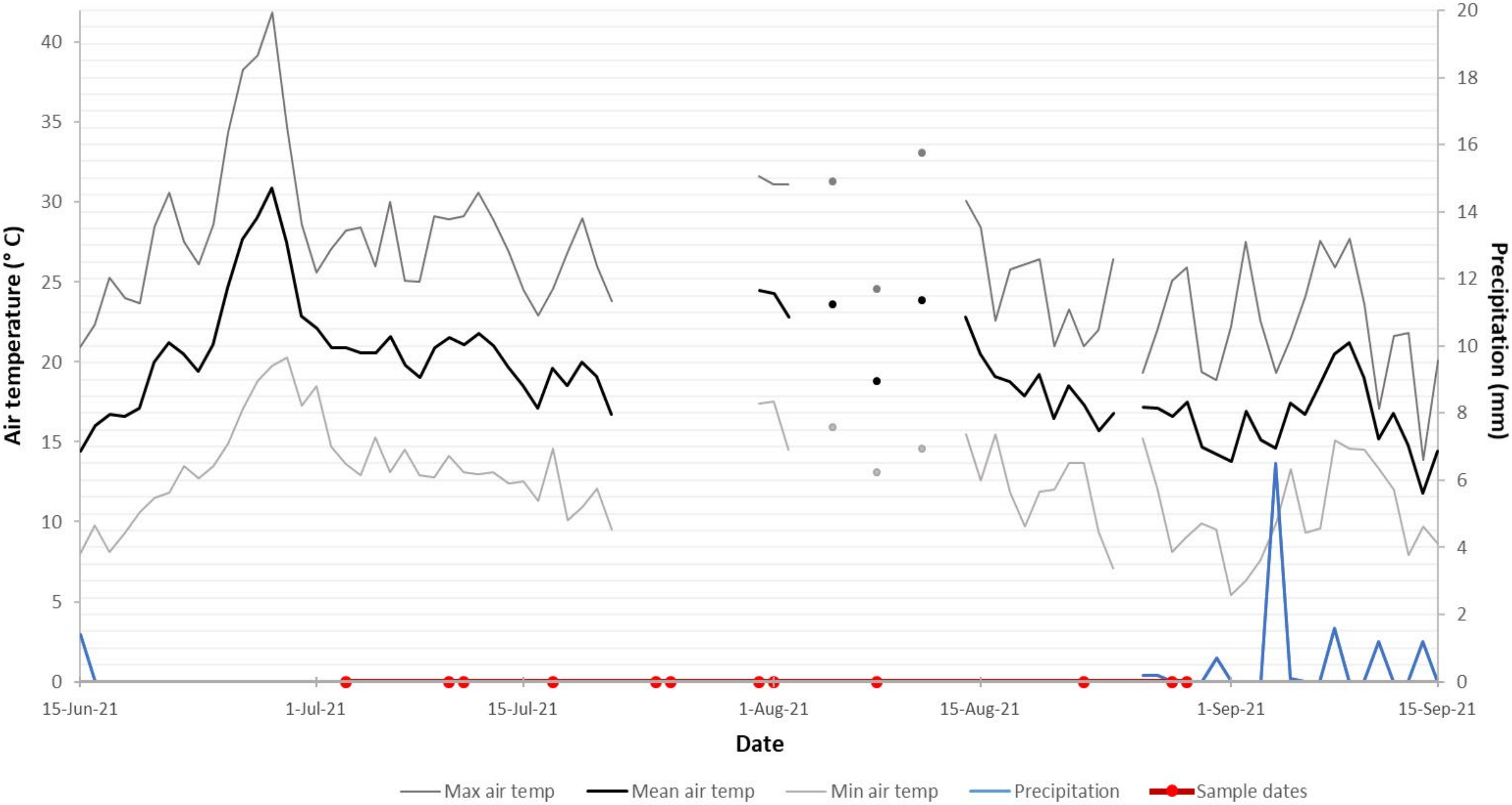

Figure 3. Precipitation (mm) and air temperature (°C) for North Cowichan, June 15–Sept 15, 2021.

Table 2. Oxybenzone concentrations (ng/L) in Cowichan River water at sites sampled in 2021.

Date	Location	Time	River km	Oxybenzone (ng/L)	Group
7/3/2021	Shaw Creek	13:00	NA	<LoD	Control
7/3/2021	Greendale Trestle	14:56	1.1	67	Rec
7/3/2021	Duck Pond	15:33	0.6	176	Rec
7/3/2021	Below Hwy Bridge	15:42	0.8	124	Rec
7/3/2021	Below Hwy Bridge - duplicate	15:42	0.8	116	Rec
7/3/2021	Little Beach	15:53	2.8	111	Rec
7/3/2021	500 m d/s TLCO	16:07	3.8	87	WWTP
7/3/2021	Mayo Road Bridge	16:28	16.5	<LoD	Rec
7/3/2021	Stoltz Pool	16:47	24.5	<LoD	Rec
7/3/2021	Sandy Pool	17:04	28	69	Rec
7/3/2021	100 m d/s JUBO	17:36	44	<LoD	WWTP
7/10/2021	Duck Pond	14:20	0.6	421	Rec
7/11/2021	Greendale Trestle	14:23	1.1	31	Rec
7/11/2021	Little Beach	14:43	2.8	101	Rec
7/11/2021	Little Beach – field blank	14:43	2.8	35	Blank
7/11/2021	Spring Pool	15:27	6.5	<LoD	Mid-River
7/17/2021	Tube Shack #1	12:42	0.5	<LoD	Rec
7/17/2021	IDA Pharmacy #2 / Foot Bridge	13:00	0.8	<LoD	Rec
7/17/2021	Greendale Trestle	13:13	1.1	<LoD	Rec
7/17/2021	Little Beach	13:28	2.8	<LoD	Rec
7/17/2021	500 m d/s TLCO	13:40	3.8	<LoD	WWTP
7/17/2021	Mayo Road Bridge	14:09	16.5	<LoD	Rec
7/17/2021	Stoltz Pool	14:39	24.5	<LoD	Rec
7/17/2021	Stoltz Pool - duplicate	14:39	24.5	<LoD	Rec
7/17/2021	Sandy Pool	15:02	28	<LoD	Rec
7/17/2021	100 m d/s JUBO	15:34	44.5	<LoD	WWTP
7/17/2021	Duck Pond	15:45	0.6	<LoD	Rec
7/24/2021	Tube Shack #1	14:25	0.5	30	Rec
7/25/2021	Duck Pond	12:05	0.6	40	Rec
7/25/2021	Greendale Trestle	14:25	1.1	92	Rec
7/25/2021	Little Beach	14:45	2.8	177	Rec
7/25/2021	Little Beach - duplicate	14:45	2.8	125	Rec
7/25/2021	Spring Pool	15:34	6.5	57	Mid-River
8/28/2021	Duck Pond	13:10	0.6	318	Rec
8/29/2021	Greendale Trestle	14:30	1.1	<LoD	Rec
8/29/2021	Little Beach	14:48	2.8	<LoD	Rec
8/29/2021	Spring Pool	15:28	6.5	<LoD	Mid-River
8/1/2021	500 m d/s TLCO	8:07	3.8	Broke in holding	WWTP
8/1/2021	Spring Pool	13:55	6.5	<LoD	Mid-River
8/1/2021	Tube Shack #1	14:04	0.5	129	Rec
8/1/2021	Duck Pond	14:14	0.6	265	Rec
8/1/2021	Btwn Duck Pond and Hwy Bridge	14:23	0.7	58	Rec
8/1/2021	Below Hwy Bridge	14:27	0.8	62	Rec
8/1/2021	Btwn Hwy Bridge and Greendale us	14:33	0.9	242	Rec
8/1/2021	Btwn Hwy Bridge and Greendale ds	14:44	1.0	88	Rec
8/1/2021	Greendale Trestle	14:48	1.1	63	Rec
8/1/2021	Btwn Trestle and Little Beach us	15:04	1.5	86	Rec
8/1/2021	Btwn Trestle and Little Beach ds	15:26	2.2	62	Rec
8/1/2021	Little Beach	15:30	2.8	69	Rec
8/1/2021	Btwn Little Beach and TLCO	15:40	2.95	68	Rec
8/1/2021	100 m u/s TLCO	15:47	3.1	43	WWTP
8/1/2021	@ TLCO	15:49	3.3	67	WWTP
8/1/2021	Btwn TLCO and 500m d/s TLCO	15:55	3.5	56	WWTP
8/1/2021	500 m d/s TLCO	16:00	3.8	Broke in holding	WWTP
8/1/2021	Sandy Pool	16:40	28	149	Rec
8/1/2021	Stoltz Pool	17:10	24.5	Broke in holding	Rec
8/1/2021	Skutz Falls	17:40	30	293	Mid-River
8/1/2021	500 m d/s TLCO	20:00	3.8	276	WWTP
8/1/2021	500 m d/s TLCO - duplicate	20:00	3.8	193	WWTP
8/8/2021	Stoltz Pool	13:50	24.5	<LoD	Rec
8/8/2021	Tube Shack #1	14:25	0.5	<LoD	Rec
8/8/2021	Duck Pond	14:50	0.6	93	Rec
8/8/2021	Greendale Trestle	14:50	1.1	25	Rec
8/8/2021	Little Beach	15:10	2.8	<LoD	Rec
8/8/2021	Spring Pool	15:40	6.5	<LoD	Mid-River
8/22/2021	Tube Shack #1	13:29	0.5	<LoD	Rec
8/22/2021	Hwy Bridge	13:45	0.8	<LoD	Rec
8/22/2021	Greendale Trestle	13:58	1.1	<LoD	Rec
8/22/2021	Little Beach	14:12	2.8	<LoD	Rec
8/22/2021	Little Beach - duplicate	14:12	2.8	<LoD	Rec
8/22/2021	500 m d/s TLCO	14:24	3.8	<LoD	WWTP
8/22/2021	Mayo Road Bridge	14:53	16.5	<LoD	Rec
8/22/2021	Stoltz Pool	15:10	24.5	<LoD	Rec
8/22/2021	Sandy Pool	15:28	28	<LoD	Rec
8/22/2021	100 m d/s JUBO	16:05	44.5	<LoD	WWTP

<LoD = Detection Limit for CP-MIMS-LEI/CI (20 ng/L)

Table 3. Oxybenzone concentrations (ng/L) in Cowichan Lake water at sites sampled in 2021.

Date	Location	Time	Oxybenzone (ng/L)	Group
7/3/2021	Honeymoon Bay	14:11	<LoD	Beach
7/3/2021	Gordon Bay Provincial Park Beach	14:27	164	Beach
7/3/2021	Spring Beach Recreation Site	15:14	95	Beach
7/10/2021	Honeymoon Bay	12:41	<LoD	Beach
7/10/2021	Gordon Bay Provincial Park Beach	13:30	368	Beach
7/11/2021	Town Water Intake	10:20	97	Drinking Water
7/11/2021	Spring Beach Recreation site	10:50	<LoD	Beach
7/11/2021	Arbutus Park – field blank	13:45	<LoD	Blank
7/11/2021	Arbutus Park	13:45	1,654	Beach
7/17/2021	Spring Beach Recreation Site	11:50	<LoD	Beach
7/17/2021	Gordon Bay Provincial Park Beach - @ 5 m	14:47	544	Beach
7/17/2021	Gordon Bay Provincial Park Beach - @ 30 m	14:43	40	Beach
7/17/2021	Gordon Bay Provincial Park Beach - @ 65 m	14:45	140	Beach
7/17/2021	Gordon Bay Provincial Park Beach - @ 145 m	15:05	<LoD	Beach
7/24/2021	Arbutus Park	15:00	76	Beach
7/25/2021	Spring Beach Recreation Site	11:34	<LoD	Beach
7/25/2021	Town Water Intake	13:30	<LoD	Drinking Water
7/25/2021	Gordon Bay Provincial Park Beach	15:00	214	Beach
7/25/2021	Honeymoon Bay	15:25	52	Beach
7/31/2021	Heather Camp	10:40	<LoD	Beach
8/1/2021	Arbutus Park	13:15	297	Beach
8/1/2021	Arbutus Park - duplicate	13:15	295	Beach
8/1/2021	Gordon Bay Provincial Park Beach	13:45	1,761	Beach
8/1/2021	Honeymoon Bay	14:10	25	Beach
8/8/2021	Gordon Bay Provincial Park Beach	13:50	382	Beach
8/8/2021	Honeymoon Bay	14:15	<LoD	Beach
8/8/2021	Arbutus Park	15:00	30	Beach
8/8/2021	Spring Beach Recreation Site	15:15	<LoD	Beach
8/8/2021	Town Water Intake	15:45	<LoD	Drinking Water
8/28/2021	Honeymoon Bay	12:00	<LoD	Beach
8/28/2021	Gordon Bay Provincial Park Beach	12:20	32	Beach
8/28/2021	Spring Beach Recreation Site	16:40	<LoD	Beach
8/28/2021	Town Water Intake	17:00	<LoD	Drinking Water

<LoD = Detection Limit for CP-MIMS-LEI/CI (20 ng/L)

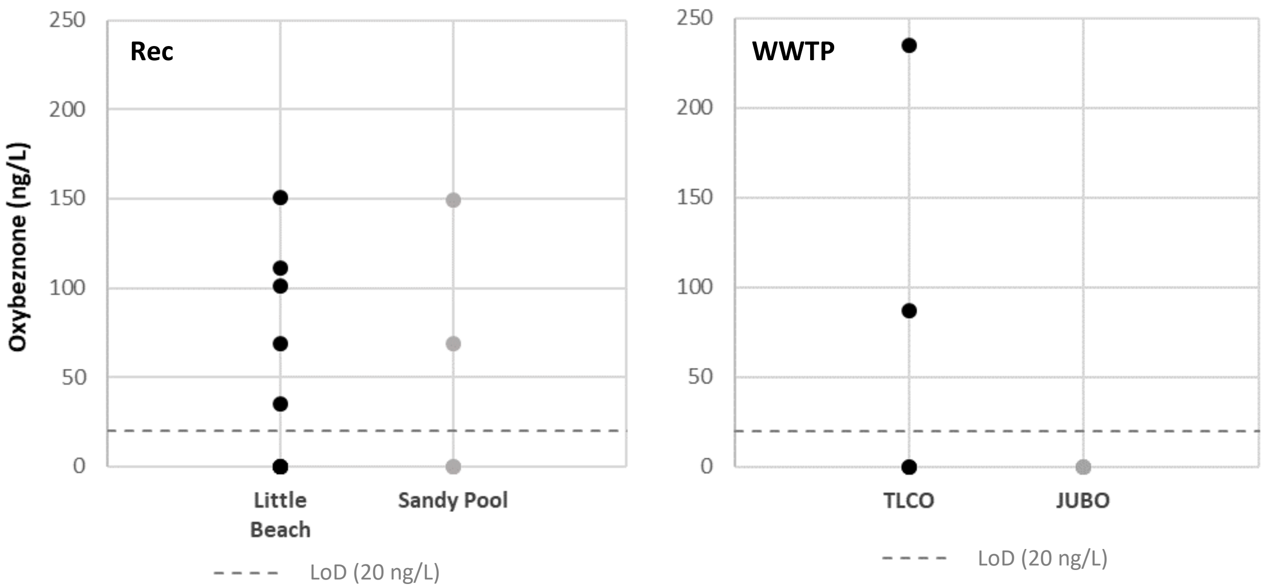

Figure 4. Comparison of oxybenzone results (ng/L) collected during the Year 2 sampling period (July 3–August 29, 2021) from Recreation (Little Beach (n=9) & Sandy Pool (n=4); left) and Wastewater (Town of Lake Cowichan outfall (n=4) & Joint Utility Board outfall (n=3); right) monitoring sites.

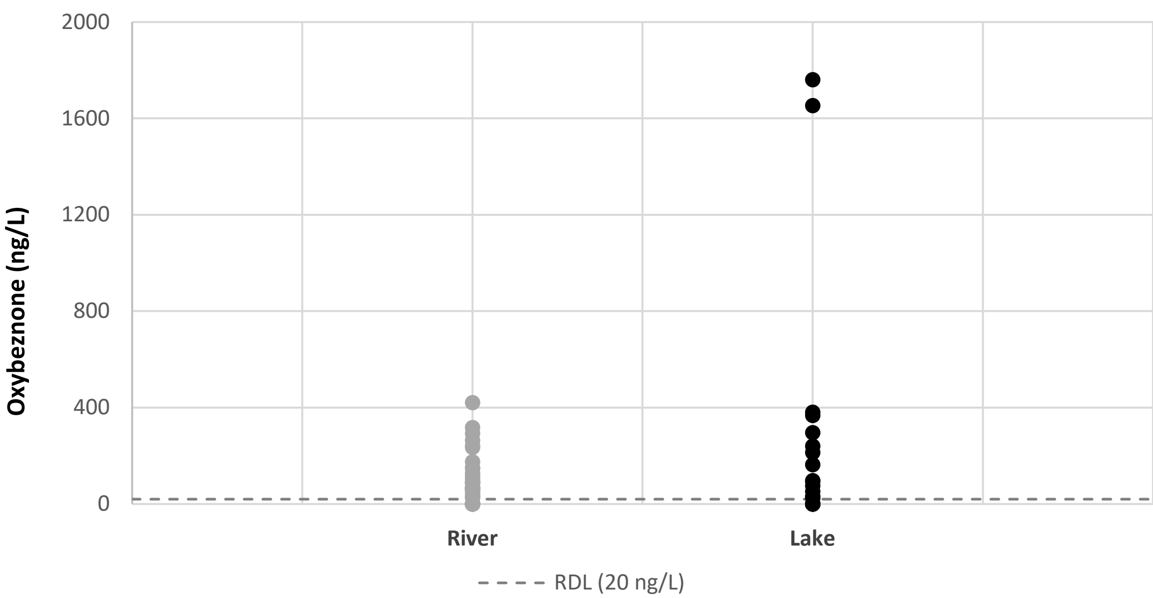

Figure 5. Comparison of oxybenzone results (ng/L) between River (n=65) and Lake (n=28) sites collected from the Cowichan watershed during the Year 2 sampling period (July 3–August 29, 2021).

4.2.2 Annual Variation

From Year 1 to Year 2, mean oxybenzone concentrations decreased at Little Beach and downstream of the JUBO, whereas mean oxybenzone concentrations increased at Sandy Pool and downstream of the TLCO (Fig. 6); however, variance remained high in both years. There was also variation in the number of samples collected from Year 1 to Year 2 due to volunteer availability and duration of the sampling window.

For a year-to-year visual variation comparison, we plotted oxybenzone results from the four sites sampled consistently since the project "Seed" year in 2019 (Duck Pond, Greendale Trestle, Little Beach, and 500 m downstream of the TLCO). River discharge was graphed to show relative differences between years (Fig. 7). Across these four sites, the average oxybenzone concentrations were highest in 2019 (M=325, SD=206, n=1), followed by 2021 (M=84, SD=110, n=1-6), then 2020 (M=71, SD=71, n=4-8). However, the number of sample dates for each site (n) is highly variable between years.

4.2.3 Quality Assurance & Control

For sampling and analysis QA/QC, two field blanks and six field duplicates were collected across a range of dates and individual samplers (Table 2 and Table 3).

Two samples at VIU-AERL were tested as depletion estimates to quantify depletion in oxybenzone over time beyond the initial date of analysis. Two samples collected from Westwood Lake on August 14 and 21, initially analyzed on August 17 and 23, respectively, were both re-analyzed on August 31.
The August 14 sample, initially analyzed at 589 ng/L after 3 days of storage in the cold and dark (<4°C), dropped to 111 ng/L after an additional 14 days of storage. The August 21 sample, initially analyzed at 229 ng/L after 2 days of storage in the cold and dark (<4°C), dropped below the limit of detection (<20 ng/L) after an additional 8 days of storage. This represented 81% total loss from the original oxybenzone concentration, with an average loss of 34 ng/L per day for the August 14 sample; and 100% total loss from the original oxybenzone concentration, with an average loss of 29 ng/L per day for the August 21 sample. Both samples were stored in the cold and dark (<4°C) between the initial and second analysis.

There were some discrepancies in QA/QC for Year 2 sampling, including a positive hit for a field blank at Little Beach on July 11 (35 ng/L, or 15 ng/L above the LoD), a calculated relative percent difference (RPD) of up to 35.3% (± 83 ng/L) in oxybenzone between duplicate samples from 500 m d/s TLCO on August 1, and 34.9% (± 53 ng/L) in oxybenzone between duplicates from Little Beach on July 25.

Other field blanks collected were <LoD as expected, and other duplicate samples collected had acceptable RPD's of <10% (Stoltz Pool, Little Beach, Arbutus Park and Below S Shore Rd (Hwy) Bridge at 0%, 0%, 1%, and 7%, respectively).

Similar to sample transport and storage challenges encountered during Year 1, three vials collected on August 1, 2021, broke while in holding at VIU-AERL due to refrigeration temperature. This resulted in complete loss of the broken samples. Thus, 105 total samples were analyzed from the Cowichan watershed.

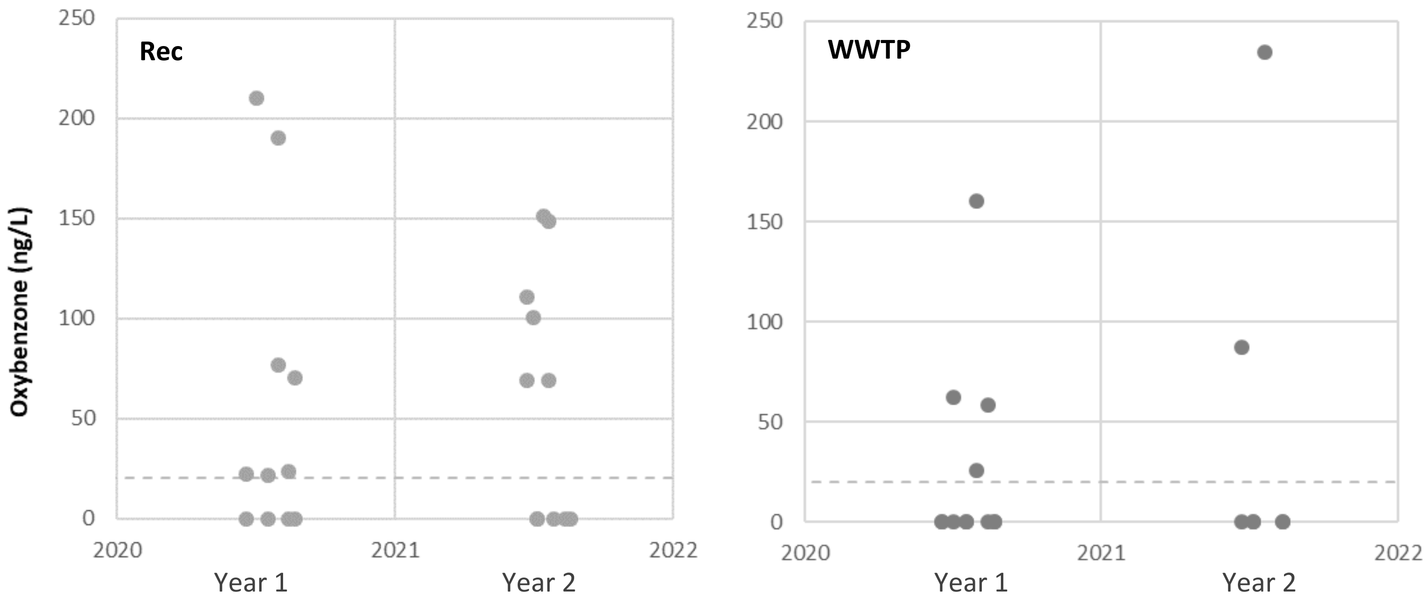

Figure 6. Comparison of oxybenzone concentrations (ng/L) between Year 1 and Year 2, as collected from the same Recreation (left; n= 11 and n=12, respectively) and Wastewater (right; n=12 and n=7, respectively) monitoring sites.

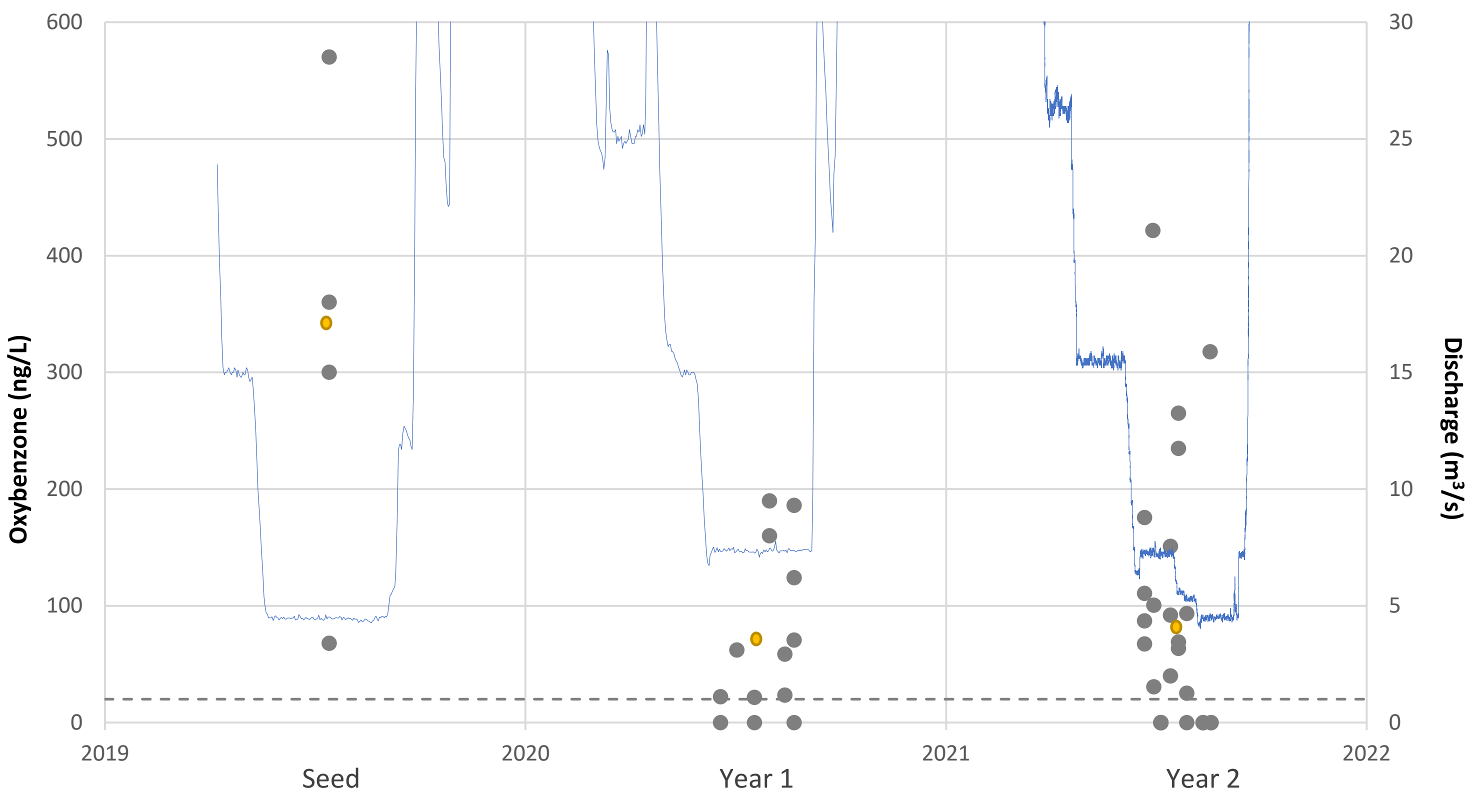

Figure 7. Oxybenzone concentrations (ng/L) as sampled across the same four sites in the upper Cowichan River, from the weir to 4 km below the weir, in 2019 (n=1 per site), 2020 (n=1-6 per site) and 2021 (n=4-8 per site). Daily river discharge (m^3/s) shown in blue; mean annual oxybenzone concentration shown in yellow. Dashed line shows limit of detection (20 ng/L).

4.3 Recreation Monitoring

4.3.1 Year 2 Results

17 river sites and 5 lake sites were monitored on 12 dates within the Year 2 sampling period (July 3–August 29, 2021). The maximum number of in-water users observed during sample collection was 125 people swimming at Gordon Bay Provincial Park Beach on July 3, 2021 (Canada Day Long Weekend). The second-highest observed number of in-water users was equal between 100 people swimming at Gordon Bay on August 1, 2021 (August Long Weekend) and 100 people swimming/floating downstream of the S Shore Rd (Hwy) Bridge in Lake Cowichan on August 1, 2021 (August Long Weekend).

Approximately 67% (n=66) of sites counted had in-water users at time of sampling, whereas 33% (n=32) had no in-water users at time of sampling (Table 4). Note: any field blanks/duplicates and Gordon Bay Provincial Park on July 17, 2021 were counted as single sites, despite the repeat measurements listed in Table 4.

Public Questionnaire

In Year 2, a total of 45 people were approached on 9 different dates with a public questionnaire (Appendix A; Fig. A1). Of those approached, 51% (n=23) were willing to answer the questionnaire in part or in full (Positive respondents), whereas 49% (n=22) were not willing to answer the questionnaire (Negative respondents); however, several of the 22 negative respondents did respond to the follow-up question ("Are you wearing sunscreen?"; n=15) and more than half allowed a photo to be taken of their sunscreen bottle for active ingredient analysis (n=13) (Figs. A2-A3).

Questionnaire responses came from Arbutus Park beach, Gordon Bay Provincial Park beach, and the Tube Shack dock. The majority (76%) of respondents assessed themselves as wearing sunscreen (Fig. A3). The most common amount of time waited between sunscreen application and bathing was 5 or fewer minutes (Fig. A4). 64% of respondents felt they were aware of the environmental impacts of sunscreen use on the environment (Fig. A5). The main motivation for sunscreen product selection was price for 56% of respondents, followed by personal health & safety (32%), then environmental concerns (12%) (Fig. A6).

The total number of products photographed for active ingredient analysis was n=19. The majority (58%) of products photographed were manufactured by multinational brand conglomerates (e.g. Johnson & Johnson, Beiersdorf, Hain Celestial); whereas 11% were produced by Canadian independent companies (e.g. Green Beaver, WILC Healthcare Inc.) (Fig. A7a-b). Of particular note, 11% of products photographed were flagged as having a Voluntary Recall Notice.

The most common sun protection factor rating was SPF 50 (Fig. A8), with the most common product application strategy split between either a cream or aerosolized spray (47% vs. 42%, respectively) (Fig. A9). The majority (79%) of products were marketed as Waterproof (Fig. A10), and the majority (68%) contained chemical UVFs (Fig. A11). A variety of marketing phrases were used to communicate "reef-safe" or "oxybenzone-free" status (Fig. A12).

The most common UVF in the ingredients list was octocrylene (n=12), followed by avobenzone (n=11) and octisalate (n=11); oxybenzone was present in only 11% of all products photographed (n=2) (Fig. A13).

Table 4. In-water counts of recreational users at time of sample collection.

RIVER

Date	Location	Time	# users
7/3/2021	Shaw Creek	13:00	0
7/3/2021	Greendale Trestle	14:56	27
7/3/2021	Duck Pond	15:33	36
7/3/2021	Below Hwy Bridge	15:42	54
7/3/2021	Below Hwy Bridge - duplicate	15:42	54
7/3/2021	Little Beach	15:53	69
7/3/2021	500 m d/s TLCO	16:07	0
7/3/2021	Mayo Road Bridge	16:28	30
7/3/2021	Stoltz Pool	16:47	9
7/3/2021	Sandy Pool	17:04	10
7/3/2021	100 m d/s JUBO	17:36	0
7/10/2021	Duck Pond	14:20	25
7/11/2021	Greendale Trestle	14:23	10
7/11/2021	Little Beach	14:43	20
7/11/2021	Little Beach – field blank	14:43	NA
7/11/2021	Spring Pool	15:27	0
7/17/2021	Tube Shack #1	12:42	0
7/17/2021	IDA Pharmacy #2 / Foot Bridge	13:00	52
7/17/2021	Greendale Trestle	13:13	3
7/17/2021	Little Beach	13:28	24
7/17/2021	500 m d/s TLCO	13:40	0
7/17/2021	Mayo Road Bridge	14:09	0
7/17/2021	Stoltz Pool	14:39	7
7/17/2021	Stoltz Pool - duplicate	14:39	7
7/17/2021	Sandy Pool	15:02	0
7/17/2021	100 m d/s JUBO	15:34	0
7/17/2021	Duck Pond	15:45	0
7/24/2021	Tube Shack #1	14:25	17
7/25/2021	Duck Pond	12:05	3
7/25/2021	Greendale Trestle	14:25	30
7/25/2021	Little Beach	14:45	37
7/25/2021	Little Beach - duplicate	14:45	37
7/25/2021	Spring Pool	15:34	0
8/28/2021	Duck Pond	13:10	10
8/29/2021	Greendale Trestle	14:30	11
8/29/2021	Little Beach	14:48	12
8/29/2021	Spring Pool	15:28	2

Date	Location	Time	# users
8/1/2021	500 m d/s TLCO	8:07	0
8/1/2021	Spring Pool	13:55	0
8/1/2021	Tube Shack #1*	14:04	30
8/1/2021	Duck Pond*	14:14	40
8/1/2021	Btwn Duck Pond and Hwy Bridge*	14:23	80
8/1/2021	Below Hwy Bridge*	14:27	60
8/1/2021	Btwn Hwy Bridge and Greendale us*	14:33	100
8/1/2021	Btwn Hwy Bridge and Greendale ds*	14:44	70
8/1/2021	Greendale Trestle*	14:48	15
8/1/2021	Btwn Trestle and Little Beach us*	15:04	30
8/1/2021	Btwn Trestle and Little Beach ds*	15:26	30
8/1/2021	Little Beach*	15:30	60
8/1/2021	Btwn Little Beach and TLCO *	15:40	4
8/1/2021	100 m u/s TLCO*	15:47	0
8/1/2021	@ TLCO*	15:49	0
8/1/2021	Btwn TLCO and 500m d/s TLCO*	15:55	0
8/1/2021	500 m d/s TLCO*	16:00	0
8/1/2021	Sandy Pool	16:40	4
8/1/2021	Stoltz Pool	17:10	5
8/1/2021	Skutz Falls	17:40	0
8/1/2021	500 m d/s TLCO	20:00	0
8/1/2021	500 m d/s TLCO	20:00	0
8/8/2021	Stoltz Pool	13:50	0
8/8/2021	Tube Shack #1	14:25	24
8/8/2021	Duck Pond	14:50	18
8/8/2021	Greendale Trestle	14:50	10
8/8/2021	Little Beach	15:10	25
8/8/2021	Spring pool	15:40	0
8/22/2021	Tube Shack #1	13:29	13
8/22/2021	Hwy Bridge	13:45	12
8/22/2021	Greendale Trestle	13:58	10
8/22/2021	Little Beach	14:12	13
8/22/2021	Little Beach - duplicate	14:12	13
8/22/2021	500 m d/s TLCO	14:24	0
8/22/2021	Mayo Road Bridge	14:53	4
8/22/2021	Stoltz Pool	15:10	0
8/22/2021	Sandy Pool	15:28	4
8/22/2021	100 m d/s JUBO	16:05	0

LAKE

Date	Location	Time	# users
7/3/2021	Honeymoon Bay	14:11	9
7/3/2021	Gordon Bay Provincial Park Beach	14:27	125
7/3/2021	Spring Beach Recreation Site	15:14	22
7/10/2021	Honeymoon Bay	12:41	3
7/10/2021	Gordon Bay Provincial Park Beach	13:30	50
7/11/2021	Town Water Intake	10:20	0
7/11/2021	Spring Beach Recreation site	10:50	4
7/11/2021	Arbutus Park – field blank	13:45	24
7/11/2021	Arbutus Park	13:45	24
7/17/2021	Spring Beach Recreation Site	11:50	0
7/17/2021	Gordon Bay Provincial Park Beach - 5 m	14:47	62
7/17/2021	Gordon Bay Provincial Park Beach - 30 m	14:43	49
7/17/2021	Gordon Bay Provincial Park Beach - 65 m	14:45	56
7/17/2021	Gordon Bay Provincial Park Beach - 145 m	15:05	0
7/24/2021	Arbutus Park	15:00	22
7/25/2021	Spring Beach Recreation Site	11:34	3
7/25/2021	Town Water Intake	13:30	0
7/25/2021	Gordon Bay Provincial Park Beach	15:00	50
7/25/2021	Honeymoon Bay	15:25	4
7/31/2021	Heather Camp	10:40	0
8/1/2021	Arbutus Park	13:15	20
8/1/2021	Arbutus Park - duplicate	13:15	20
8/1/2021	Gordon Bay Provincial Park Beach	13:45	100
8/1/2021	Honeymoon Bay	14:10	2
8/8/2021	Gordon Bay Provincial Park Beach	13:50	50
8/8/2021	Honeymoon Bay	14:15	0
8/8/2021	Arbutus Park	15:00	4
8/8/2021	Spring Beach Recreation Site	15:15	7
8/8/2021	Town Water Intake	15:45	0
8/28/2021	Honeymoon Bay	12:00	0
8/28/2021	Gordon Bay Provincial Park Beach	12:20	35
8/28/2021	Spring Beach Recreation Site	16:40	4
8/28/2021	Town Water Intake	17:00	3

* Counted during drift of tubing reach, therefore repeat-counts are expected

4.3.2 Annual Variation

In Year 2, the total number of engagements at the Cowichan Lake Visitor Center (CLVC) was just under 3,000 people, with just over 5,000 people engaged via the mobile outreach wagon; this is roughly 78% of total engagements in Year 1, and 15% of total engagements in the project Seed year (Table 5)[1].

Table 5. Cowichan Lake Visitor Center and mobile outreach wagon engagements from 2019 – 2021[1].

	Seed 2019	Year 1 2020	Year 2 2021
Visitor Center	35,125	9,945	2,866
Mobile Wagon	16,920	337	5,126
Total	**52,045**	**10,282**	**7,992**

Visitation trends for Year 2 indicate engagements at the Cowichan Lake District Chamber of Commerce's mobile outreach wagon peaked in July and August (Fig. 9).

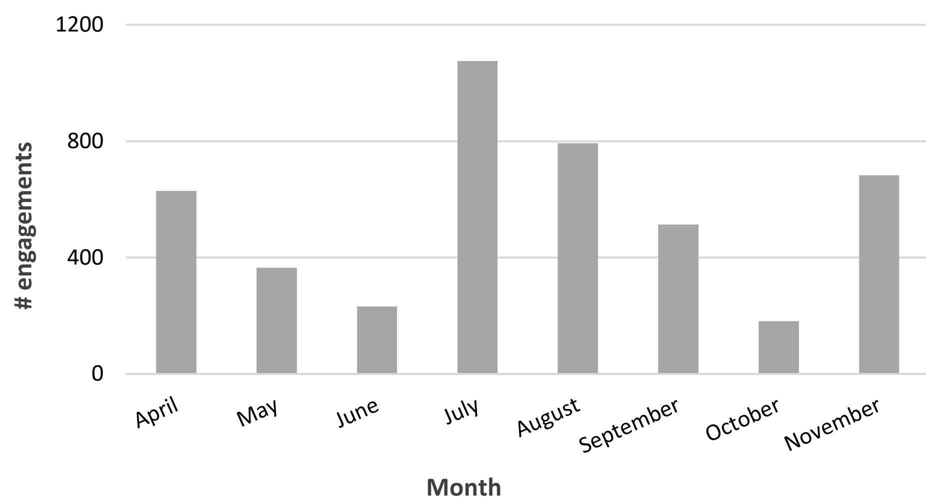

Figure 8. Total engagements at the Chamber of Commerce mobile outreach wagon in Year 2 (2021)[1].

Tube rentals from the Tube Shack local business increased by 14% in Year 2 relative to Year 1[2], suggesting the upper 4 km of the Cowichan River could have had a greater potential for oxybenzone inputs in Year 2. The business provided 19 litres of Stream2Sea "reef-safe" sunscreen to customers.

Across all sample dates, the average count of in-water users at River sites was nine people (n=55) in Year 1, compared to seventeen people (n=66) in Year 2. This is an increase of nearly 1.9x from Year 1 to Year 2, which is in agreement with the Tube Shack results.

There is insufficient data to compare in-water users at Lake sites between years; however, in Year 2 the average count of in-water users at Lake sites was 24 people (n=31), or approximately 1.4x greater than the average count of people in water at River sites.

[1] – Data supplied by the Cowichan Lake District Chamber of Commerce (K. Worsley, pers. comm., Feb 2021 and Feb 2022).

[2] – Data supplied by the Tube Shack (A. Frisby, pers. comm., Sept. 2020 and Feb 2021).

4.4 Sediment Sampling

No oxybenzone was detected in either sediment sample submitted to ALS Global. Samples produced a low yield (<32%) of surrogate Oxybenzone-13C6; when spiked with 50 µg/kg oxybenzone, the Lab Control produced a 102% recovery of 51.1 µg/kg (Table 6). Based on the method and historical data, the recoveries observed by ALS Global were in the range expected for this procedure (Appendix B).

Samples from Spring Beach were predominantly sand, whereas samples from Gordon Bay were predominantly silt and organics; this is reflected in the results from ALS. Samples were dried and weighed to obtain % solids and % moisture; the Gordon Bay sample had a 48% higher moisture content than Spring Beach, indicative of smaller grain size at Gordon Bay (Table 6).

Table 6. Oxybenzone concentrations (ng/L) in sediment at sites sampled in Year 2 (2021).

Sample Date	Extraction Date	Analysis Date	Location	Method	Oxybenzone (µg/kg)	Total Solids (%)	Total Moisture (%)
18-Jul	23-Jul	28-Jul	Spring Beach	1694 – Dry Sediment	Not Detected		
18-Jul	23-Jul	28-Jul	Spring Beach (Lab Duplicate)	1694 – Dry Sediment	Not Detected		
18-Jul	23-Jul	28-Jul	Gordon Bay	1694 – Dry Sediment	Not Detected		
N/A	23-Jul	28-Jul	Method Blank	1694 – Dry Sediment	Not Detected		
N/A	23-Jul	28-Jul	Lab Control	1694 – Dry Sediment	51.1		
18-Jul	N/A	22-Jul	Spring Beach	160.3 Modified – Sediment		69.0	31.0
18-Jul	N/A	22-Jul	Spring Beach (Lab Duplicate)	160.3 Modified – Sediment		67.5	N/A
18-Jul	N/A	22-Jul	Gordon Bay	160.3 Modified – Sediment		20.5	79.5

Samples submitted to VIU-AERL for method comparison with ALS similarly produced no detectable yields of oxybenzone. The additional sample collected from Westwood Lake was provided to VIU-AERL with an anticipated positive result, but there was no detection of oxybenzone.

Unfortunately, samples could not be analyzed within the appropriate time window to be comparable to ALS. Samples provided to ALS were found to have a relatively coarser grain size than the marine sediments tested at VIU-AERL (see 3.3.2). When spiked with varying concentrations of oxybenzone (µg/kg), the marine test sediments at VIU-AERL produced an average 73% recovery (Fig. 10).

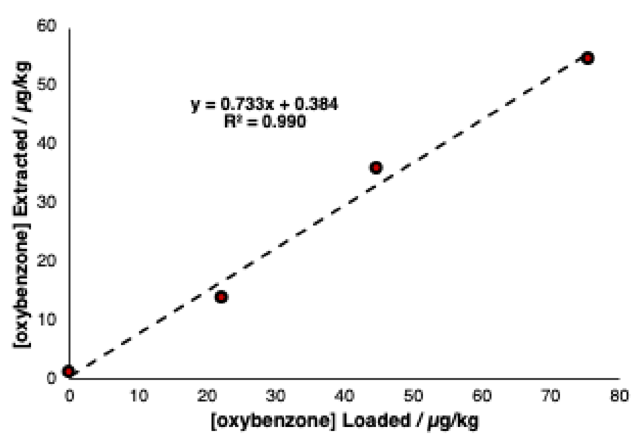

Figure 9. VIU-AERL marine sediment tests showing a 73% extraction recovery rate.

4.5 Fish Sampling

Rainbow/steelhead trout (*O. mykiss*) (n=14) were opportunistically collected from various sampling locations in the upper 20 km of the Cowichan River during steelhead stock assessment projects in the early fall of Year 2, for future tissue analysis. The mean fork length and weight of samples collected were 59.6 mm and 3.2 g (Table 7).

Brown trout (n=3) and cutthroat trout (n=1) were collected during targeted angling in the winter of Year 2, for future tissue analysis. The mean fork length and weight of adult samples collected were 410 mm and 598 g (Table 8).

Juvenile fish from Year 1 (2020) and Year 2 (2021) were grouped into composite samples according to fork length. Five composite samples (A, B, C, D, E) were produced for Year 1, while four composite samples (J, K, L, M) were produced for Year 2 (Table 9).

Table 7. Fish collected from the upper reaches of the Cowichan River in September 2021.

Date	Location	River km	Species	Fork Length (mm)	Weight (g)
Sep 10	Saysells Pool	2.9	RB	28	0.3
Sep 10	Saysells Pool	2.9	RB	37	0.7
Sep 10	Saysells Pool	2.9	RB	43	1
Sep 10	Saysells Pool	2.9	RB	49	1.5
Sep 10	Saysells Pool	2.9	RB	52	1.1
Sep 10	Saysells Pool	2.9	RB	81	6.8
Sep 10	Saysells Pool	2.9	RB	82	6.9
Sep 10	u/s Skutz Falls	16	RB	51	1.6
Sep 10	u/s Skutz Falls	16	RB	64	3.2
Sep 10	u/s Skutz Falls	16	RB	64	3.1
Sep 10	u/s Skutz Falls	16	RB	58	2.5
Sep 10	u/s Skutz Falls	16	RB	68	3.9
Sep 10	u/s Skutz Falls	16	RB	71	4.5
Sep 10	u/s Skutz Falls	16	RB	86	7.2

RB = juvenile rainbow/steelhead trout

Table 8. Adult fish sampled in February 2022.

Date	Location	River km	Species	Fork Length (mm)	Weight (g)*	Sex	Maturity
9-Feb	70.2 Mile	8.5	BT	435	614	F	M
10-Feb	Three Firs	9.5	BT	415	637	F	M
9-Feb	u/s Skutz Falls	14	BT	420	685	F	M
9-Feb	u/s Skutz Falls	15	CT	370	456	F	M

BT = brown trout; CT = cutthroat trout * Bled weight

VIU-AERL has completed sample extractions from fish tissue for method development but has not yet been able to run these extractions using CP-MIMS-LEI/CI. Alternate analysis methods using GC-MS are being explored, and method development is in process.

Eight commercial labs have been contacted as of February 2022; however, none can analyze tissue for oxybenzone as a comparison. Two labs have indicated the potential for analysis at the scoping level for future work (ALS Global and SGS/Axys), but at a significantly higher cost than VIU-AERL.

Table 9. Composite samples for juvenile trout collected in Year 1 (2020) and Year 2 (2021).

Composite	Year collected	n=	%FL	Group weight (g)	Notes	Species	Fork Length (mm)	Weight (g)	Location	River km
A	2020	N/A	N/A	17.9		BT	112	17.9	70.2 Mile	8.5
B	2020	3	91.2%	5.6		RB	57	2.1	Three Firs	9.5
						RB	53	1.9	Three Firs	9.5
						RB	52	1.6	Three Firs	9.5
C	2020	N/A	N/A	3.6		BT	66	3.6	Three Firs	9.5
D	2020	2	98.4%	6.4		RB	63	3.2	Horseshoe	14.5
						RB	64	3.2	Horseshoe	14.5
E	2020	N/A	N/A	7.1		BT	86	7.1	Horseshoe	14.5
F	2020	N/A	N/A	N/A	*Not to analyze (not enough weight)*	RB	49	1.4	u/s Skutz	16
G	2020	N/A	N/A	N/A	*Not to analyze (not enough weight)*	RB	40	0.9	Saysells	2.9
H	2021	2	75.6%	N/A	*Not to analyze (not enough weight)*	RB	28	0.3	Saysells	2.9
						RB	37	0.7	Saysells	2.9
J	2021	3	82.7%	3.6		RB	43	1	Saysells	2.9
						RB	49	1.5	Saysells	2.9
						RB	52	1.1	Saysells	2.9
K	2021	2	98.7%	13.7		RB	81	6.8	Saysells	2.9
						RB	82	6.9	Saysells	2.9
L	2021	4	79.7%	10.4		RB	51	1.6	u/s Skutz	16
						RB	64	3.2	u/s Skutz	16
						RB	64	3.1	u/s Skutz	16
						RB	58	2.5	u/s Skutz	16
M	2021	3	79.0%	15.6		RB	68	3.9	u/s Skutz	16
						RB	71	4.5	u/s Skutz	16
						RB	86	7.2	u/s Skutz	16

BT = juvenile brown trout; RB = juvenile rainbow/steelhead trout

4.6 Data Analysis

4.6.1 Relationship between recreational use and oxybenzone

A generalized linear model (glm) was used to assess whether the number of recreational users was an effective predictor of oxybenzone concentrations across six different sites (n=4 River, n=2 Lake) in Year 2. Sites were selected based on the presence of recreational users in water and representative of a range of conditions. Data were analyzed by site to ensure homogeneity across sampling conditions (Fig. 11).

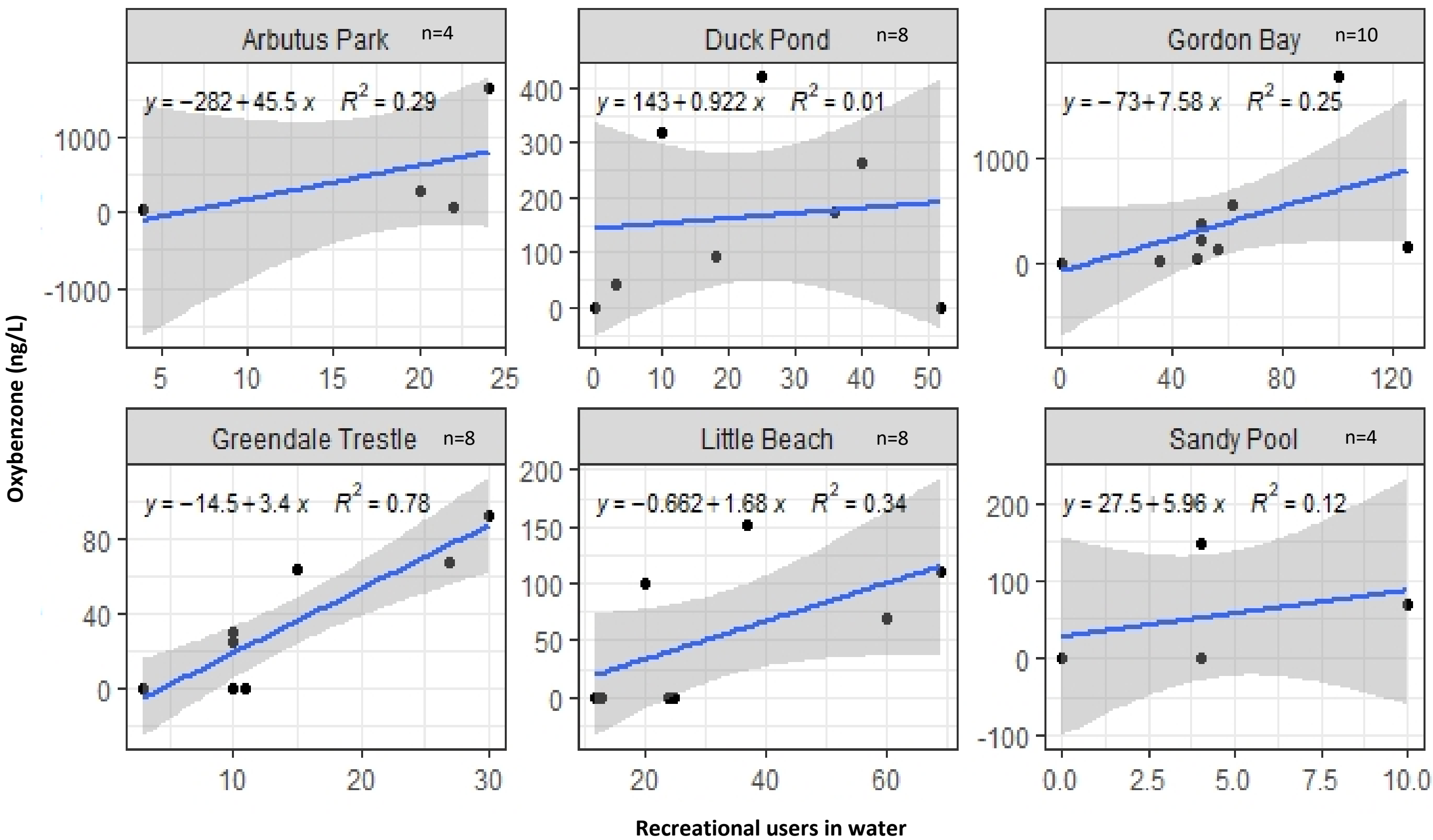

Figure 10. Generalized linear regression analysis between number of recreational users in water and oxybenzone concentration in water, for six sites sampled in 2021. Grey bars represent standard error.

With the exception of the Greendale Trestle ($r(6)=.89$, $p=.003$), Pearson's correlation showed a very weak linear relationship for all other sites ($|r| < .60$, $p > .10$) in Year 2.

4.6.2 Relationship between recreational use and air temperature

Recreational use was influenced by air temperature in Year 2 (Fig. 8). When daily maximum air temperatures reached above 28°C, the point-in-time counts of in-water users were elevated; comparatively, when daily maximum air temperatures remained below 22°C, the point-in-time counts of in-water users remained low.

The positive, linear relationship between maximum air temperature and in-water users was considered statistically significant ($r(85)=.28$, $p=.009$).

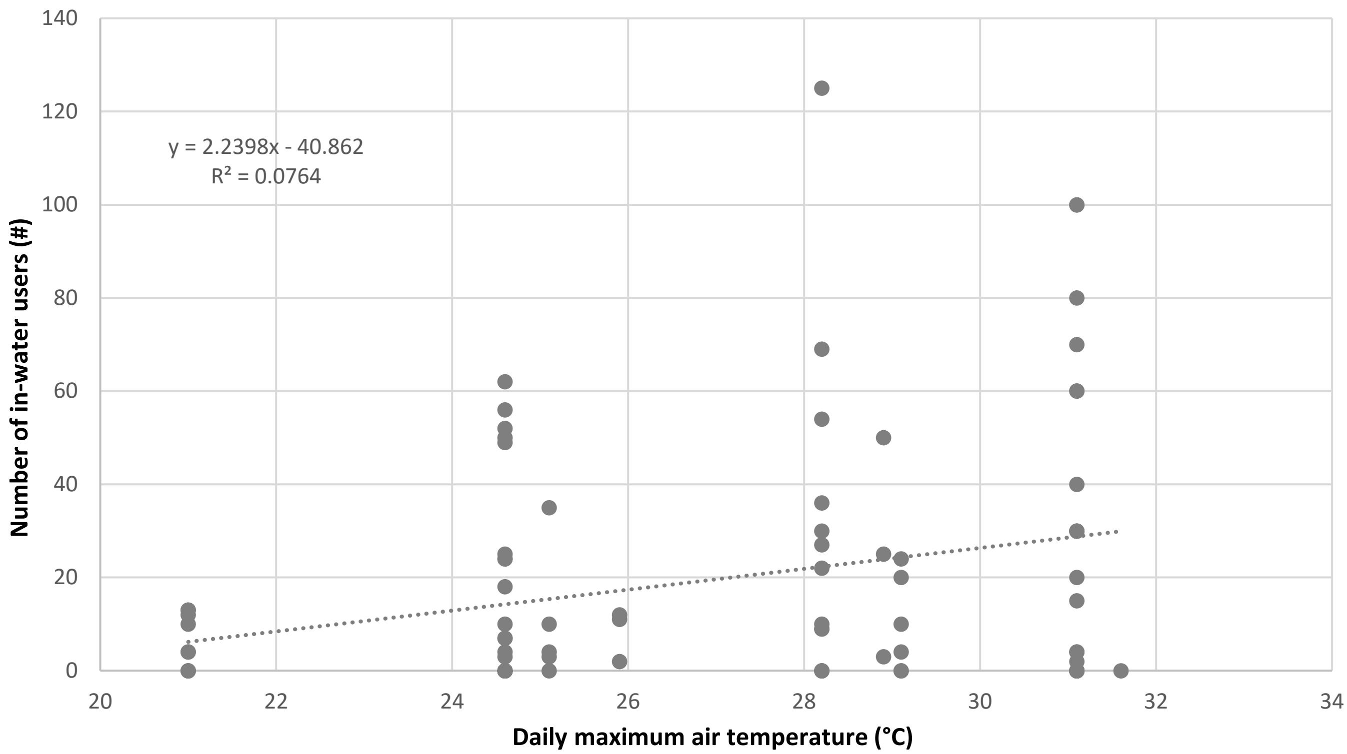

Figure 11. Daily maximum air temperature (°C) at North Cowichan climate station vs. (a) oxybenzone concentrations (ng/L) and (b) number of in-water users, for all sites sampled in the Cowichan watershed during Year 2 (*p*<.01).

4.6.3 Wastewater

A Levene's test for equality of variances was used to describe spatiotemporal variation in oxybenzone at the long-term Recreation vs. Wastewater monitoring sites (Fig. 1; in pink) in Year 2; this indicated the assumption of equal variances was met for the sites ($p>.05$) but not for the sampling dates ($p<0.001$) (Appendix B; Table B1).

A one-way analysis of variance (ANOVA) was conducted between sites (Table B2), while a Welch's F test (Welch's ANOVA) was conducted between sampling dates (Table B3), both at α=0.05 level of significance.

Among the four long-term Recreation vs. Wastewater monitoring sites in Year 2, the mean oxybenzone concentrations were highest downstream of the Town of Lake Cowichan outfall and lowest downstream of the JUB outfall (Fig. 4); however, a one-way ANOVA showed the differences between all sites were not statistically significant ($F(3,11)=0.68$, $p=.581$).

4.6.4 Lake vs. River

A Shapiro-Wilk test was conducted to determine normality of distribution for Lake and River samples in Year 2. Both sample groups were found to be not normally distributed ($p<0.01$) (Table B4).

A nonparametric Mann-Whitney U test was calculated to determine whether there was a significant difference in oxybenzone results between Lake and River samples in 2021 (Table B5).

Despite the high point-in-time values recorded above 1,500 ng/L at swim beaches around the lake in 2021, the median oxybenzone results were slightly higher in River samples (*Mdn*=30.1, *IQR*=87) than in Lake samples (*Mdn*=27.7, *IQR*=176.2). However, the range of oxybenzone results was high for both groups, and the difference in oxybenzone concentrations between Lake and River calculated by the U test was not considered statistically significant ($U(N_{river}=65, N_{lake}=28)=835.5$, $z=(-0.62)$, $p=.533$) (Table A5).

5.0 DISCUSSION

To understand the potential impacts of contaminants, it is necessary to understand their sources and sinks within an aquatic ecosystem (Bashir *et al.* 2020). A confirmed, direct source of UVF contamination to the Cowichan River ecosystem is recreational use, while a potential indirect source is wastewater (Rodgers *et al.* 2021). Potential sinks for UVF contamination include transport and/or transformation in air or water, adsorption to sediment, and/or uptake by biota.

In Year 1 (2020), we demonstrated that despite being a potential sink for oxybenzone, there was no build-up over time within the water column of the Cowichan River; status remained unknown for swim beaches around Cowichan Lake and for other UVFs of concern besides oxybenzone. Additionally, we demonstrated consistently little- to no- detection of oxybenzone downstream of the Joint Utility Board (JUB) outfall in the City of Duncan. However, detection of oxybenzone was notable downstream of the Town of Lake Cowichan outfall (TLCO). It was undetermined whether this was due to the TLCO directly or caused by recreational activity upstream.

In Year 2 (2021), we aimed to continue describing the presence and variability of oxybenzone and other UVFs of concern in water for both Cowichan River and Cowichan Lake, in addition to sediment and biota.

Below, we discuss the progress made towards these objectives and some specific results obtained in Year 2. We also address aspects of ongoing method development at VIU-AERL, some emerging studies about risks to aquatic life and degradation of UVFs (with options for treatment or mitigation), and we provide updated information about contaminant regulations being applied around the world.

5.1 Year 2 Results

5.1.1 River Discharge & Precipitation

River discharge and water temperature data reflect the hot and dry summer season experienced across the east coast of Vancouver Island in Year 2. In late June, a “heat dome” event and lack of significant precipitation further exacerbated the summer drought season. Mean daily river discharge was 30% lower in August 2021 than in August 2020 (4.92 vs. 7.08, respectively; ECCC 2021a), which may have been a contributing factor to the different oxybenzone results observed between years as higher river discharge has greater potential to dilute contaminant inputs (Turunen *et al.* 2020).

Precipitation was lower during the Year 2 sampling period, and air temperature was higher than the Year 1 sampling period. Average summer precipitation is not expected to alter river discharge significantly due to flow control at the Cowichan Lake weir, but precipitation and air temperature both impact users’ willingness to sunbathe or recreate in water, as well as users’ perceived need to wear sunscreen when outdoors (Turrisi *et al.* 1999).

Historical data (2000 – 2019) indicates the mean daily discharge for the Cowichan River is lowest throughout the months of July – September, with August typically experiencing the lowest discharge (ECCC 2021a). This coincides with the occurrence of drought, high air temperatures, high UV index, and peak summer tourism in the Town of Lake Cowichan (ECCC 2021b; K. Worseley, pers. comm, Feb 2022).

Catalyst Paper Excellence operates the weir at the outlet of Cowichan Lake (Slater 2018) with a license requiring a minimum flow of 7.08 m^3/s into the river when the weir control gates are fully opened. This minimum flow was not able to be met during the project Seed year (2019) (B. Houle, pers. comm., Feb 2021) nor again in Year 2 due to severe drought conditions observed in the watershed.

5.1.2 Water Quality

In Year 2, more water samples than anticipated were able to be collected, a result of volunteer support and the cost-effective analysis method. Unfortunately, due to instrument repairs in early summer delaying method development at VIU-AERL, only oxybenzone was able to be analyzed again during the Year 2 sampling window.

Spatial differences were assessed between sample sites, while temporal differences were assessed at the same sites through time. Differences between sites and dates were observed, but there was no clear pattern, and these differences were not considered statistically significant.

Spatially, the highest concentrations of oxybenzone were detected at swim beaches around the lake and at sites in the upper river where recreation is concentrated. Studies worldwide have found that sunscreen by-products do accumulate in recreational swimming areas and describe a contaminant "plume" which decreases with distance from the swimming area (Downs *et al.,* 2022; Labille *et al.,* 2020; Torres-Bejarano *et al.,* 2018). A similar distribution of oxybenzone was observed at Gordon Bay Provincial Park on July 17, 2021, when water samples were collected at an increasing distance from the beach swimming area (Table 3).

Mean oxybenzone results were highest for Cowichan Lake, with the maximum point-in-time result obtained from Gordon Bay Provincial Park (1,761 ng/L). Other studies worldwide have reported oxybenzone values in the range of 0.3 – 5,390 ng/L in river water (up to 44,000 in vicinity of wastewater treatment plants (WWTP); 0.8 – 200 in lake water; 4 – 4,500 ng/L in swimming pool water; and 10 – 9,900 ng/L in shower water (Ramos *et al.* 2015). Values above 1,000 ng/L are typically associated with either direct recreational inputs, or WWTP discharges (Ramos *et al.* 2015).

Comparison to past results

Across all water samples, the maximum oxybenzone concentration collected in Year 2 (1,760 ng/L) was more than eight times the maximum collected in Year 1 (211 ng/L) and three times the maximum collected in the project Seed year (570 ng/L). This result could have been influenced by the adjusted sampling plan developed for Year 2 (adjusted to capture peak use times, and more samples collected from Lake sites).

At sites in the upper 4 km of Cowichan River (Duck Pond, Greendale Trestle, Little Beach, and 500 m d/s TLCO), oxybenzone was highest in the project Seed year, followed by Year 2, then Year 1. This difference is likely influenced by several factors, including a smaller sample size in the Seed year, higher peak visitation clustered around weekends in the Seed year (pre-COVID-19 pandemic), changes to visitation trends since Year 1 due to COVID-19 pandemic restrictions, and variations in river discharge between years.

Year-to-year variation in weather patterns likely impacts the input and degradation of UVFs in the Cowichan River ecosystem as weather can influence recreational use and other environmental factors that affect molecule degradation (e.g., solar intensity) (Carstensen *et al.* 2022). Oxybenzone concentrations were observed to fluctuate significantly, both within and between sampling years.

Past findings suggest oxybenzone does not accumulate within the water column of the Cowichan River; the processes of molecule degradation were described in detail in the Year 1 report (Rodgers *et al.* 2021) in section 5.1.6. This was observed again in Year 2, at both River and Lake sites in the Cowichan watershed, giving confidence to the assumption that oxybenzone does not accumulate within the water column over time.

Quality Assurance

The high variation in oxybenzone results can be attributed to a number of factors, including but not limited to: varied inputs over time (e.g., fluctuating numbers of people wearing oxybenzone-containing sunscreens), varied mixing within the water column (potentially influenced by site characteristics, river discharge, water temperature, human disturbances, etc.), and human error (e.g., having multiple samplers across multiple sites).

We were unable to control for variations in sunscreen products applied by recreational users, mixing conditions, and human error. We attempted to understand the potential variation in ingredients by administering and collecting public surveys at select sampling locations (Appendix A); to control for mixing conditions by selecting a consistent sampling depth (30 cm) and location (middle of swim area (Lake), or downstream of target location as close to the point of obvious mixing as possible (River); and to control for human error by providing volunteer sample training, utilizing a standardized sampling protocol, and providing the same equipment, data sheets, and site directions to volunteers.

Quality Control

As addressed in Year 1, small sample sizes can skew results in favour of strong correlations. There is some difficulty in assessing results for the Cowichan watershed due to small sample sizes, different sampling designs and different analysis methods between project years. Sample design replication in future sampling years will help improve the statistical quality of analyses and comparisons.

Field blank samples were collected to determine the existence or magnitude of any contamination problem associated with sample containers, collection, handling, and transport (BC Ministry of Environment 2013). There was a detectable result for one field blank processed at Little Beach on July 11, 2021, indicating a contamination or analysis issue for this sample. A review of protocol was undertaken with the sampler and concluded that the most likely source of error was via aerosolized particles encountering the vial while uncapped (low likelihood) or via a lab reporting issue (higher likelihood). An additional field blank, sampled at Arbutus Park on the same date and also analyzed at VIU-AERL on the same date as the erroneous field blank, was below detection limits, as expected.

Duplicate samples were collected to measure the precision of sampling analysis, and environmental heterogeneity; a large difference between duplicate results may indicate samples were not representative of the background water (BC Ministry of Environment 2013). Duplicates not close in value could indicate either: a) oxybenzone was not adequately mixed throughout the water column during sampling, b) contamination between samples had occurred, or c) the analysis method was inaccurate between samples.

A relative percent difference (RPD) of up to 35% was calculated between duplicate samples; while other duplicate samples collected had acceptable RPD's of <10% (see 4.2.3). RPD values >20% typically indicate a possible problem and >50% a definite problem, most likely either contamination or lack of sample representativeness (BC Ministry of Environment 2013).

The moderate RPD for the two upper Cowichan River samples in Year 2 indicates a possible sample contamination, uneven mixing at time of sample collection, or variation in the analysis method. Duplicate samples collected from Cowichan Lake had much lower RPD (0-1%) than duplicates from the river (7-35%), possibly pointing to a mixing issue caused by river transport and water turbulence.

Further samples comparing River vs. Lake duplicates, with inclusion of results for lab duplicates and lab controls, will help improve results analysis and interpretation in future years.

Sample Transport & Storage

Two out of three vials broken during Year 2 were part of a temporal assessment of downstream TLCO concentrations (8 AM, 4 PM, 8 PM); this impacted the planned evaluation of TLCO discharge over time for Year 2. Two separate attempts were made to contact the Town of Lake Cowichan public works department to gain access to the wastewater treatment plant for sample comparison after this issue was discovered, but a response was not received within the timeframe of the sample analysis window. Future attempts should be made to confirm the concentration of oxybenzone in any treated discharges to the Cowichan River.

Future sample storage should be monitored for acceptable placement distance from refrigeration input to ensure homogeneous storage temperatures and avoid breakage due to frozen samples. Additionally, future samples collected should leave a space at the top of the vial for sample expansion in the rare event of sample freezing.

5.1.3 Recreation Monitoring

Despite the high numbers of observed swimmers, tubers, and recreationists on the August long weekend in Year 2, oxybenzone concentrations did not reach above 265 ng/L in the upper 4 km of river (also known as the popular "tubing reach" in the Town of Lake Cowichan), where the highest numbers of river users were counted. However, on the same date, oxybenzone concentrations reached a maximum of 1,761 ng/L at Gordon Bay Provincial Park on Cowichan Lake, where the highest number of lake users were counted.

The influence of air temperature on recreational behaviour is an expected result. However, it does highlight the importance of sampling during anomalous periods of high air temperature that may fall outside of the standard summer sampling window (e.g., the "heat dome" event in June 2021). This may also help narrow target sampling times in order to capture peak instantaneous UVF inputs in future years. Air temperature data may also be used to refine the start- and end-dates of the Year 3 sampling window, e.g., to begin and end sampling only when maximum daily air temperatures are above 20-22°C.

Taking into account metrics of tube rentals and total point-in-time counts of in-water users, the Year 2 results suggest that tourism levels may be returning to pre-COVID-19 levels in the Town of Lake Cowichan. Despite this potential impact on water quality for Year 2, there was no increase in oxybenzone sampled at Little Beach relative to Year 1 (Fig. 4-5); and, although the Duck Pond site had higher point-in-time measurements in Year 2 relative to Year 1, the mean oxybenzone concentration at this site decreased by 12% in Year 2 (n=8) relative to Year 1 (n=1).

Conversely, the data collected via visitor center engagements suggests that face-to-face interactions were down. Reduction in engagement numbers could be attributed to disruptions in Year 2 due to the operational transitions mentioned in section 3.0, and ongoing COVID-19 impacts since Year 1. This result may also partially explain why several individuals approached and asked to participate in the public questionnaire opted not to respond—the willingness for products to be photographed rather than providing verbal questionnaire responses was surprising, and indicates that alternative tactics for receiving responses should be explored in future project years.

In future, several factors will dictate total recreational pressure experienced in the watershed – including weather patterns and public health measures (e.g., travel restrictions, border closures). However, data from Year 2 indicates that popular swim beaches around Cowichan Lake do experience higher recreational pressures, with only a few select sites (e.g., the upper tubing reach) on the Cowichan River experiencing levels of recreational pressure within range of the lake sites.

Site conditions

Although the relationship between recreational users and oxybenzone concentrations was not as strongly positively correlated in Year 2, the data does suggest there may be a site-specific relationship that is dependent on either physical site characteristics and/or user behaviour (Fig. 11).

Further sampling to determine whether evidence for a site-specific relationship exists, and/or to quantify annual variation in the relationship between recreational users and UVF concentrations at each site, should be completed in Year 3. If evidence of site-specific relationships between recreational users and UVF concentrations are present, this could help prompt investigation of a critical "recreational use threshold" for each site. Monitoring for such a threshold could assist with focusing contaminant mitigation efforts by triggering public outreach about river-safe sunscreen alternatives when a recreational use threshold is reached at a specific site.

Potential UVF loading

Labille *et al.* (2020) performed public use surveys and found that 68% of surveyed beachgoers applied sunscreen while at the beach. The average user applied ~17.5 grams of sunscreen in a typical day, re-applying an average of 2.6 times. Labille *et al.* (2020) also calculated a recovery factor for the UVFs of concern released into water, which was about 30%.

We attempted to develop a similar estimate of UVF loading for the Cowichan River. Through recreation monitoring and local business data captured as part of this project, we estimated that the upper 20 km of the Cowichan River sees between 20 – 1,000 tubers and swimmers per day, contingent on whether it is a weekend or weekday, and which month of the summer season it is. Throughout the peak recreation season (62 days, July – August inclusive), this amounts to between 1,240 – 62,000 recreational users (or an average estimate of ~30,500 people for the season).

Multiplying the estimated total number of river users by 17.5 g sunscreen each gives a range of 21.7–1,085 kg of sunscreen potentially applied; this amounts to approximately 6.5–325 kg of sunscreen potentially released (or an average of ~160 kg over one summer recreation season). With a UVF recovery factor of 30%, an average of 48kg of UVFs could potentially be making their way into the Cowichan watershed each season.

However, it is necessary to consider that actual recovery values in the Labille *et al.* (2020) study were much lower than their modelled concentrations of UVFs, which they proposed could have been due to uptake through the skin barrier or partial photodegradation of UVFs in the environment. Additionally, this assumes that products are applied as creams, and not as aerosol sprays.

Public questionnaire results from the Cowichan watershed indicated almost half of the sunscreen products surveyed were applied as an aerosol spray (Appendix A; Fig. A9), which would influence the potential UVF load as estimated here. Two of these aerosol-type sunscreens surveyed in Year 2 were part of a Voluntary recall notice issued in 2021, due to potentially elevated benzene concentrations (Neutrogena and Coppertone aerosol sprays) within the product (Rendall, J., 2021).

Understanding an approximate range for UVF loading, as in the above example, allows for the development of more informed outreach strategies, and improves quantification of mitigation impacts.

5.1.4 Sediment

No oxybenzone was detected in Cowichan Lake sediments analyzed by either a commercial lab (ALS Global) or VIU-AERL. A review of existing literature (Ramos *et al.,* 2015) revealed one German study that also sampled lake sediments and similarly had no detection of oxybenzone (Rodil & Moeder, 2008). The maximum detection was 27 ng/g dry weight (d.w.), collected from river sediments in Spain located downstream of a wastewater treatment plant (WWTP) (Gago-Fererro *et al.,* 2011).

Other UVFs of concern, including octocrylene and enzacamene, have also been detected in river sediments and found to range from 0.4 – 2,400 ng/g d.w. and 4 – 8 ng/g d.w., respectively (the highest values being located downstream of WWTP) (Ramos *et al.,* 2015).

Further sampling of additional UVFs in sediment may reveal information about potential UVF sinks in the Cowichan River ecosystem; however, at this time the data does not point to lake sediment as a strong sink for oxybenzone. Future project years should endeavour to sample river sediments and perhaps also target peak use times (e.g. August long weekend) and non-recreation times (e.g. early April, late September) for a thorough overview of sediment conditions during fish rearing and spawning windows.

5.1.5 Fish Sampling

Life history varies within and between fish species (McPhail, 2007). Some species emerge from the river bed and migrate to the ocean within a few months (e.g. pink and Chinook salmon), while other species spend at least one year rearing in the freshwater natal environment (e.g. coho salmon) before migrating to saltwater.

The majority of resident rainbow, cutthroat and brown trout in the Cowichan River spend their entire lives within the freshwater environment (McPhail, 2007). Fish spending the majority of their life in freshwater are the focus for UVF impacts in the Cowichan River ecosystem, as there is a risk of exposure to UVFs from recreational use during periods of peak metabolism (e.g. during summer months when feeding and growth rates are highest) (Campana, 1999).

Electrofishing surveys indicate the upper 20 km of Cowichan River contains the highest densities of rainbow trout/steelhead fry and parr (McCulloch and Atkinson, 2019), with the highest concentrations occurring between 7–8.5 km (a few kilometres downstream from the most recreationally-intensive areas of the upper river (0–4 km). The majority of brown trout spawn in creeks that are tributary to the upper 2 km of the Cowichan River; fry, parr, and adults migrate to the mainstem at various ages (B. Anderson, pers. comm., 2021). Brown trout likely spend most of their lives rearing within the upper 20 km (B. Anderson, pers. comm., 2021). The upper Cowichan River is thus critical habitat for these two trout species, and there is overlap between resident trout rearing habitat and areas of significant recreation/UVF contamination.

Many studies have acknowledged the potential risks to aquatic life of long-term or chronic exposure to lower, environmentally-relevant concentrations of UVFs during sensitive life stages, e.g. juvenile rearing, or under conditions that enhance biotic stress response, e.g. high water temperatures (Campos *et al.,* 2017; Kim *et al.* 2014; Muniz-Gonzalez and Martinez-Guitarte, 2020; Ozàez, Martinez-Guitarte and Morcillo, 2013; Scheil, Tiebskorn and Kohler, 2008).

Due to a lack of primary literature about chronic exposure to UVFs at environmentally-relevant concentrations within freshwater systems, especially ecosystems within North America, developing a reliable method to assess UVF accumulation in fish tissues continues to be a major objective for this project. Progress on method development is described in section 5.2, below.

5.2 Method Development

A technical summary of analysis method development at VIU-AERL is included in Appendix C. Methods were previously refined during Year 1 for oxybenzone in water (Vandergrift *et al.*, 2022).

5.2.1 Other UVFs of concern in water

During winter 2022, the UVFs enzacamene (4-MBC), octinoxacte (EHMC) and octisalate were able to be detected and calibrated in the low ng/L range using a modified version of the CP-MIMS-LEI/CI protocol used in Vandergrift *et al.* (2022). Increased sensitivity was also achieved for oxybenzone (<20 ng/L). Octocrylene was tested, but not found to be compatible with analysis using the CP-MIMS-LEI/CI method.

This advancement in analysis methods will allow for future water samples to be analyzed for all four UVFs of concern at one time, using the same sample volume, with an average rate of 7.5 samples per hour.

5.2.2 Sediment

During mid-summer 2021, marine test sediments were collected to aid in method development at VIU-AERL. An initial calibration curve for oxybenzone in sediment yielded 73% recovery. Additional tests in winter of 2022 with a lab standard sediment were run over a ~40-minute period, spiking the stirred sediment slurry with known concentrations of oxybenzone and activated carbon at different times in order to observe oxybenzone adsorption to the sediment slurry.

No notable changes were observed in oxybenzone concentrations when added to the sediment slurry other than noise, suggesting minimal uptake by the lab standard sediments; whereas, oxybenzone concentrations dropped immediately after adding activated carbon to the sediment slurry, as expected.

These results suggest oxybenzone did not adsorb well to sediments tested. Method refinement and positive control samples are still needed to obtain reportable results for oxybenzone in sediment through VIU-AERL. Possible strategies include changing the pH of the sediment slurry, and/or using river- or lake-based sediments for the spiking tests rather than the lab standard.

While not yet comparable to the HPLC/MS/MS method in use by ALS Global, further method development by VIU-AERL will yield a rapid and cost-effective analysis method for sediment.

5.2.3 Fish Tissue

VIU-AERL completed sample extractions from fish test tissue to aid in method development, but have not yet been able to run these extractions using CP-MIMS-LEI/CI due to issues with probe immersion in high-concentration lipids. Alternate analysis methods are being explored using gas chromatography (GC-MS), and sample analysis is in process.

Due to the nature of the CP-MIMS-LEI/CI method, solutions must be buffered to pH<7 for optimal extraction; however, lower pH increases the probability of fatty acids crossing the probe membrane and damaging the instrument. This was the cause of the spring and early summer instrument repairs/delays at VIU-AERL. Due to the heavy reliance on equipment across all university courses and other clients, the risks of further membrane damage must be mitigated. Options are currently being investigated within VIU-AERL; at this time, tissue method refinement is expected to take, at minimum, an additional 6-12 months.

5.3 Emerging Studies

Despite a growing body of evidence showing sub-lethal impacts across many different aquatic species, the scientific community appears to remain divided about the urgency and toxicity of UVFs in the aquatic environment. Recent studies have suggested there is low environmental concern in freshwater environments of the USA (Burns *et al.,* 2021), while other studies highlight a need for increased investigation, especially in high-use coastal environments (Kwon and Choi, 2021; Levine, 2021).

A common statement within the literature is that environmentally-relevant risks are difficult to quantify in a strictly laboratory setting, and require further environmental investigation. Given this, the field of UVF research has been rapidly intensifying at a global scale, with a specific focus on human and aquatic ecosystem health.

5.3.1 Risks to Aquatic Life

Potential impacts to aquatic life demonstrated via lab-controlled experiments and peer-reviewed studies include endocrine disruption, bioaccumulation, and biomagnification at high concentrations of exposure and across varying types of UVFs (Gago-Ferrero *et al.,* 2015; Tsui *et al.,* 2014).

In the Year 1 report (Rodgers *et al.* 2021), specific risks of UVFs were described in detail in section 5.6, but no specific values were reported for chronic or acute ecotoxicological thresholds. Additionally, several studies have been published or recently become accessible online since the Year 1 report, some of which are summarized below.

Toxicity thresholds

Previously reported Predicted No-Effect Concentrations (also known as PNEC) of oxybenzone for aquatic organisms ranged from 1,320–1,800 ng/L (Kim and Choi, 2014; Du *et al.,* 2017). More recent literature, published within the last twelve months, suggests a new PNEC of 73,300 ng/L may be more appropriate (Jung *et al.,* 2021).

The large range in possible PNEC values stems from different methods used to calculate the PNEC (assessment factor (AF) method vs. species sensitivity distribution (SSD) method), and different species studied (e.g., a range of model organisms such as green algae (*Chlorella vulgaris, Pseudokirchneriella subcapitata*), water fleas (*Daphnia magna, Moina macrocopa*), and fish including zebrafish (*Danio rerio*), Japanese medaka (*Oryzias latipes*), and Eurasian carp (*Cyprinus carpio*)).

For both the AF and the SSD assessment methods, method performance depends on data quality (e.g. sample size, variation) and species sensitivity to the compound being assessed. Both methods have garnered criticism and support among researchers (Sorgog & Kamo, 2019): whereas AF tends to be derived from ecotoxicological endpoints observed on a species-specific scale, SSD considers toxicity data across a range of different species and uses AF results in its analysis (Sorgog & Kamo, 2019; Jung *et al.,* 2021). This makes the SSD method potentially more reliable, both due to its use of confidence intervals and because it improves the range of possible species applicability (Sorgog & Kamo, 2019).

While a "one-size-fits-all" approach such as SSD may be helpful for applying toxicity thresholds from a regulatory perspective, it may not be the most appropriate method from a site-specific standpoint such as the Cowichan watershed, especially given many studies report varying degrees of stress responses across different species. Focusing on studies quantifying *in vivo* impacts to freshwater rainbow trout (a model organism) will be most directly relevant to the Cowichan watershed.

Unfortunately, few studies to date have been produced with specific relevance to the same fish species found in the Cowichan, and/or the specific UVFs that VIU-AERL is currently capable of analyzing in water (Buser *et al.,* 2006; Coronado *et al.*, 2008).

The majority (96%) of water samples with detectable oxybenzone concentrations collected in Year 2 were well below any reported PNECs. Only 4% of detectable samples (*i.e.*, 2 of 56), both collected from swim beaches on Cowichan Lake, were at or near the previously reported PNECs of 1,320–1,800 ng/L (Kim and Choi, 2014; Du *et al.,* 2017; Jung *et al.,* 2021).

No samples were close to any of the previously reported thresholds for acute toxicity (e.g., LC_{50})[3], in any of the studies reviewed (generally concentrations >100,000 ng/L).

Multigenerational Effects of UVFs in Daphnia

A study by de Paula *et al.* (2022) exposed water fleas to low concentrations of UVFs and observed impacts through multiple generations. Exposure to environmentally-relevant concentrations (170 – 4,400 ng/L) of four different UVFs (oxybenzone, octinoxate, avobenzone and octocrylene) were tested by exposing two consecutive generations of the water flea, *Daphnia magna.* Each generation (parents & offspring) was exposed for 21 days (de Paula *et al.,* 2022).

Findings showed the concentrations were not lethal but could produce "sublethal effects", like reproductive interference, in the second-generation *D. magna*. Among the four UVFs analyzed, oxybenzone showed the highest chronic toxicity potential. At concentrations of 170 ng/L, oxybenzone was capable of reducing reproduction rates and inducing antioxidant defence activity in the second-generation *D. magna*.

Multigenerational studies, as opposed to single generation lab studies, consider longer-term impacts through the life cycle of affected organisms and may be a more practical approach to understanding the environmental effects of UVFs.

Daphnia magna is most commonly found in Eurasia, but many *Daphnia* species are found worldwide and are known to provide a food source for larger invertebrates such as midges, true bugs, and other insects commonly associated with the diets of fish and mammals (Ebert, 2005; Klecka & Boukal, 2012).

Bioaccumulation of UVFs in the food web of a freshwater lake

Lyu *et al.* (2022) examined the bioaccumulation and trophic transfer of 12 UVFs in wild species found in China's fifth-largest freshwater lake (Lake Chaohu, located southwest of Nanjing). They found most compounds analyzed were present in all species studied, with concentrations ranging from 5.4–131 ng/g, d.w.

Findings showed the UVF enzacamene (also known as 4-MBC) increased with species' trophic levels. The study authors assigned it a "trophic magnification factor" of 3.79. Other UVFs studied had no significant relationship or were assigned a "trophic dilution factor" of <1.

This highlights enzacamene as a compound of interest to study in the Cowichan watershed; fortunately, methods of analysis are now available through VIU-AERL.

[3] – LC_{50}: lethal exposure concentration at which 50% of the sample population experiences mortality.

Effects of UVF mixtures on Chironomus midges

A study by Ozàez *et al.* (2016) attempted to evaluate the toxicity of UVF compounds when applied as a mixture vs. in isolation. They combined enzacamene (4-MBC), octinoxate, and oxybenzone – ingredients commonly blended in sunscreens and certainly found in mixture in recreational waters – and tested the effect on survival of the larval stage of freshwater midges, *Chironomus riparius*, when exposed to very high concentrations (> 0.1 mg/L, or 100,000 ng/L).

Findings showed UVF mixtures had a similar impact on mortality as individual compounds. The mRNA for the heat shock protein gene, *hsp70*, increased after exposure to all mixtures and individual compounds (a potential indicator of metabolic stress). However, mRNA for the ecdysone receptor gene, *EcR*, increased under exposure to the lowest concentration (100,000 ng/L) of 4-MBC alone then returned to normal when exposed to the UVF mixtures. This highlights the complexity of UVF mixture impacts.

These concentrations of oxybenzone are not known to exist in the Cowichan watershed, although concentrations of enzacamene in Cowichan water have yet to be tested. Additionally, some species of chironomids are relatively tolerant of pollution, while others can be sensitive to pollution. Chironomids are known to reside and feed in close contact with sediments and are a major source of food for freshwater fish (Klecka & Boukal, 2012).

5.4.2 UVF degradation

The scope of this report does not investigate specific timing of UVF degradation within the Cowichan River ecosystem, but literature can be used to infer a range of expected values for persistence in the environment.

Because oxybenzone degradation is driven by molecular reactions with the free hydroxy radical, OH^- (Semones *et al.,* 2017), the time it takes to break down in the natural environment depends on several factors including temperature, microbial activity, intensity of solar radiation, and the nature and concentration of dissolved organic matter (De Laurentiis *et al.,* 2013; Li *et al.,* 2016; Ramos *et al.,* 2015; Semones *et al.,* 2017; Vione *et al.,* 2013).

Degradation of oxybenzone will happen more readily at the water's surface (high solar energy) and/or when compounds that promote the production of OH^- are present (such as dissolved organic matter (DOM) or nitrate (NO_3^-), (Semones *et al.,* 2017); both of which can be found in treated wastewater effluent, or naturally throughout a watershed).

Oxybenzone into derivative metabolites

Oxybenzone is readily degraded to its metabolites (e.g. BP-1, BP-2, BP-8, 4-hydroxybenzophenone). These metabolites may be further broken down by fungal or microbial degradation (Gago-Ferrero *et al.,* 2012b), but little is known about their potential presence or accumulation within the Cowichan watershed.

There is concern that the complex metabolites produced by degrading UVFs may have toxic impacts on aquatic life, and may themselves be less readily degraded than their parent compounds (Carstensen *et al.,* 2022). BP-1 in particular has been found to have a more substantial estrogenic impact than oxybenzone.

Octocrylene into derivative benzophenone

Downs *et al.* (2021) aimed to determine whether concentrations of benzophenone (of which oxybenzone is itself a derivative metabolite) would increase in a sunscreen product over time and whether octocrylene was the likely source of that benzophenone. After a six-week stability aging protocol, the study found 16 octocrylene-based products had greater concentrations of benzophenone than at the start of the aging protocol (average of 75 mg/kg, as compared to 39 mg/kg at the start). Benzophenone was also detected in a pure standard of octocrylene but was not detectable in a product that did not contain octocrylene.

The authors called for a rapid review of octocrylene as an approved SPF ingredient due to the risk of exposing humans to absorption of benzophenones through the skin. This study also highlights the complexity of molecule transformations that may be occurring in the natural environment.

5.4.3 Options for treatment/mitigation

Treatment of wastewater

Wastewater treatment facilities have been identified as a major source of indirect UVF inputs to freshwater systems worldwide (Balmer *et al.,* 2005; Evans, 2019; Ramos *et al.,* 2015). Untreated wastewater is the highest potential source of UVF contamination, because the treatment process naturally enhances degradation of benzophenone compounds by exposing them to physical (filtration, UV light) and chemical (chlorine) processes, as well as exposing UVFs to high concentrations of organic matter (Semones *et al.,* 2017).

Oxybenzone concentrations were higher downstream of the Town of Lake Cowichan wastewater treatment outfall (TLCO) than downstream of the Joint Utility Board outfall (JUBO) in Year 2. The TLCO currently treats wastewater using direct filtration, chlorine, and ultraviolet disinfection (soon to add soda ash for pH adjustment) (McGonigle, 2020). The JUBO treats wastewater using direct filtration, oxidation, alum and chlorine (Reitsma, 2018). The difference in oxybenzone results between TLCO and JUBO may be due to upstream recreation at Little Beach, as the TLCO sample site was less than 2 km downstream of Little Beach.

An attempted survey of TLCO concentrations at three stages during the day (8 AM, 4 PM, 8 PM) was undertaken on August 1, 2021. Early-morning and mid-afternoon samples were collected as single samples, whereas the evening sample was collected in duplicate. Sample transport and delivery to VIU-AERL went as planned, but the early-morning and mid-afternoon samples were both lost in sample storage due to low refrigeration temperatures and placement of vials that resulted in breakage due to sample freezing.

Questions therefore remain whether the concentrations of oxybenzone downstream of the TLCO are due to the TLCO or are a result of upstream recreation. This should be pursued again in Year 3, with advance arrangement to sample influent and effluent from the Town of Lake Cowichan wastewater treatment facility.

The removal efficiency of oxybenzone and other UVFs (e.g. octocrylene) from treated wastewater effluent has been reported as high (68–100%) (Balmer *et al.,* 2005; Palmiotto *et al.,* 2018), but other UVFs such as BP-4 and phenylbenzimidazole sulfonic acid (PBSA) may persist even after wastewater treatment (average removal efficiencies of 28% and 16%, respectively; Palmiotto *et al.,* 2018).

If other UVFs of concern are tested for in future project years, samples should continue to be collected downstream of WWTPs. Further efforts should be undertaken early in the season to secure access for sampling of influent and treated effluent from the Town of Lake Cowichan WWTP.

Options for mitigation: regulatory

Mitigation options to reduce recreational inputs of UVFs to the Cowichan watershed include both regulatory and public awareness measures.

From a regulatory perspective, oxybenzone and many UVFs in use today have been acknowledged by several countries as emerging environmental pollutants. In 2019, the US Food and Drug Administration removed several chemicals commonly found in sunscreens from the Category I GRASE ("generally recognized as safe and effective"). Twelve of these were demoted to Category III, including oxybenzone, homosalate, octocrylene, avobenzone and other UVFs. This was done on the basis that the currently available literature raises questions about endocrine impacts, absorption, and systemic exposure, with limited or no data characterizing their absorption (US Food and Drug Administration, 2019).

The State of Hawaii famously made headlines in 2018 as the first to pass a law banning the sale of over-the-counter sunscreens containing oxybenzone and octinoxate; this law has now come into effect as of January 2021 (Wu, 2020a). Other states and countries followed suit after this ban. Companion bills are proposed for 2023 that would limit sunscreen products to only those containing GRASE ingredients in order to be sold or distributed in Hawaii (Wu, 2020b).

The European Parliament includes 28 common UVFs (including oxybenzone, octocrylene, homosalate, and PBSA) under cosmetic regulation, limiting their maximum concentrations in cosmetic products (European Commission, 2018). Products containing oxybenzone are required to contain the warning of "Contains Benzophenone-3" unless the concentration is <0.5 % and used only for product protection purposes (European Commission, 2018).

Despite the growing body of research and the mounting concern about the impacts of UVFs on human and environmental health, no consistent regulations are limiting maximum concentrations in urban wastewater effluent or within the natural environment to protect aquatic life (European Commission, 2018).

Part of this stems from the complicated fact that there are many varieties of UVFs in use today, all with different chemical properties, and many with limited scientific studies quantifying impacts to aquatic and terrestrial species. However, as a rapidly emerging area of research with many innovative technological advancements, studies published over the past 10–20 years have considerably broadened the knowledge of UVFs across the scientific community.

In Canada, sunscreen regulation is a blend of both European and US regulations (Pirotta, 2015), and sunscreen ingredients are regulated under the *Food and Drugs Act* (Health Canada 2017). Health Canada currently states: "more information is needed to determine if the ingredients in sunscreens pose a safety risk when absorbed in humans", and encourages people not to stop using sunscreen because of its health benefits of helping prevent skin cancer and protecting against premature aging and sunburn.

The recommendation from Health Canada is to continue using sunscreen and wearing protective clothing to mitigate UV exposure, mentioning the agency "will continue to work closely with international partners, including the FDA, to review any new studies on the safety of sunscreens" (Health Canada, 2017).

Encouraging stronger environmental regulation by promoting the inclusion of oxybenzone as a regulated contaminant in the BC Provincial Water Quality Guidelines (WQGs) for the Protection of Aquatic Life (BC Ministry of Environment, 2019) is an intended outcome of this project. WQGs are based on the most current scientific information available and provide direction and information to decision-makers. WQGs can be used as a basis for wastewater management and can address both long-term chronic and short-term acute contaminant scenarios (BC Ministry of Environment, 2019).

Options for mitigation: awareness

Casas-Beltran *et al.* (2021) studied tourist behaviour and sunscreen contamination in Mexico. The study authors concluded that, in the absence of strong regulations and improved labelling practices, the health of the aquatic ecosystems would depend on individual tourists' responsibility to make environmentally friendly choices. Moreover, they found that tourists struggled to make ecologically responsible decisions due to a lack of clear guidance about which types/brands of sunscreen minimize risk to aquatic ecosystems or which ingredients should be omitted. This type of societal barrier is difficult to address within the scope of our project work, but we can incorporate this into public outreach to help educate consumers about reading labels and which ingredients can be avoided.

A case study from an outreach and education campaign held in Kahaluu Bay, Hawaii between April 2018– November 2019 showed the campaign resulted in a significant decrease in environmental oxybenzone levels within the bay (Levine, 2021). Oxybenzone levels dropped ≥ 93% at four out of five water sampling sites, coinciding with a local education and outreach campaign by the Kahaluu Bay Education Center to reduce beachgoer use of chemical-based sunscreens. Outreach strategies included signage, outreach booths with educational brochures/materials, sunscreen-swapping events, and more than 10,000 "reef-safe" sunscreen samples. The volunteer efforts encouraged the use of sunscreens containing only non-nano zinc oxide or titanium dioxide-based alternatives to chemical sunscreens containing UVFs like oxybenzone (Levine, 2021).

Matouskova & Vandenberg (2022) presented six core principles relevant to environmental health decision-making and investigated oxybenzone pollution in aquatic environments using that framework. Oxybenzone was examined through six lenses to evaluate human need (protection from ultraviolet damage) versus environmental cost. The authors concluded the risk outweighed the benefit of continued use and identified alternatives to using oxybenzone, including: 1. non-technical solutions, e.g. shirts, hats (preferred), 2. safer chemical UVFs (where necessary), and 3. inorganic UVFs, e.g. zinc and aluminum oxides (to be used with caution).

Community-based social marketing (CBSM) is a strategy that, when used correctly, can help change human behaviours. Frequently it is used by non-profit organizations, government, the healthcare industry, and marketers to connect with audiences, shift mentality and change behaviour. The core of this strategy is the idea that "a new behaviour should have a seemingly higher value than the current behaviour" (McKenzie-Mohr, 2000). Future project years should employ specific CBSM tactics in environmental education and public awareness campaigns.

Environmental education can facilitate knowledge transfer from the scientific community to the broader public, helping influence human behaviours. Admittedly, achieving reductions in human-caused impacts is likely non-linear and may take years to accomplish; however, a recent study conducted a systematic review of environmental education outcomes (Ardoin *et al.,* 2020). This review concluded that successful environmental education programs, *i.e.* those that documented direct impacts, included: 1. a focus on localized issues or locally relevant dimensions of broader issues; 2. collaboration with scientists, resource managers, and/or community organizations; 3. integrated action elements; and, 4. integrated intentional measurement and reporting structures.

This project includes several of these aspects already, e.g. presenting relevant findings from the local watershed as concrete results and explaining how local species (e.g. trout) are impacted; highlighting lab partnership and research funding attained through VIU-AERL; and, continuing to partner with resource managers and volunteer societies.

The sunscreen currently provided by the Tube Shack local business in Lake Cowichan is Stream2Sea, an 8.8% non-nanotized titanium dioxide based product whose marketing includes "No oxybenzone, avobenzone, octinoxate and octocrylene". This product should be explored as a potential option for leveraging existing outreach, and for providing samples to recreationists in future years.

Future project years should incorporate aspects of integrated action elements and intentional measurement/reporting by providing and tracking amounts of sample products used, asking for individual commitments to product and behavioural change, giving a follow-up survey about thoughts on the alternative product, and comparing water quality results pre-and post- outreach efforts. All of this will contribute to the success of ongoing future project work.

6.0 RECOMMENDATIONS

This report summarizes results from the second full year of collaborative UV Filter (UVF), *i.e.* sunscreen, monitoring in the Cowichan watershed. Project goals included: describing the nature and extent of UVF contamination within the aquatic ecosystem, improving understanding of potential impacts to resident aquatic organisms, and encouraging public education, outreach and regulatory measures to help mitigate UVF inputs to the watershed.

Specific objectives for Year 2 were to continue describing oxybenzone contamination at primarily recreational sites in the watershed, to investigate other UVFs of concern in water, and to analyze both sediment and biotic tissues for oxybenzone.

Partly a result of instrument repair and delays in matching research funds, these objectives were partially completed in Year 2 and are summarized within this report. Methods are now in place to analyze for oxybenzone and other UVFs of concern (enzacamene, octinoxate and octisalate) in water, as well as oxybenzone in sediment. While tissue methods have been delayed, advancements are in progress using alternate approaches.

With the current Snow-Water Equivalent for Heather Mountain (Station ID # 3B24P) at only 87% of the long-term median for March 1 (BC River Forecast Center, 2022a & 2022b), there is potential for another hot, dry summer season with low discharges in the Cowichan River heading into Year 3.

1) Continued monitoring for Year 3 should focus on high-impact recreational sites in the lake and river, as identified in Years 1 & 2 (e.g. Gordon Bay Provincial Park, Arbutus Park, upper 4km of river, Sandy Pool). Future analysis must include a broader range of UVF contaminants, for which methods were developed in 2021.

2) Influent/effluent sampling at the Town of Lake Cowichan wastewater treatment plant must be attempted again in Year 3 to determine if UVFs are discharged at the TLCO; sampling should include all four UVFs of concern.

3) Biota and sediment sampling should continue in Year 3. Sediment should be collected from downstream of the TLCO and at specific river recreation sites. Tissue analysis in partnership with VIU-AERL must be finalized in order to assess potential bioaccumulation in the Cowichan River's resident aquatic species and compare results to frozen tissue samples from prior years.

4) Expanded outreach and education is planned for Years 4 and 5 of this project, but preliminary public education should continue to be pursued in partnership with project stakeholders and local businesses in the Town of Lake Cowichan.

ACKNOWLEDGEMENTS

We would like to recognize the Habitat Conservation Trust Foundation and the anglers, hunters, trappers and guides who contribute to the Trust, for the ongoing financial support for this project. We would also like to recognize the RBC Foundation for their continued financial support. The Regional District of Nanaimo supported a project expansion to select RDN watersheds in 2021, which provided comparisons for the Cowichan samples. In 2022, Mitacs partnered with BCCF and VIU-AERL to provide significant support for method development and sample analysis via an Accelerate grant.

Thanks are due to the staff, volunteers and community partners who assisted with this project in Year 2: Mike Patrick, Dianne Flood, Jim Deck, Karen Deck, Marley Bates, Henry James, Rebekah Aplin, Ally Badger, Jeff Strohm, Aaron Androsoff, Eli Riedl, Joseph Monaghan, Harry Tassell, Ian Knipe, Judy Wickland, Kathy Doyle, Iain Doyle, Emma Simard-Provencal, Alex Lupin.

Thank you to Cowichan Tribes, Peter Leacock, and Joe Saysell for land access to collect water quality samples from the Cowichan River. Thank you also to the Cowichan Lake and River Stewardship Society for continued support and involvement, and to Aaron Frisby and the staff at the Tube Shack in Lake Cowichan for data sharing and participation, and promoting education about river-safe sunscreen to your customers. Thanks to the Cowichan Lake District Chamber of Commerce for sharing data in support of this project.

Thank you to the staff and students at VIU-AERL research lab for all the analysis, and development of analysis methods in 2021. We are looking forward to continuing this partnership in 2022 and beyond.

The British Columbia Conservation Foundation (BCCF) is a charitable non-profit society, dedicated to promoting and assisting in the conservation and stewardship of British Columbia's fish, wildlife, and their habitats. For more information or to become involved, please visit: www.bccf.com

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PERSONAL COMMUNICATION

Aaron Frisby Owner, The Tube Shack. Lake Cowichan, BC.

- E-mail and text message communication with T. Rodgers, September 1-30 2020.
- E-mail communication with T. Rodgers, March 1 2022.

Brian Houle Manager, Environment. Catalyst Paper Excellence Canada. Crofton, BC.

- E-mail communication with T. Rodgers, February 5 2021.
- E-mail bulletin: “Special update re: pumping from Cowichan Lake”, October 22 2019.

Brendan Anderson Senior Fisheries Biologist, Fish and Wildlife. MFLNRORD Region 1. Nanaimo, BC.

- E-mail communication with J. Atkinson, February 5 2021.

Katherine Worsley Coordinator, Cowichan Lake District Chamber of Commerce. Lake Cowichan, BC.

- E-mail communication with T. Rodgers, February 6 2021.
- E-mail communication with T. Rodgers, February 18 2022.

APPENDIX A – PUBLIC SURVEY RESULTS

BC CONSERVATION FOUNDATION July 2021 trodgers@bccf.com

UV Filter (Sunscreen) Questionnaire

Sampler name: ________________________ **Location:** ________________________

Hello, we are conducting a sunscreen usage study in partnership with VIU, and are wondering if you would be willing to share some information for our study? I have 4 questions, and it will take about 5 minutes

Your responses will be anonymous, and will be used to better understand the impacts of sunscreen on the aquatic environment.

Wearing sunscreen? Yes / No / NA

1. What is the brand of sunscreen in use today?

Brand Name: ____________________________ Chemical / Physical / NA (circle)

☐ Photo taken: ______________ ☐ Waterproof? Spray / Lotion (circle)

Active Ingredients: ______________________________ % ________

______________________________ % ________

______________________________ % ________

2. Thinking about today, or a typical beach / river day:

   How many minutes do you wait before bathing after you apply sunscreen? ________ or NA

   How many times do you re-apply during the day? ________ or NA

3. Are you aware, either via the news or other sources, about any of the environmental impacts of sunscreen?

   Yes / No / NA

4. Does the idea of a "fish-safe" sunscreen product appeal to you?

   Yes / No / NA

Care to elaborate? ____

____

____

Figure A1. Public questionnaire used in Year 2.

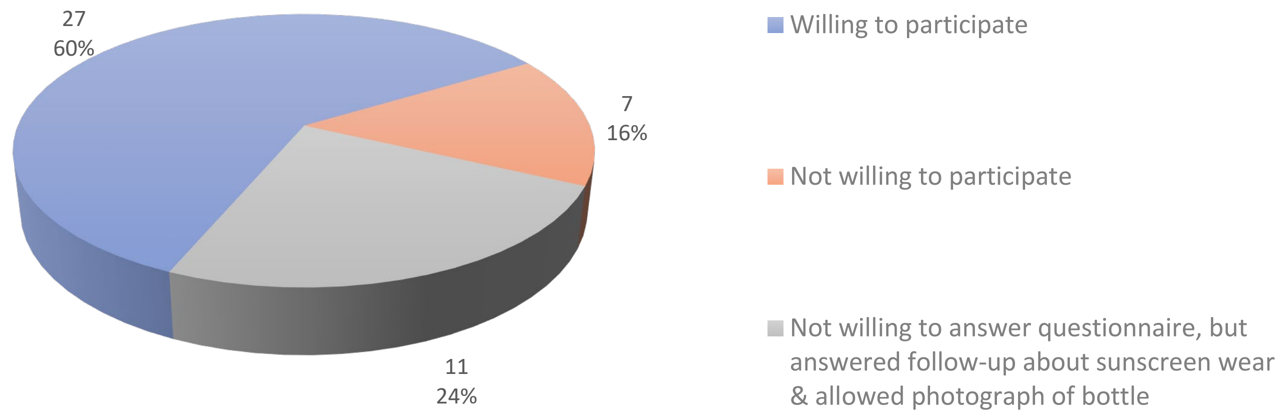


Figure A2. People approached with a public questionnaire, and their responses (n=45).

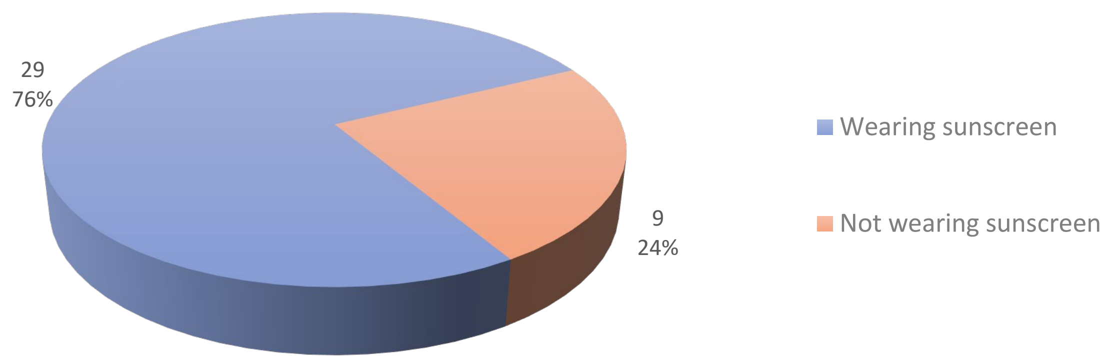


Figure A3. Respondents' self-assessment of sunscreen wear (n=38).

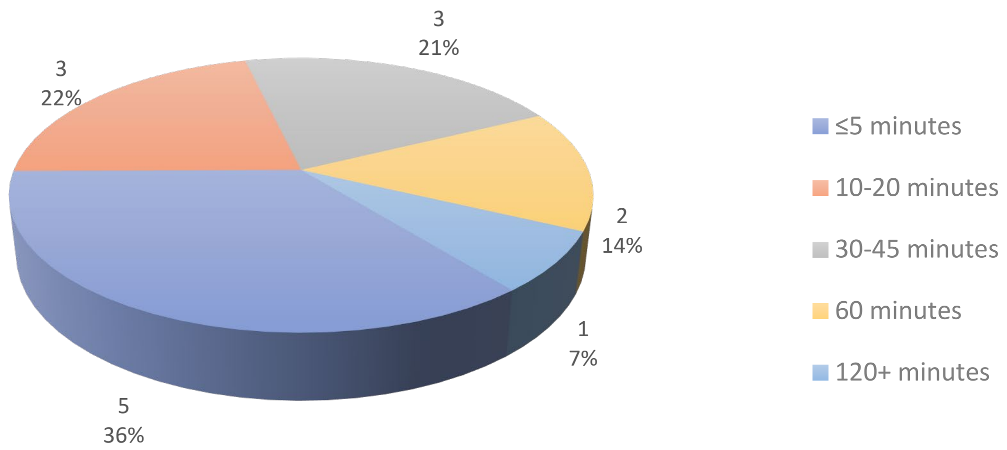


Figure A4. Respondents' self-assessed time between applying sunscreen and bathing in water (river or lake) (n=14).

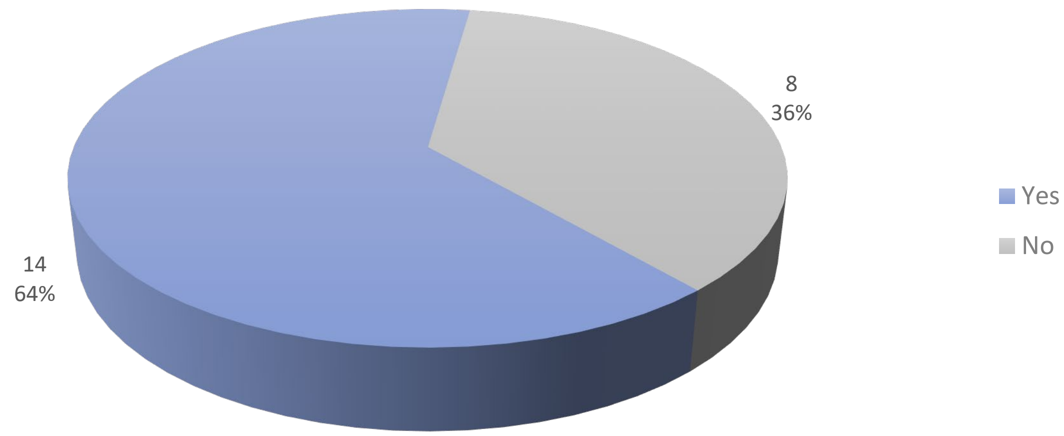


Figure A5. Respondents' self-assessed awareness of environmental impacts of sunscreens.

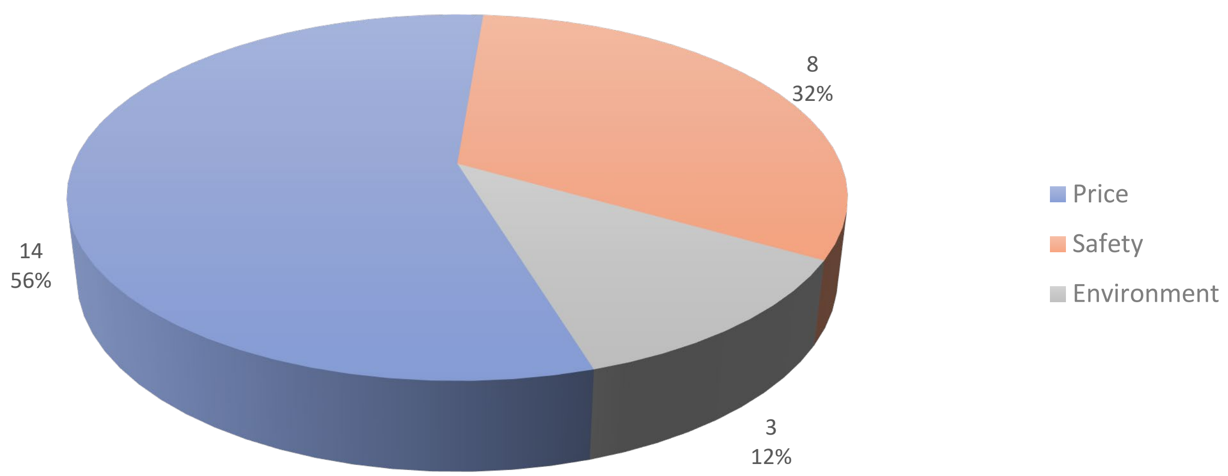


Figure A6. Respondents' main motivation for product selection (n=25).

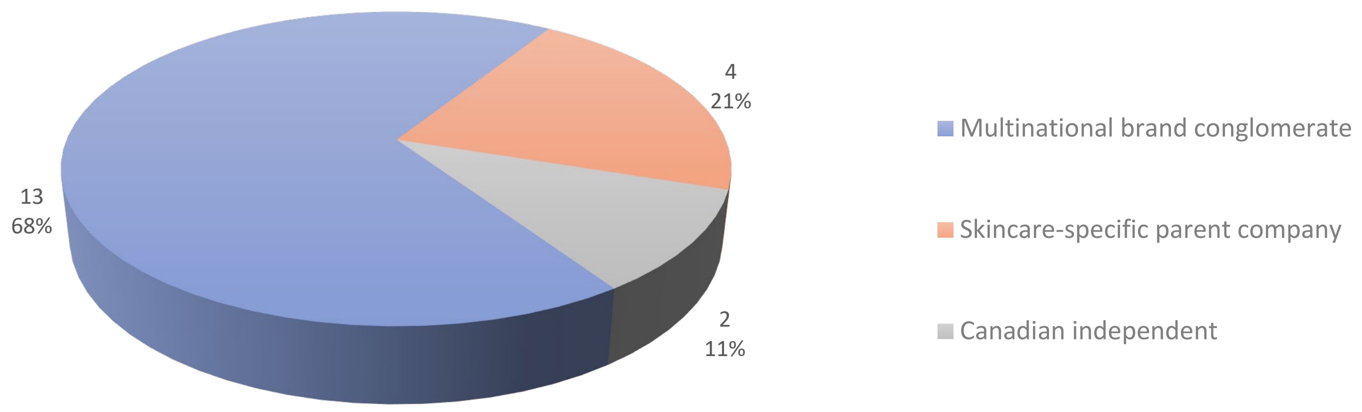


Figure A7a. Classification of product manufacturer type for sunscreen products in use (n=19).

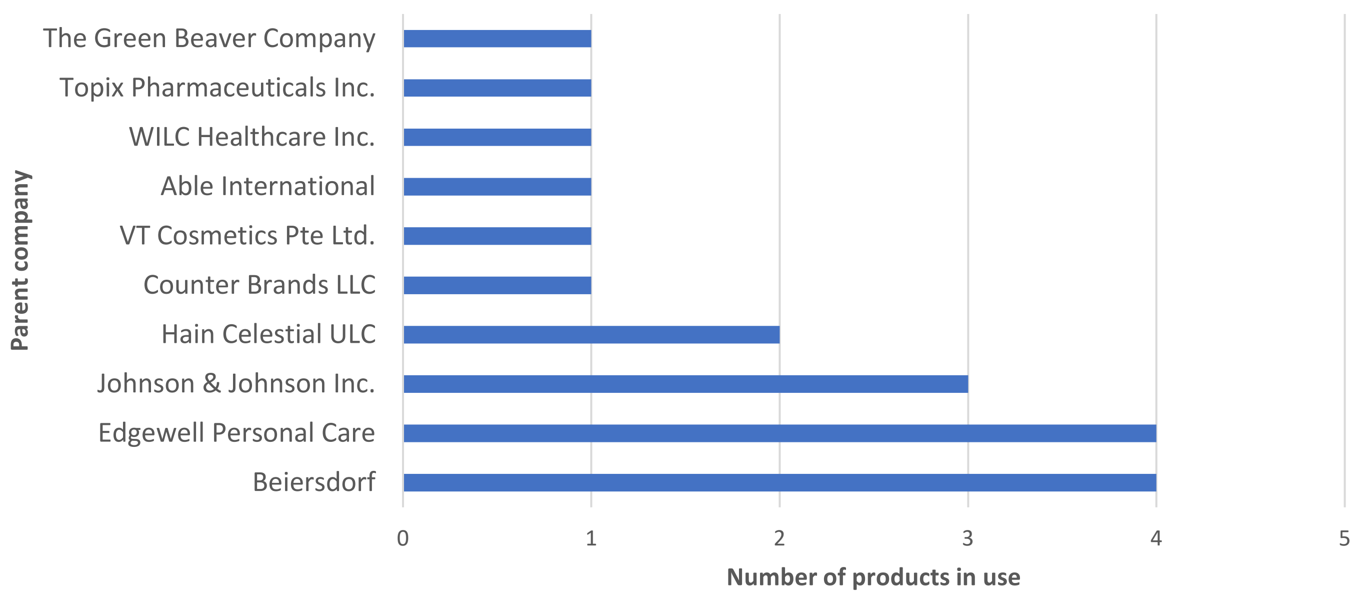


Figure A7b. Classification of product manufacturer name for sunscreen products in use (n=19).

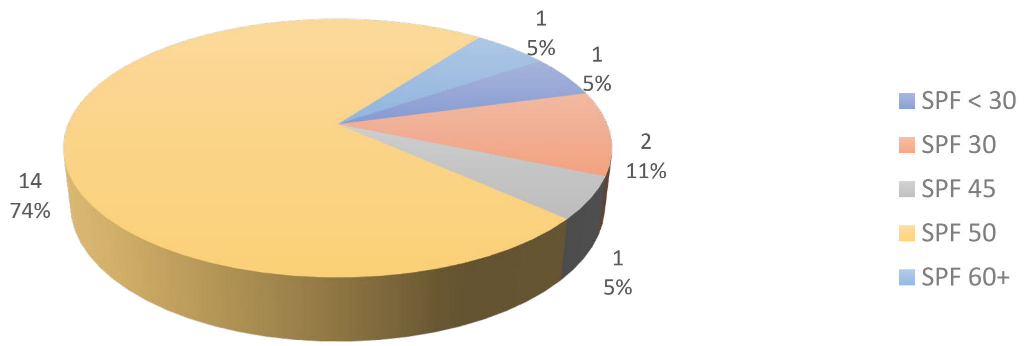


Figure A8. SPF rating of sunscreen products in use (n=19).

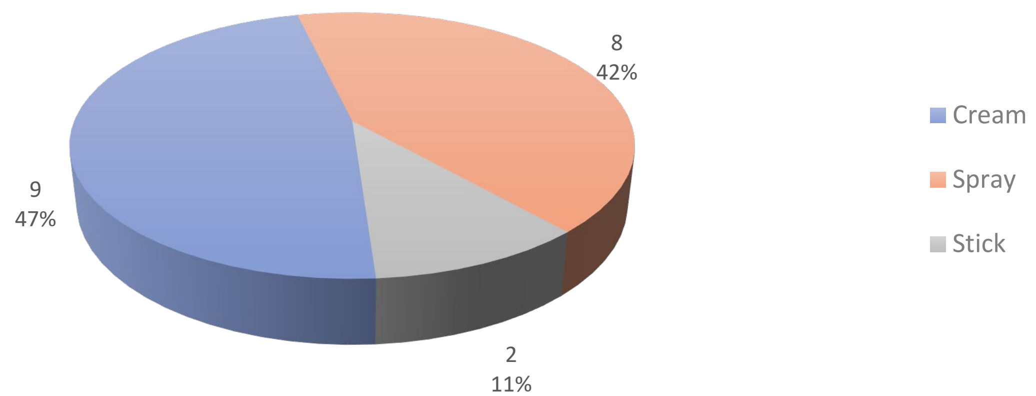


Figure A9. Type of application strategy for sunscreen products in use (n=19).

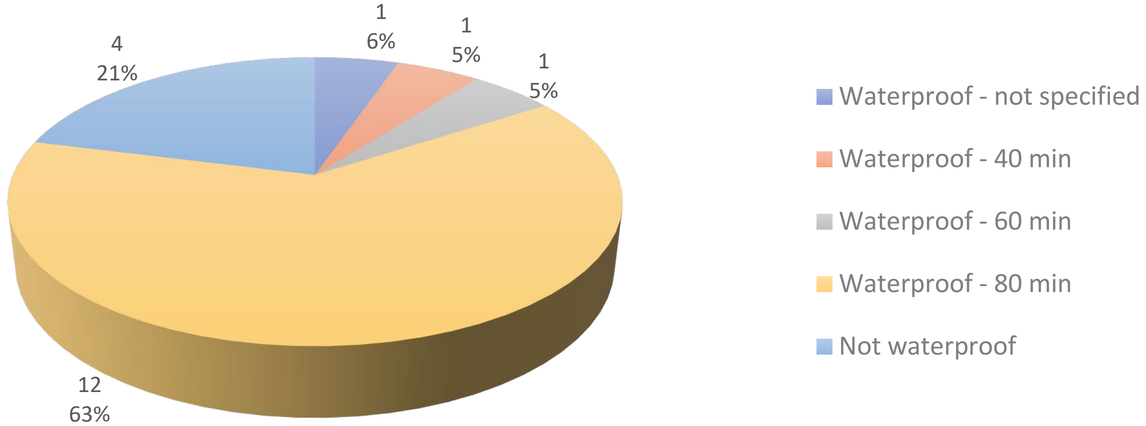


Figure A10. Waterproof rating for sunscreen products in use (n=19).

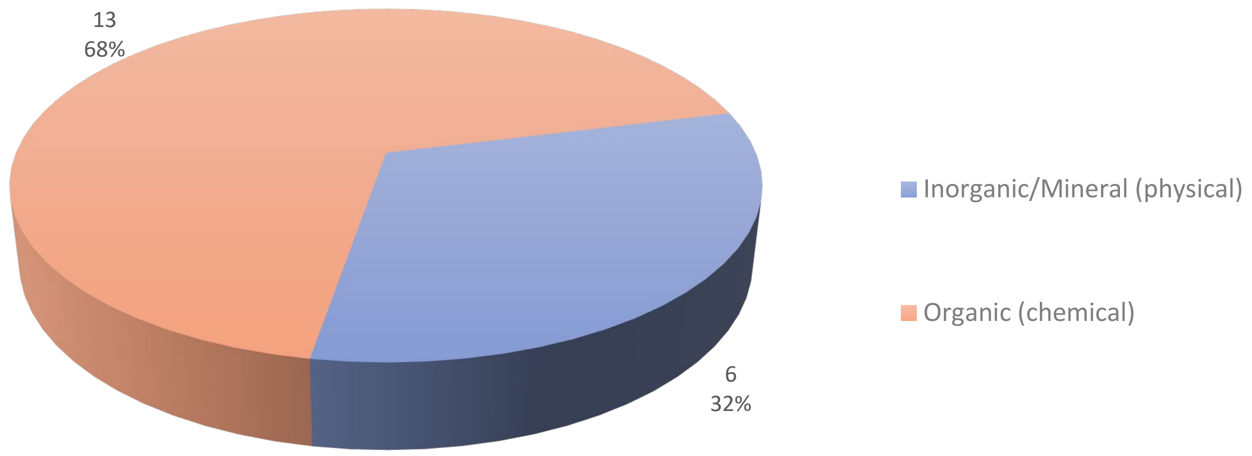


Figure A11. UVF type (physical vs. chemical) for sunscreen products in use (n=19).

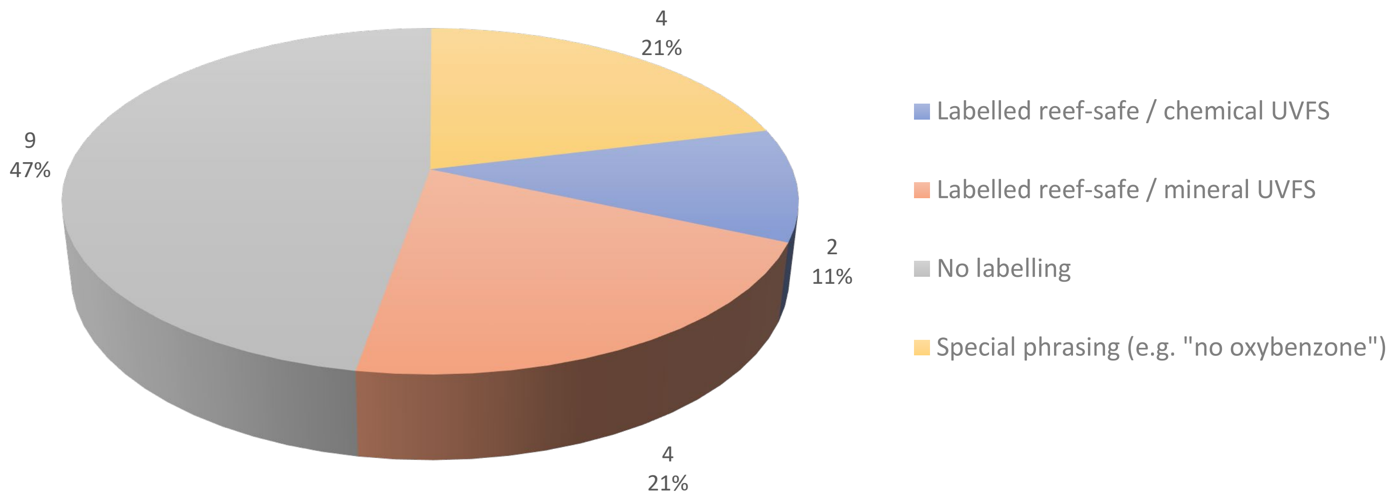


Figure A12. Presence of "reef-safe" marketing strategy for sunscreen products in use (n=19).

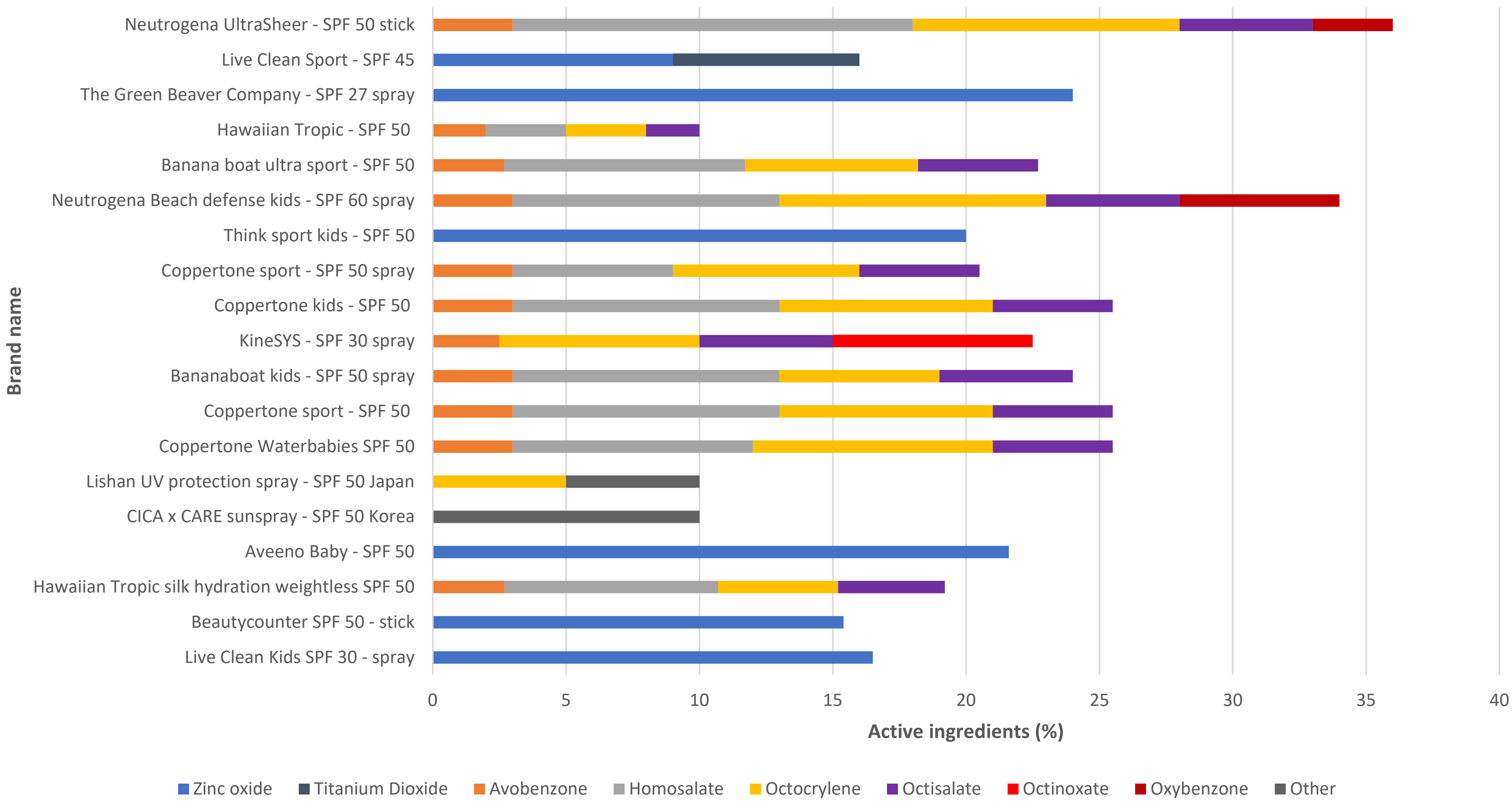


Figure A13. Summary of active ingredients (common physical or chemical UVFs) for sunscreen products in use (n=19).

APPENDIX B – RAW DATA

Table B1. Levene's test results checking assumption of equal variances between repeat sample sites and repeat sample dates.

	df	F-value	P-value	F crit	Assumption met?	ANOVA used
Between sites	11	2.7676	**0.0919**	3.5874	Yes	Standard one-way
Between dates	11	4.4106	**0.0287**	3.5874	No	Welch's

Table B2. One-way ANOVA comparing oxybenzone concentrations between repeat sample sites.

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Little Beach	4	179.76	44.94	2983.39
TLCO	4	321.98	80.49	12290.91
Sandy Pool	4	218	54.5	5027
JUBO	3	0	0	0

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11342.16	3	3780.72	0.6828	**0.5808**	3.5874
Within Groups	60903.92	11	5536.72			
Total	72246.08	14				

Table B3. Welch's ANOVA comparing oxybenzone concentrations between repeat sample dates.

SUMMARY				
Groups	*Count*	*Sum*	*Mean*	*Variance*
3-Jul	4	267.00	66.7	2269
17-Jul	3	0.00	0	0
1-Aug	4	453.00	151.0	6892
22-Aug	4	0.00	0	0

Welch's ANOVA				
Source	df num	df den	F-value	P-value
Dates	3	4.9934	0.6529	**0.6218**

Table B4. Shapiro-Wilk test of normality for oxybenzone results from Lake and River sample groups.

	df	*SS*	*b*	*W*	*P-value*	*Normal distribution?*
Lake	27	5291599.28	1583.48	0.47	<0.01	No
River	64	518888.07	612.22	0.72	<0.01	No

Table B5. Mann-Whitney U test comparing oxybenzone results between Lake and River sample groups.

	Lake			River					
	Median	*IQR*	*N*	*Median*	*IQR*	*N*	*U*	*z*	*P-value*
Oxybenzone	27.66	176.23	28.00	30.09	86.98	65.00	835.50	-0.6239	**0.5327**

Table B6. Pearson's correlation and residual output results between recreational users and oxybenzone for Little Beach samples.

SUMMARY	
Regression Statistics	
Multiple R	0.581052969
R Square	0.337622553
Adjusted R Square	0.227226312
Standard Error	54.27868657
Observations	8

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	9010.228872	9010.229	3.058279	0.130908801
Residual	6	17677.05489	2946.176		
Total	**7**	26687.28376			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.66160175	36.64105578	-0.01806	0.986179	-90.31903539	88.99583189
X Variable 1	1.679579076	0.960421501	1.748794	0.130909	-0.670487678	4.029645829

RESIDUAL OUTPUT		
Observation	Predicted Y	Residuals
1	21.17292623	-21.17292623
2	61.48282404	89.49460081
3	19.49334716	-19.49334716
4	41.32787514	-41.32787514
5	39.64829606	-39.64829606
6	32.92997976	67.65024809
7	100.1131428	-31.03165514
8	115.2293545	-4.470749161

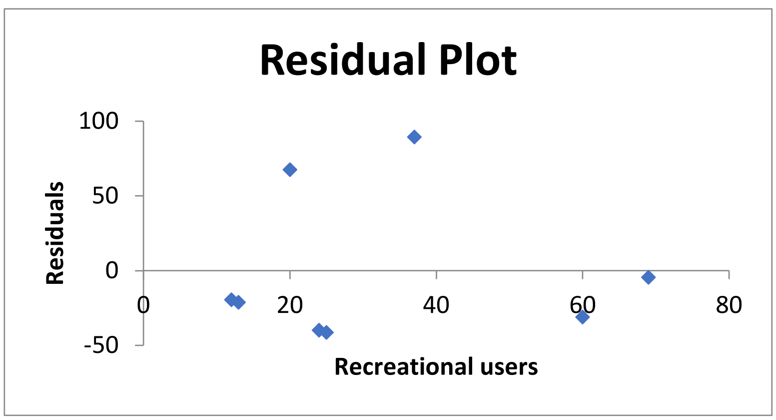


Table B7. Pearson's correlation and residual output results between recreational users and oxybenzone for Sandy Pool samples.

SUMMARY	
Regression Statistics	
Multiple R	0.34750697
R Square	0.120761094
Adjusted R Square	-0.318858359
Standard Error	81.25405595
Observations	4

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	1813.59469	1813.59469	0.27469461	0.65249303
Residual	2	13204.44322	6602.221609		
Total	**3**	15018.03791			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	27.54242539	65.36073551	0.421390995	0.714438831	-253.6821217	308.7669725
X Variable 1	5.963277639	11.37784363	0.524113165	0.65249303	-42.99163231	54.91818759

RESIDUAL OUTPUT		
Observation	Predicted Y	Residuals
1	51.39553595	-51.39553595
2	27.54242539	-27.54242539
3	51.39553595	97.29957826
4	87.17520178	-18.36161693

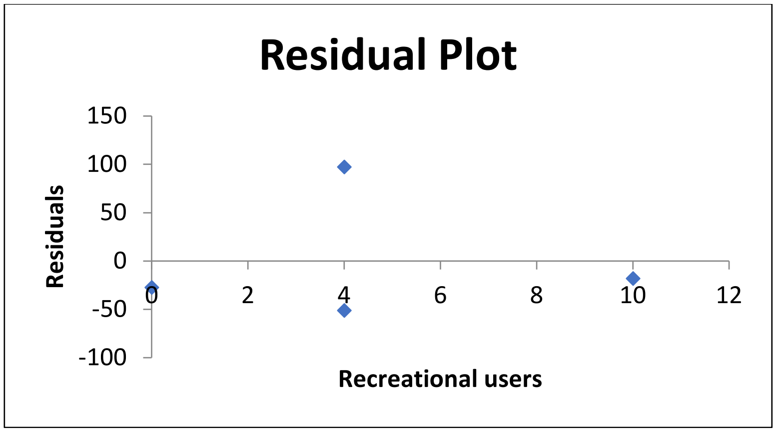


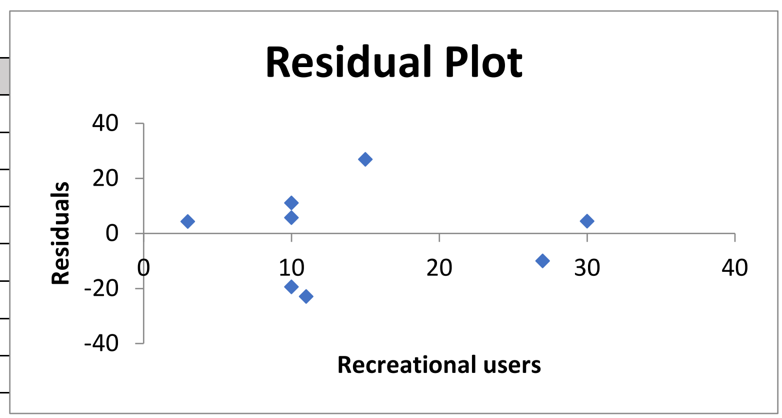
Table B8. Pearson's correlation and residual output results between recreational users and oxybenzone for Greendale Trestle samples.

SUMMARY	
Regression Statistics	
Multiple R	0.885432645
R Square	0.78399097
Adjusted R Square	0.747989465
Standard Error	17.89452598
Observations	8

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	6973.178787	6973.178787	21.77661651	0.003443806
Residual	6	1921.28436	320.2140601		
Total	**7**	8894.463148			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-14.53632492	12.32324291	-1.17958601	0.282812331	-44.69021404	15.61756421
X Variable 1	3.403432601	0.729326431	4.666542244	0.003443806	1.618835113	5.188030089

RESIDUAL OUTPUT		
Observation	Predicted Y	Residuals
1	19.49800109	-19.49800109
2	22.90143369	-22.90143369
3	19.49800109	5.681589065
4	-4.326027117	4.326027117
5	36.51516409	26.90541946
6	19.49800109	11.05312869
7	87.5666531	4.430309561
8	77.3563553	-9.997039118



August 13, 2021 Service Request No:K2108502

Thea Rodgers
BC Conservation Fountation (BCCF)
#1-7217 Lantzville Rd
Lantzville, BC V0R 2H0

Laboratory Results for: 1694

Dear Thea,

Enclosed are the results of the sample(s) submitted to our laboratory July 22, 2021
For your reference, these analyses have been assigned our service request number **K2108502.**

Analyses were performed according to our laboratory's NELAP-approved quality assurance program. The test results meet requirements of the current NELAP standards, where applicable, and except as noted in the laboratory case narrative provided. For a specific list of NELAP-accredited analytes, refer to the certifications section at www.alsglobal.com. All results are intended to be considered in their entirety, and ALS Group USA Corp. dba ALS Environmental (ALS) is not responsible for use of less than the complete report. Results apply only to the items submitted to the laboratory for analysis and individual items (samples) analyzed, as listed in the report.

Please contact me if you have any questions. My extension is 3350. You may also contact me via email at Kelley.Lovejoy@alsglobal.com.

Respectfully submitted,

ALS Group USA, Corp. dba ALS Environmental

Kelley Lovejoy

Kelley Lovejoy
Project Manager

ADDRESS 1317 S. 13th Avenue, Kelso, WA 98626
PHONE +1 360 577 7222 | FAX +1 360 636 1068
ALS Group USA, Corp.
dba ALS Environmental

Narrative Documents

ALS Environmental—Kelso Laboratory
1317 South 13th Avenue, Kelso, WA 98626
Phone (360) 577-7222 Fax (360) 425-9096
www.alsglobal.com

1317 South 13th Ave, Kelso, WA 98626 | 1-360-577-7222 | www.alsglobal.com

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Received:**	07/22/2021
Sample Matrix:	Sediment		

CASE NARRATIVE

All analyses were performed consistent with the quality assurance program of ALS Environmental. This report contains analytical results for samples for the Tier II level requested by the client.

Sample Receipt:

Two sediment samples were received for analysis at ALS Environmental on 07/22/2021. Any discrepancies upon initial sample inspection are annotated on the sample receipt and preservation form included within this report. The samples were stored at minimum in accordance with the analytical method requirements.

Metals:

No significant anomalies were noted with this analysis.

Organic LC:

Method 1694, 07/28/2021:The recovery of Oxybenzone-13C6 in sample Sed 5 (Gordon Bay) was outside the control limits listed in the results summary. The limits are default values temporarily in use until sufficient data points are generated to calculate statistical control limits. Based on the method and historic data, the recoveries observed were in the range expected for this procedure. No further corrective action was taken.

Approved by Kelley Lovejoy   Date 08/13/2021

SAMPLE DETECTION SUMMARY

CLIENT ID: Sed 3 (Spring Beach)	Lab ID: K2108502-001					
Analyte	**Results**	**Flag**	**MDL**	**MRL**	**Units**	**Method**
Solids, Total	69.0				Percent	160.3 Modified
Moisture	31.0				Percent	Calculation

CLIENT ID: Sed 5 (Gordon Bay)	Lab ID: K2108502-002					
Analyte	**Results**	**Flag**	**MDL**	**MRL**	**Units**	**Method**
Solids, Total	20.5				Percent	160.3 Modified
Moisture	79.5				Percent	Calculation

Sample Receipt Information

ALS Environmental—Kelso Laboratory
1317 South 13th Avenue, Kelso, WA 98626
Phone (360) 577-7222 Fax (360) 425-9096
www.alsglobal.com

Client: BC Conservation Fountation (BCCF) **Service Request:** K2108502
Project: 1694

SAMPLE CROSS-REFERENCE

SAMPLE #	CLIENT SAMPLE ID	DATE	TIME
K2108502-001	Sed 3 (Spring Beach)	7/18/2021	1210
K2108502-002	Sed 5 (Gordon Bay)	7/18/2021	1427

K2108502

ALS Environmental

www.alsglobal.com

Chain of Custody (COC) / Analytical Request Form

Canada Toll Free: 1 800 668 9878

Affix ALS barcode label here (lab use only)

COC Number: 15 -

Report To	Contact and company name below will appear on the final report
Company:	BC Conservation Foundation
Contact:	Thea Rodgers
Phone:	250-390-2525 ext. 104
	Company address below will appear on the final report
Street:	#105-1885 Boxwood Road
City/Province:	Nanaimo BC, Canada
Postal Code:	V9S 5X9

Report Format / Distribution	
Select Report Format:	☑ PDF ☑ EXCEL ☐ EDD (DIGITAL)
Quality Control (QC) Report with Report	☑ YES ☐ NO
☐ Compare Results to Criteria on Report - provide details below if box checked	
Select Distribution:	☑ EMAIL ☐ MAIL ☐ FAX
Email 1 or Fax	trodgers@bccf.com
Email 2	
Email 3	

Select Service Level Below - Please confirm all E&P TATs with your AM - surcharges will apply

Regular [R]	☑ Standard TAT if received by 3 pm - business days - no surcharges apply	
PRIORITY (Business Days)	4 day [P4] ☐	
	3 day [P3] ☐	
	2 day [P2] ☐	
EMERGENCY	1 Business day [E1] ☐	
	Same Day, Weekend or Statutory holiday [E0] ☐	
Date and Time Required for all E&P TATs:	dd-mmm-yy hh:mm	

For tests that can not be performed according to the service level selected, you will be contacted.

Invoice To		
	Same as Report To	☑ YES ☐ NO
	Copy of Invoice with Report	☑ YES ☐ NO
Company:	BC Conservation Foundation	
Contact:	Lisa Limerick	

Invoice Distribution	
Select Invoice Distribution:	☑ EMAIL ☐ MAIL ☐ FAX
Email 1 or Fax	llimerick@bccf.com
Email 2	trodgers@bccf.com

Project Information	
ALS Account # / Quote #:	
Job #:	
PO / AFE:	1132007
LSD:	
ALS Lab Work Order # (lab use only)	

Oil and Gas Required Fields (client use)	
AFE/Cost Center:	PO#
Major/Minor Code:	Routing Code:
Requisitioner:	
Location:	
ALS Contact: Kelley Lovejoy	Sampler: TR

Analysis Request — Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (lab use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	Oxybenzone (Method 1694)	Number of Containers
	Sed 3 (Spring Beach)	18-Jul-21	12:10	Sediment	R	1
	Sed 5 (Gordon Bay)	18-Jul-21	14:27	Sediment	R	1

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System? ☐ YES ☐ NO

Are samples for human drinking water use? ☐ YES ☐ NO

Special Instructions / Specify Criteria to add on report by clicking on the drop-down list below (electronic COC only)

Special: Oxybenzone in sediment test.

SAMPLE CONDITION AS RECEIVED (lab use only)

Frozen ☐ SIF Observations Yes ☐ No ☐
Ice Packs ☐ Ice Cubes ☐ Custody seal intact Yes ☐ No ☐
Cooling Initiated ☐

INITIAL COOLER TEMPERATURES °C | FINAL COOLER TEMPERATURES °C

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (lab use only)			FINAL SHIPMENT RECEPTION (lab use only)		
Released by: TR	20-Jul-21	Time: 900	Received by: [signature]	Date: 7/22/21	Time: 0930	Received by:	Date:	Time:

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION WHITE - LABORATORY COPY YELLOW - CLIENT COPY

1. If any water samples are taken from a Regulated Drinking Water (DW) System, please submit using an Authorized DW COC form.

PM KL

Cooler Receipt and Preservation Form

Client BC Conservation Foundation Service Request K21 08502

Received: 7/22/21 Opened: 7/22/21 By: CG Unloaded: 7/22/21 By: CG

1. Samples were received via? *USPS* (*Fed Ex*) *UPS* *DHL* *PDX* *Courier* *Hand Delivered*
2. Samples were received in: (circle) (*Cooler*) *Box* *Envelope* *Other* ______ *NA*
3. Were custody seals on coolers? NA Y (N) If yes, how many and where? ______
   If present, were custody seals intact? Y N If present, were they signed and dated? Y N
4. Was a Temperature Blank present in cooler? NA Y (N) If yes, notate the temperature in the appropriate column below:
   If no, take the temperature of a representative sample bottle contained within the cooler; notate in the column "Sample Temp":
5. Were samples received within the method specified temperature ranges? NA Y (N)
   If no, were they received on ice and same day as collected? If not, notate the cooler # below and notify the PM. NA Y (N)

If applicable, tissue samples were received: *Frozen* *Partially Thawed* *Thawed*

Temp Blank	Sample Temp	IR Gun	Cooler #/COC ID / (NA)	Out of temp indicate with "X"	PM Notified If out of temp	Tracking Number NA	Filed
/	14.7	IR02		X	X	2816 5288 0907	

6. Packing material: *Inserts* *Baggies* *Bubble Wrap* (*Gel Packs*) *Wet Ice* *Dry Ice* *Sleeves* Foam padding
7. Were custody papers properly filled out (ink, signed, etc.)? NA (Y) N
8. Were samples received in good condition (unbroken) NA (Y) N
9. Were all sample labels complete (ie, analysis, preservation, etc.)? NA (Y) N
10. Did all sample labels and tags agree with custody papers? NA (Y) N
11. Were appropriate bottles/containers and volumes received for the tests indicated? NA (Y) N
12. Were the pH-preserved bottles (*see SMO GEN SOP*) received at the appropriate pH? *Indicate in the table below* (NA) Y N
13. Were VOA vials received without headspace? *Indicate in the table below.* (NA) Y N
14. Was C12/Res negative? (NA) Y N

Sample ID on Bottle	Sample ID on COC	Identified by:

Sample ID	Bottle Count Bottle Type	Head-space	Broke	pH	Reagent	Volume added	Reagent Lot Number	Initials	Time

Notes, Discrepancies, Resolutions: ______

Miscellaneous Forms

ALS Environmental—Kelso Laboratory
1317 South 13th Avenue, Kelso, WA 98626
Phone (360) 577-7222 Fax (360) 425-9096
www.alsglobal.com

Inorganic Data Qualifiers

- * The result is an outlier. See case narrative.
- # The control limit criteria is not applicable. See case narrative.
- B The analyte was found in the associated method blank at a level that is significant relative to the sample result as defined by the DOD or NELAC standards.
- E The result is an estimate amount because the value exceeded the instrument calibration range.
- J The result is an estimated value.
- U The analyte was analyzed for, but was not detected ("Non-detect") at or above the MRL/MDL. *DOD-QSM 4.2 definition* : Analyte was not detected and is reported as less than the LOD or as defined by the project. The detection limit is adjusted for dilution.
- i The MRL/MDL or LOQ/LOD is elevated due to a matrix interference.
- X See case narrative.
- Q See case narrative. One or more quality control criteria was outside the limits.
- H The holding time for this test is immediately following sample collection. The samples were analyzed as soon as possible after receipt by the laboratory.

Metals Data Qualifiers

- # The control limit criteria is not applicable. See case narrative.
- J The result is an estimated value.
- E The percent difference for the serial dilution was greater than 10%, indicating a possible matrix interference in the sample.
- M The duplicate injection precision was not met.
- N The Matrix Spike sample recovery is not within control limits. See case narrative.
- S The reported value was determined by the Method of Standard Additions (MSA).
- U The analyte was analyzed for, but was not detected ("Non-detect") at or above the MRL/MDL. *DOD-QSM 4.2 definition* : Analyte was not detected and is reported as less than the LOD or as defined by the project. The detection limit is adjusted for dilution.
- W The post-digestion spike for furnace AA analysis is out of control limits, while sample absorbance is less than 50% of spike absorbance.
- i The MRL/MDL or LOQ/LOD is elevated due to a matrix interference.
- X See case narrative.
- \+ The correlation coefficient for the MSA is less than 0.995.
- Q See case narrative. One or more quality control criteria was outside the limits.

Organic Data Qualifiers

- * The result is an outlier. See case narrative.
- # The control limit criteria is not applicable. See case narrative.
- A A tentatively identified compound, a suspected aldol-condensation product.
- B The analyte was found in the associated method blank at a level that is significant relative to the sample result as defined by the DOD or NELAC standards.
- C The analyte was qualitatively confirmed using GC/MS techniques, pattern recognition, or by comparing to historical data.
- D The reported result is from a dilution.
- E The result is an estimated value.
- J The result is an estimated value.
- N The result is presumptive. The analyte was tentatively identified, but a confirmation analysis was not performed.
- P The GC or HPLC confirmation criteria was exceeded. The relative percent difference is greater than 40% between the two analytical results.
- U The analyte was analyzed for, but was not detected ("Non-detect") at or above the MRL/MDL. *DOD-QSM 4.2 definition* : Analyte was not detected and is reported as less than the LOD or as defined by the project. The detection limit is adjusted for dilution.
- i The MRL/MDL or LOQ/LOD is elevated due to a chromatographic interference.
- X See case narrative.
- Q See case narrative. One or more quality control criteria was outside the limits.

Additional Petroleum Hydrocarbon Specific Qualifiers

- F The chromatographic fingerprint of the sample matches the elution pattern of the calibration standard.
- L The chromatographic fingerprint of the sample resembles a petroleum product, but the elution pattern indicates the presence of a greater amount of lighter molecular weight constituents than the calibration standard.
- H The chromatographic fingerprint of the sample resembles a petroleum product, but the elution pattern indicates the presence of a greater amount of heavier molecular weight constituents than the calibration standard.
- O The chromatographic fingerprint of the sample resembles an oil, but does not match the calibration standard.
- Y The chromatographic fingerprint of the sample resembles a petroleum product eluting in approximately the correct carbon range, but the elution pattern does not match the calibration standard.
- Z The chromatographic fingerprint does not resemble a petroleum product.

ALS Group USA Corp. dba ALS Environmental (ALS) - Kelso
State Certifications, Accreditations, and Licenses

Agency	Web Site	Number
Alaska DEH	http://dec.alaska.gov/eh/lab/cs/csapproval.htm	UST-040
Arizona DHS	http://www.azdhs.gov/lab/license/env.htm	AZ0339
Arkansas - DEQ	http://www.adeq.state.ar.us/techsvs/labcert.htm	88-0637
California DHS (ELAP)	http://www.cdph.ca.gov/certlic/labs/Pages/ELAP.aspx	2795
DOD ELAP	http://www.denix.osd.mil/edqw/Accreditation/AccreditedLabs.cfm	L16-58-R4
Florida DOH	http://www.doh.state.fl.us/lab/EnvLabCert/WaterCert.htm	E87412
Hawaii DOH	http://health.hawaii.gov/	-
ISO 17025	http://www.pjlabs.com/	L16-57
Louisiana DEQ	http://www.deq.louisiana.gov/page/la-lab-accreditation	03016
Maine DHS	http://www.maine.gov/dhhs/	WA01276
Minnesota DOH	http://www.health.state.mn.us/accreditation	053-999-457
Nevada DEP	http://ndep.nv.gov/bsdw/labservice.htm	WA01276
New Jersey DEP	http://www.nj.gov/dep/enforcement/oqa.html	WA005
New York - DOH	https://www.wadsworth.org/regulatory/elap	12060
North Carolina DEQ	https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/laboratory-certification-branch/non-field-lab-certification	605
Oklahoma DEQ	http://www.deq.state.ok.us/CSDnew/labcert.htm	9801
Oregon – DEQ (NELAP)	http://public.health.oregon.gov/LaboratoryServices/EnvironmentalLaboratoryAccreditation/Pages/index.aspx	WA100010
South Carolina DHEC	http://www.scdhec.gov/environment/EnvironmentalLabCertification/	61002
Texas CEQ	http://www.tceq.texas.gov/field/qa/env_lab_accreditation.html	T104704427
Washington DOE	http://www.ecy.wa.gov/programs/eap/labs/lab-accreditation.html	C544
Wyoming (EPA Region 8)	https://www.epa.gov/region8-waterops/epa-region-8-certified-drinking-water-	-
Kelso Laboratory Website	www.alsglobal.com	NA

Analyses were performed according to our laboratory's NELAP-approved quality assurance program. A complete listing of specific NELAP-certified analytes, can be found in the certification section at www.ALSGlobal.com or at the accreditation bodies web site.
Please refer to the certification and/or accreditation body's web site if samples are submitted for compliance purposes. The states highlighted above, require the analysis be listed on the state certification if used for compliance purposes and if the method/anlayte is offered by that state.

Acronyms

ASTM	American Society for Testing and Materials
A2LA	American Association for Laboratory Accreditation
CARB	California Air Resources Board
CAS Number	Chemical Abstract Service registry Number
CFC	Chlorofluorocarbon
CFU	Colony-Forming Unit
DEC	Department of Environmental Conservation
DEQ	Department of Environmental Quality
DHS	Department of Health Services
DOE	Department of Ecology
DOH	Department of Health
EPA	U. S. Environmental Protection Agency
ELAP	Environmental Laboratory Accreditation Program
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantitation
LUFT	Leaking Underground Fuel Tank
M	Modified
MCL	Maximum Contaminant Level is the highest permissible concentration of a substance allowed in drinking water as established by the USEPA.
MDL	Method Detection Limit
MPN	Most Probable Number
MRL	Method Reporting Limit
NA	Not Applicable
NC	Not Calculated
NCASI	National Council of the Paper Industry for Air and Stream Improvement
ND	Not Detected
NIOSH	National Institute for Occupational Safety and Health
PQL	Practical Quantitation Limit
RCRA	Resource Conservation and Recovery Act
SIM	Selected Ion Monitoring
TPH	Total Petroleum Hydrocarbons
tr	Trace level is the concentration of an analyte that is less than the PQL but greater than or equal to the MDL.

Analyst Summary report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694/		

Sample Name:	Sed 3 (Spring Beach)	**Date Collected:**	07/18/21
Lab Code:	K2108502-001	**Date Received:**	07/22/21
Sample Matrix:	Sediment		

Analysis Method	Extracted/Digested By	Analyzed By
160.3 Modified		JGRIMES
1694	LILLIANSMITH	CCONOVER
Frz Dry		CLUKKEN

Sample Name:	Sed 5 (Gordon Bay)	**Date Collected:**	07/18/21
Lab Code:	K2108502-002	**Date Received:**	07/22/21
Sample Matrix:	Sediment		

Analysis Method	Extracted/Digested By	Analyzed By
160.3 Modified		JGRIMES
1694	LILLIANSMITH	CCONOVER
Frz Dry		CLUKKEN

Sample Results

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Organic Compounds by HPLC/MS/MS

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Analytical Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Collected:**	07/18/21 12:10
Sample Matrix:	Sediment	**Date Received:**	07/22/21 09:30
Sample Name:	Sed 3 (Spring Beach)	**Units:**	ug/Kg
Lab Code:	K2108502-001	**Basis:**	Dry, per Method

Steroids and Endocrine Disrupting Compounds

Analysis Method: 1694
Prep Method: Method

Analyte Name	Result	MRL	Dil.	Date Analyzed	Date Extracted	Q
Oxybenzone	ND U	4.9	1	07/28/21 02:53	7/23/21	

Surrogate Name	% Rec	Control Limits	Date Analyzed	Q
Oxybenzone-13C6	31	30 - 130	07/28/21 02:53	

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Analytical Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Collected:**	07/18/21 14:27
Sample Matrix:	Sediment	**Date Received:**	07/22/21 09:30
Sample Name:	Sed 5 (Gordon Bay)	**Units:**	ug/Kg
Lab Code:	K2108502-002	**Basis:**	Dry, per Method

Steroids and Endocrine Disrupting Compounds

Analysis Method: 1694
Prep Method: Method

Analyte Name	Result	MRL	Dil.	Date Analyzed	Date Extracted	Q
Oxybenzone	ND U	4.9	1	07/28/21 03:35	7/23/21	

Surrogate Name	% Rec	Control Limits	Date Analyzed	Q
Oxybenzone-13C6	23	30 - 130	07/28/21 03:35	*

General Chemistry

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Analytical Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Collected:**	07/18/21 12:10
Sample Matrix:	Sediment	**Date Received:**	07/22/21 09:30
Sample Name:	Sed 3 (Spring Beach)	**Basis:**	As Received
Lab Code:	K2108502-001		

General Chemistry Parameters

Analyte Name	Analysis Method	Result	Units	MRL	Dil.	Date Analyzed	Q
Moisture	Calculation	**31.0**	Percent	-	1	07/22/21 12:43	
Solids, Total	160.3 Modified	**69.0**	Percent	-	1	07/22/21 12:43	

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Collected:**	07/18/21 14:27
Sample Matrix:	Sediment	**Date Received:**	07/22/21 09:30
Sample Name:	Sed 5 (Gordon Bay)	**Basis:**	As Received
Lab Code:	K2108502-002		

General Chemistry Parameters

Analyte Name	Analysis Method	Result	Units	MRL	Dil.	Date Analyzed	Q
Moisture	Calculation	**79.5**	Percent	-	1	07/22/21 12:43	
Solids, Total	160.3 Modified	**20.5**	Percent	-	1	07/22/21 12:43	

QC Summary Forms

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QA/QC Report

Client: BC Conservation Fountation (BCCF) **Service Request:** K2108502
Project: 1694
Sample Matrix: Sediment

SURROGATE RECOVERY SUMMARY
Steroids and Endocrine Disrupting Compounds

Analysis Method: 1694
Extraction Method: Method

Sample Name	Lab Code	Oxybenzone-13C6 30-130
Sed 3 (Spring Beach)	K2108502-001	31
Sed 5 (Gordon Bay)	K2108502-002	23*
Sed 3 (Spring Beach)	KQ2113826-01	32
Method Blank	KQ2113826-03	45
Lab Control Sample	KQ2113826-02	45

QA/QC Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project	1694	**Date Collected:**	07/18/21
Sample Matrix:	Sediment	**Date Received:**	07/22/21
		Date Analyzed:	07/28/21

Replicate Sample Summary
Steroids and Endocrine Disrupting Compounds

Sample Name:	Sed 3 (Spring Beach)	**Units:**	ug/Kg
Lab Code:	K2108502-001	**Basis:**	Dry, per Method

Analyte Name	Analysis Method	MRL	Sample Result	Duplicate Sample KQ2113826-01 Result	Average	RPD	RPD Limit
Oxybenzone	1694	4.9	ND U	ND U	NC	NC	30

Results flagged with an asterisk (*) indicate values outside control criteria.

Results flagged with a pound (#) indicate the control criteria is not applicable.

Percent recoveries and relative percent differences (RPD) are determined by the software using values in the calculation which have not been rounded.

Analytical Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Collected:**	NA
Sample Matrix:	Sediment	**Date Received:**	NA
Sample Name:	Method Blank	**Units:**	ug/Kg
Lab Code:	KQ2113826-03	**Basis:**	Dry, per Method

Steroids and Endocrine Disrupting Compounds

Analysis Method: 1694
Prep Method: Method

Analyte Name	Result	MRL	Dil.	Date Analyzed	Date Extracted	Q
Oxybenzone	ND U	5.0	1	07/28/21 02:32	7/23/21	

Surrogate Name	% Rec	Control Limits	Date Analyzed	Q
Oxybenzone-13C6	45	30 - 130	07/28/21 02:32	

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QA/QC Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project:	1694	**Date Analyzed:**	07/28/21
Sample Matrix:	Sediment	**Date Extracted:**	07/23/21

Lab Control Sample Summary
Steroids and Endocrine Disrupting Compounds

Analysis Method:	1694	**Units:**	ug/Kg
Prep Method:	Method	**Basis:**	Dry, per Method
		Analysis Lot:	732942

Lab Control Sample
KQ2113826-02

Analyte Name	Result	Spike Amount	% Rec	% Rec Limits
Oxybenzone	51.1	50.0	102	30-130

General Chemistry

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QA/QC Report

Client:	BC Conservation Fountation (BCCF)	**Service Request:**	K2108502
Project	1694	**Date Collected:**	07/18/21
Sample Matrix:	Sediment	**Date Received:**	07/22/21
		Date Analyzed:	07/22/21

Replicate Sample Summary
Inorganic Parameters

Sample Name:	Sed 3 (Spring Beach)	**Units:**	Percent
Lab Code:	K2108502-001	**Basis:**	As Received

Analyte Name	Analysis Method	MRL	Sample Result	Duplicate Sample K2108502-001DUP Result	Average	RPD	RPD Limit
Solids, Total	160.3 Modified	-	69.0	67.5	68.3	2	20

Results flagged with an asterisk (*) indicate values outside control criteria.

Results flagged with a pound (#) indicate the control criteria is not applicable.

Percent recoveries and relative percent differences (RPD) are determined by the software using values in the calculation which have not been rounded.

APPENDIX C – METHOD DEVELOPMENT

Updated methodology and detection of oxybenzone and other UV filters.

UV filters enzacamene, octyl methoxycinnamate (octinoxate) and octyl salicylate (octisalate) were able to be detected and calibrated in the low ng L^{-1} range. Also, an increased sensitivity was achieved for oxybenzone, using a modified version of the CP-MIMS set-up used in Vandergrift *et al.* [1]. The acceptor phase solution was pumped at a rate of 50 µL min^{-1} using an Agilent 1100 Series HPLC pump. A zero dead volume stainless steel tee junction was used as a passive flow splitter pre-membrane, reducing the acceptor phase flowrate before passing through the membrane and flowing to the LEI interface. The CP-MIMS immersion probe used was modified from that of Monaghan *et al*. [2] with a membrane length of 4.0 cm.

UV filter calibration standards were made by spiking a methanolic UV filter combined standard into deionized water with ~10 mM glycine pH 3 buffer to ensure oxybenzone and octyl methoxycinnamate were neutral and therefore able to cross the membrane for the sample phase into the acceptor phase. Baseline signal was acquired using deionized water and then the membrane was immersed into the calibration standard for 3 minutes. After 3 minutes, the membrane was placed back into deionized water for 5 minutes to allow the signal to return to baseline before putting on the next calibration standard. Calibration curves were made for oxybenzone from 0 ng L^{-1} to ~300 ng L^{-1} and for enzacamene, octyl methoxycinnamate and octyl salicylate from 0 ng L^{-1} to ~900 ng L^{-1}. All signals were corrected using *m/z* 231 -> 153 (CID @ 17.0 eV) as a null channel according to equation 1.

$$S_{corrected} = \frac{S_{UV\ filter\ standard}}{\left(\frac{S_{null}}{S_{null\ max}}\right)} - \frac{S_{UV\ filter\ baseline}}{\left(\frac{S_{null}}{S_{null\ max}}\right)} \quad (1)$$

Table 1: Summary of calibration curves for UV filters using CP-MIMS-LEI/CI.

Compound/Mass transition	Slope (L ng^{-1})	R2	SD of blank (n=3)	LoD (ng L^{-1})[a]
Oxybenzone				
(*m/z* 229.0 -> 151.0) CID @ 17.0 eV	0.0151	0.98	0.0362	7
Enzacamene				
(*m/z* 255.2 -> 105.1) CID @ 20.0 eV	0.0025	0.98	0.1224	145
Octyl methoxycinnamate				
(*m/z* 193.1 -> 133.1) CID @ 30.0 eV	0.0135	0.996	0.3618	80
(*m/z* 193.1 -> 161.1) CID @ 10.0 eV	0.0279	0.993	0.3171	34
Octyl salicylate				
(*m/z* 251.2 -> 121.0) CID @ 20.0 eV	0.0109	0.994	0.0372	10
(*m/z* 251.2 -> 139.0) CID @ 5.0 eV	0.0231	0.996	0.1275	17

[a] Limit of detection calculated as 3 times the standard deviation of the matrix blank signal (n=3) divided by the slope of the calibration curve.

CP-MIMS-LEI/CI full scans of UV filters

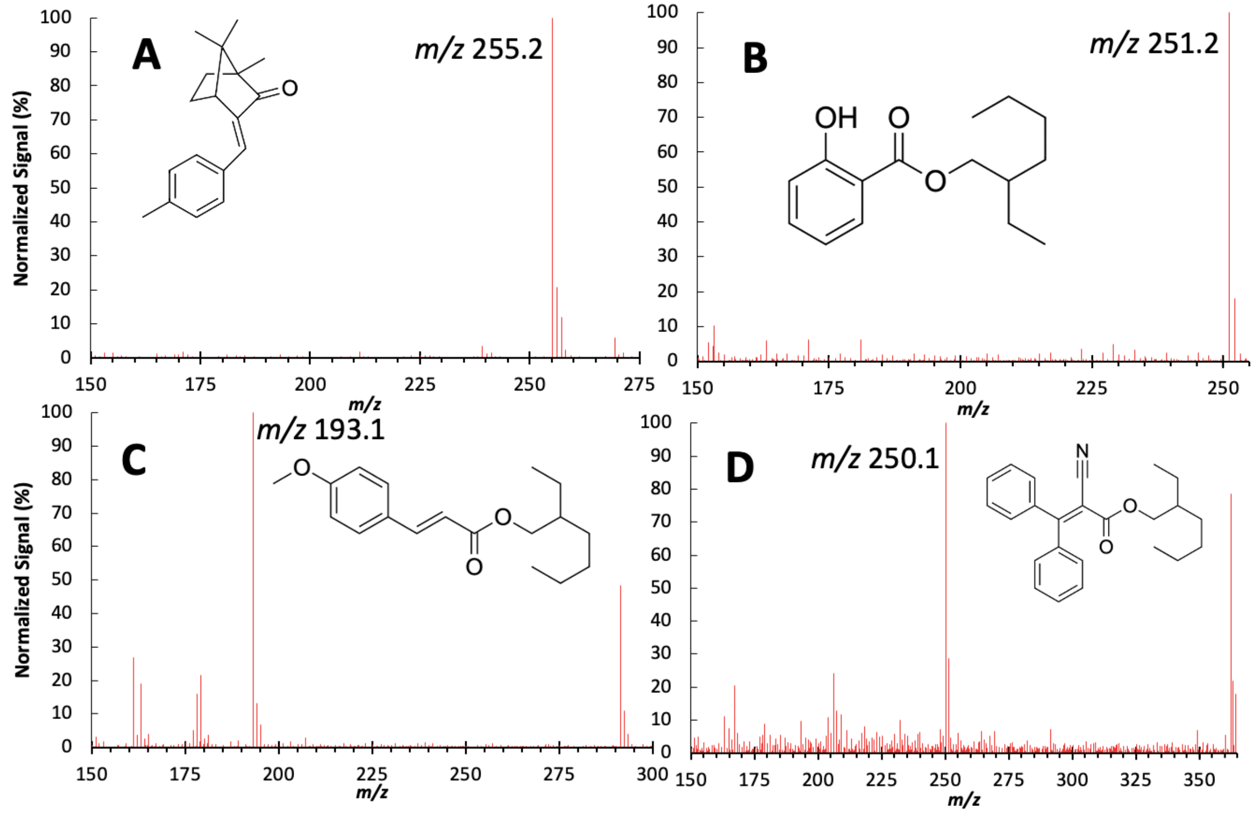

Figure 1: CP-MIMS-LEI/CI full scan mass spectra (normalized to 100%) of A) enzacamene, B) octyl salicylate, C) octyl methoxycinnamate and D) octocrylene.

CP-MIMS-LEI/CI product scans of UV filters

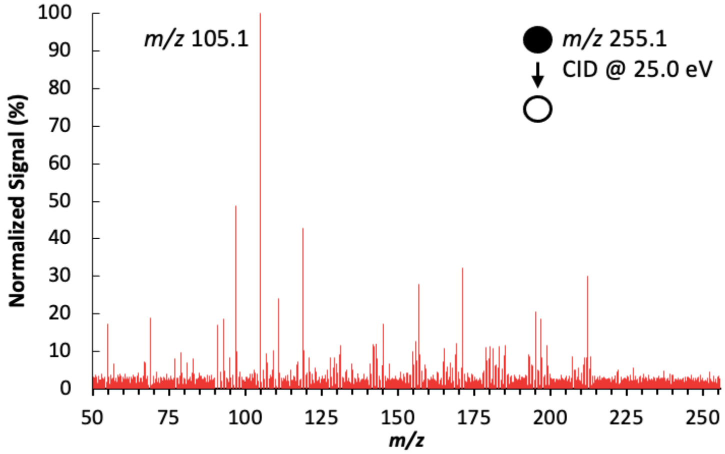

Figure 2: CP-MIMS-LEI/CI product scan of enzacamene using precursor ion *m/z* 255.1 with collision induced dissociation (CID) energy at 25.0 eV.

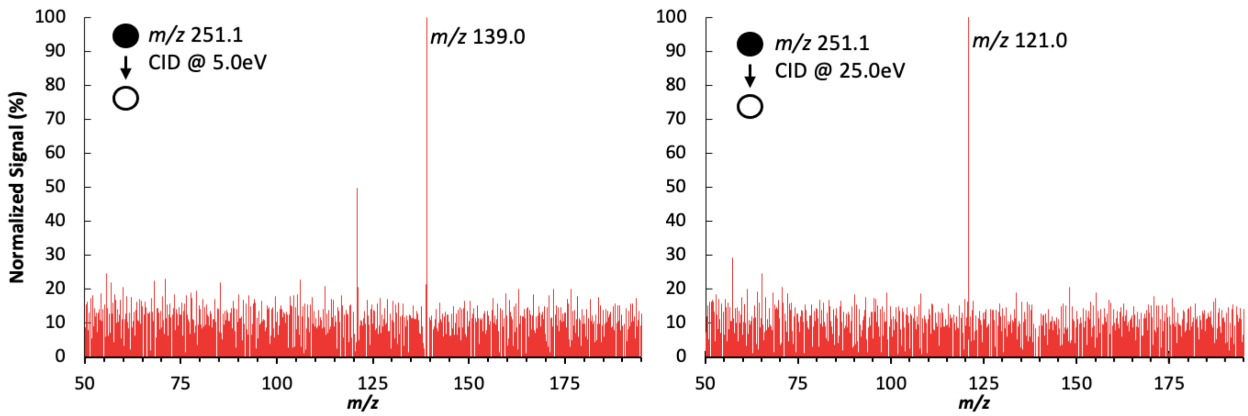

Figure 3: CP-MIMS-LEI/CI product scans of octyl salicylate using precursor ion *m/z* 251.1 with CID energy at 5.0 eV and 25.0 eV.

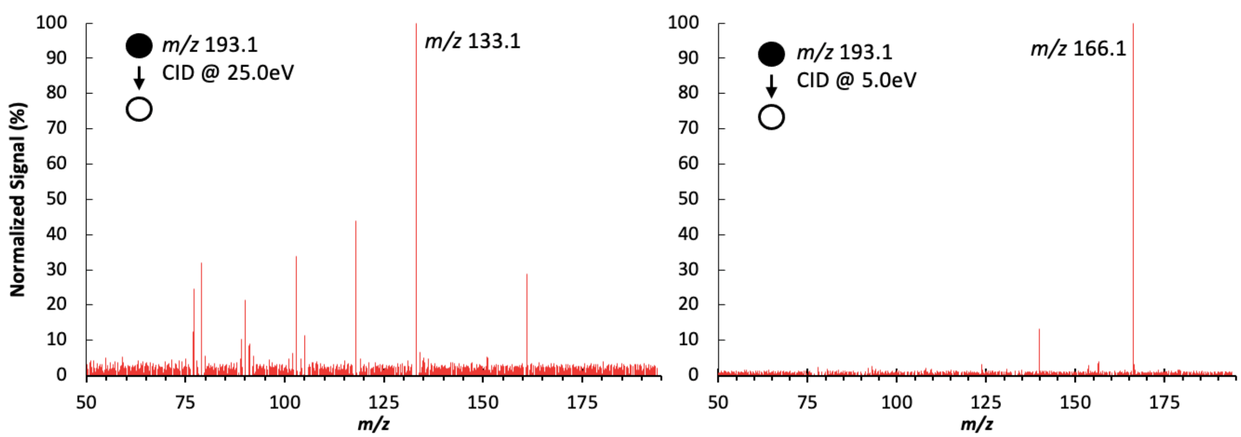

Figure 4: CP-MIMS-LEI/CI product scans of octyl methoxycinnamate using precursor ion *m/z* 193.1 with CID energy at 25.0 eV and 5.0 eV.

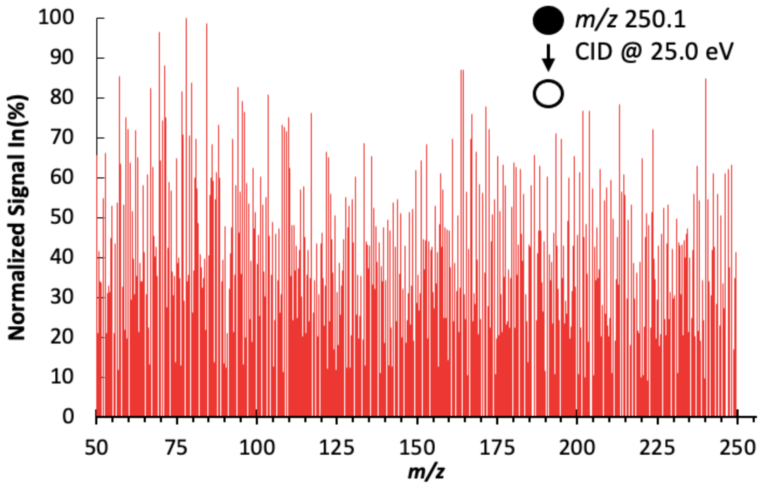

Figure 5: CP-MIMS-LEI/CI product scans of octocrylene using precursor ion *m/z* 250.1 with CID energy at 25.0 eV. A discrete mass transition for octocrylene was unable to be identified.

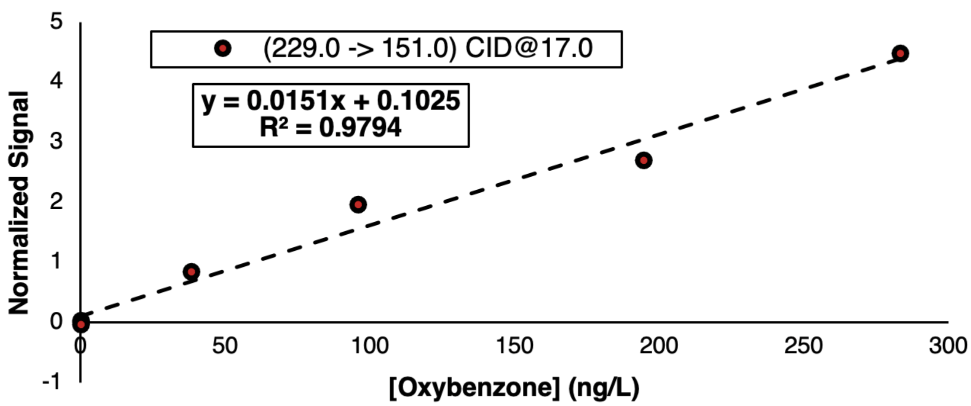

Figure 6: Calibration curve for oxybenzone using CP-MIMS-LEI/CI.

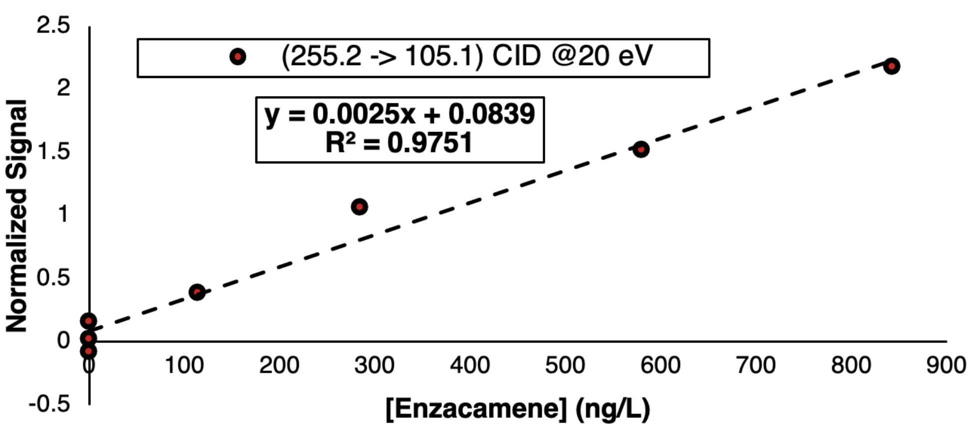

Figure 7: Calibration curves for enzacamene using CP-MIMS-LEI/CI.

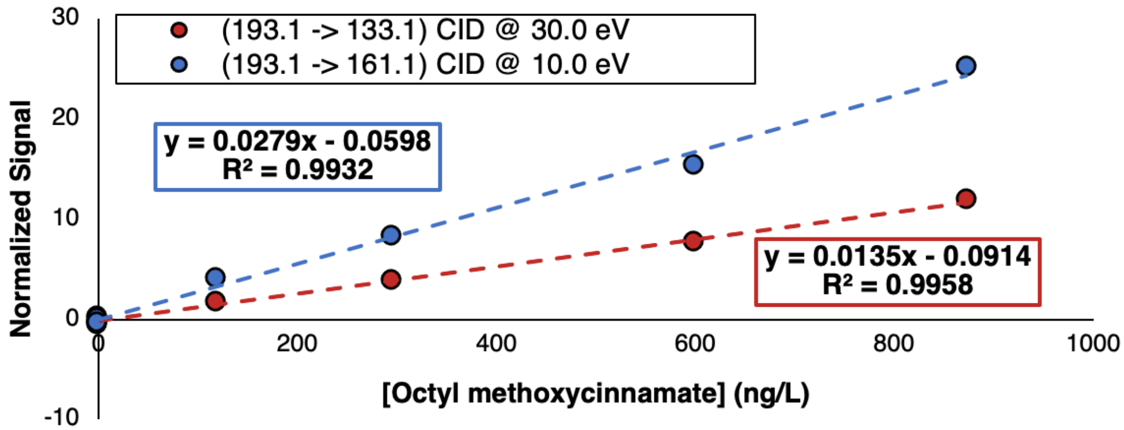

Figure 8: Calibration curves for octyl methoxycinnamate using CP-MIMS-LEI/CI

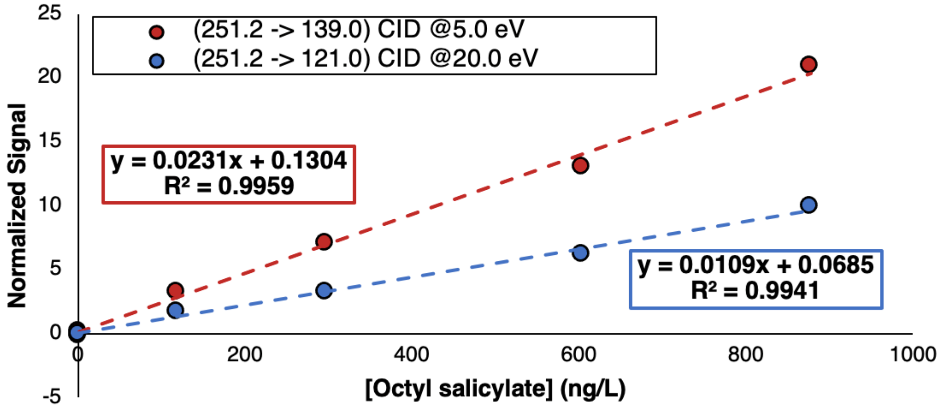

Figure 9: Calibration curve for octyl salicylate using CP-MIMS-LEI/CI.

References:

1. Vandergrift, G. W.; Lattanzio-Battle, W.; Rodgers, T. R.; Atkinson, J. B.; Krogh, E. T.; Gill, C. G. Geospatial assessment of trace-level benzophenone-3 in a fish-bearing river using direct mass spectrometry. *ACS EST Water*, **2022**, *2*(2), 262–267.

2. Monaghan, J.; Jaeger, A.; Agua, A. R.; Stanton, R. S.; Pirrung, M.; Gill, C. G.; Krogh, E. T. A direct mass spectrometry method for the rapid analysis of ubiquitous tire-derived toxin N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine quinone (6-PPDQ). *Environ. Sci. Technol. Lett.*, **2021**, *8*(12), 1051–1056.

Sediments

To determine the propensity of UV filters to adsorb to sediments, a signal for oxybenzone was allowed to reach steady-state and then clean sandy loam was added to the solution (Figure 1). Signal would be expected to decline upon adding the sorbent as free oxybenzone would adsorb to the sediment and be unable to cross the membrane, however no significant decrease in oxybenzone signal was observed over the 10 minutes of measurement. A positive control using activated carbon shows signal decay down to near baseline upon adding the sorbent to the solution (Figure 2). Longer term adsorption studies are planned to determine if adsorption to relevant sediments occurs on the time scale of days.

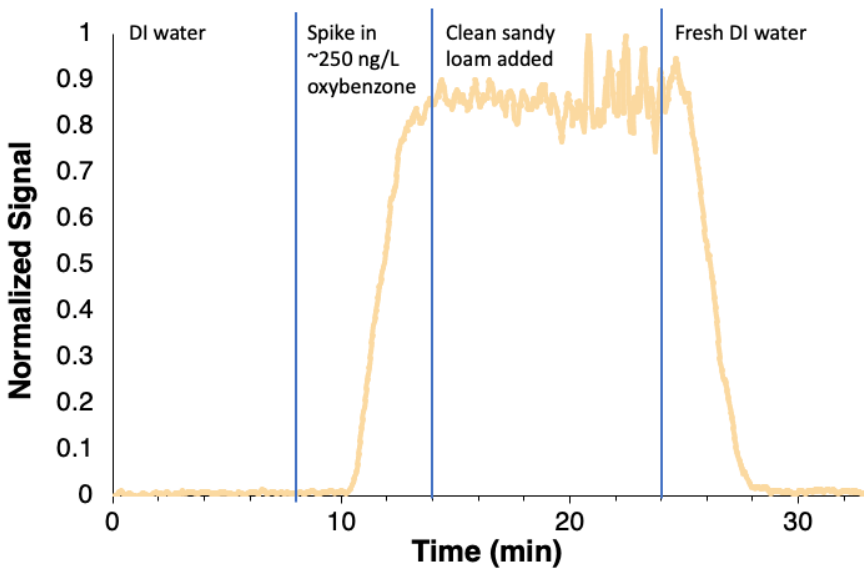

Figure 1: Real-time monitoring of oxybenzone signal upon adding clean sandy loam sorbent.

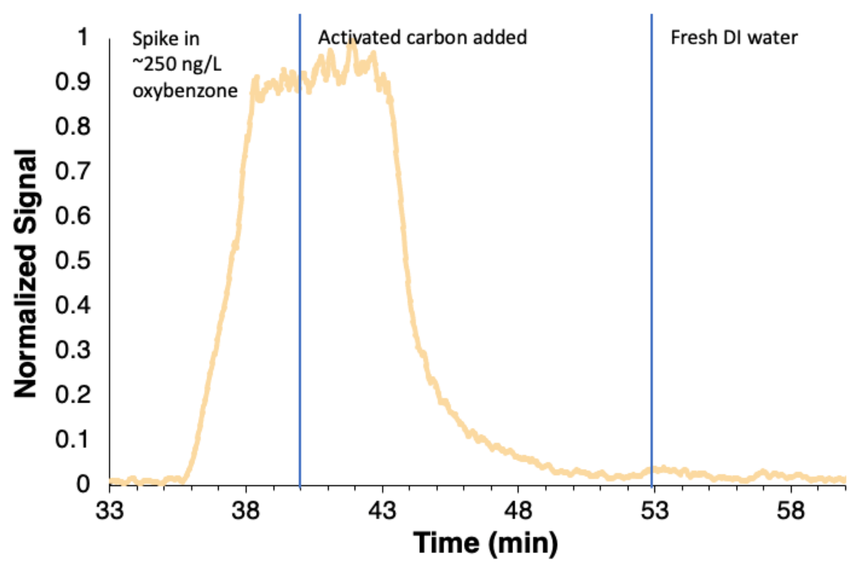

Figure 2: Real-time monitoring of oxybenzone signal upon adding activated carbon sorbent.

Fish Tissue

The measurement of UV filters in fish tissues by CP-MIMS-LEI/CI is currently being explored. Because of the high fatty acid content of fish tissues and the potential for instrument contamination, preliminary experiments are in process to determine the fatty acid content of extracts from fish tissue acquired by off-line CP-MIMS and analyzing the extracts by GC-MS.

APPENDIX D – PHOTOS

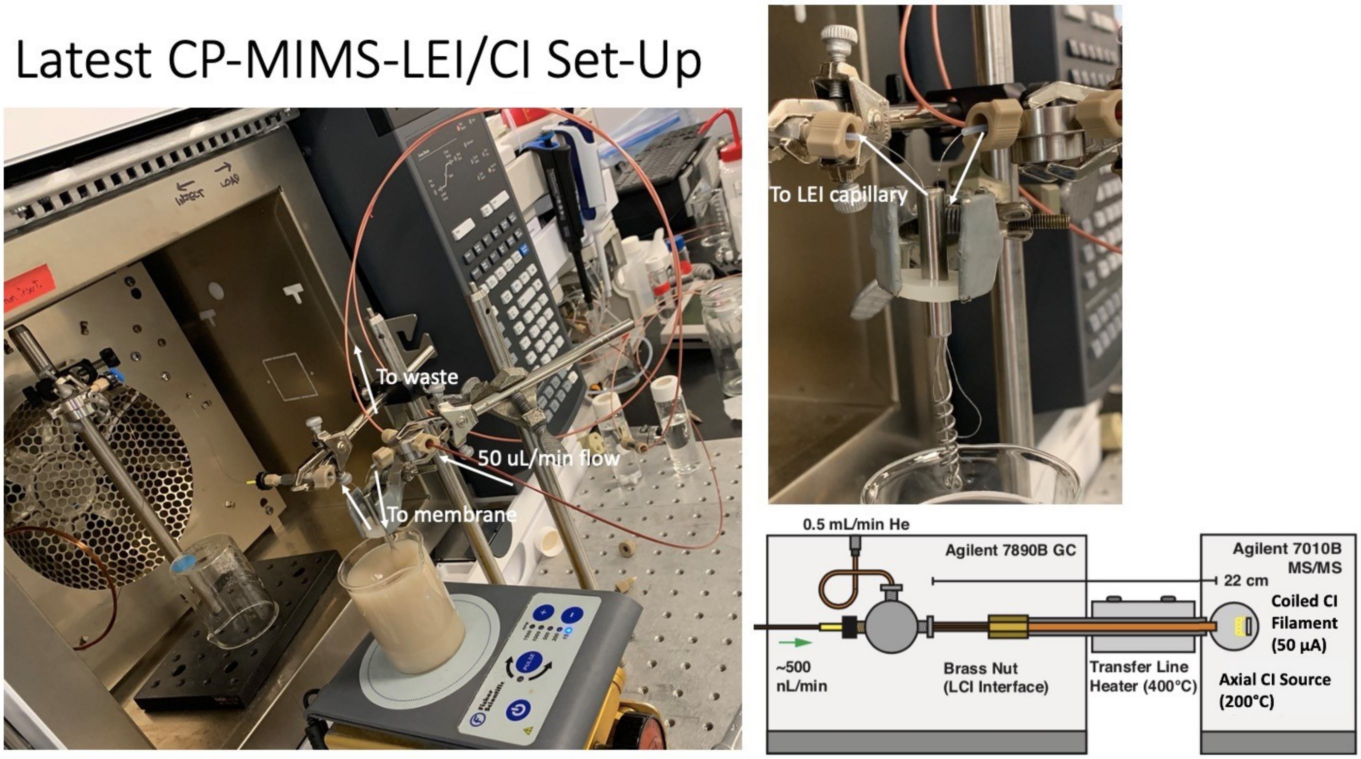

Figure D1. Analysis of sediment slurry at VIU-AERL using CP-MIMS-LEI/CI modified method.

Figure D2. Community volunteer Jim Deck collects a water sample from Arbutus Park, in Youbou.

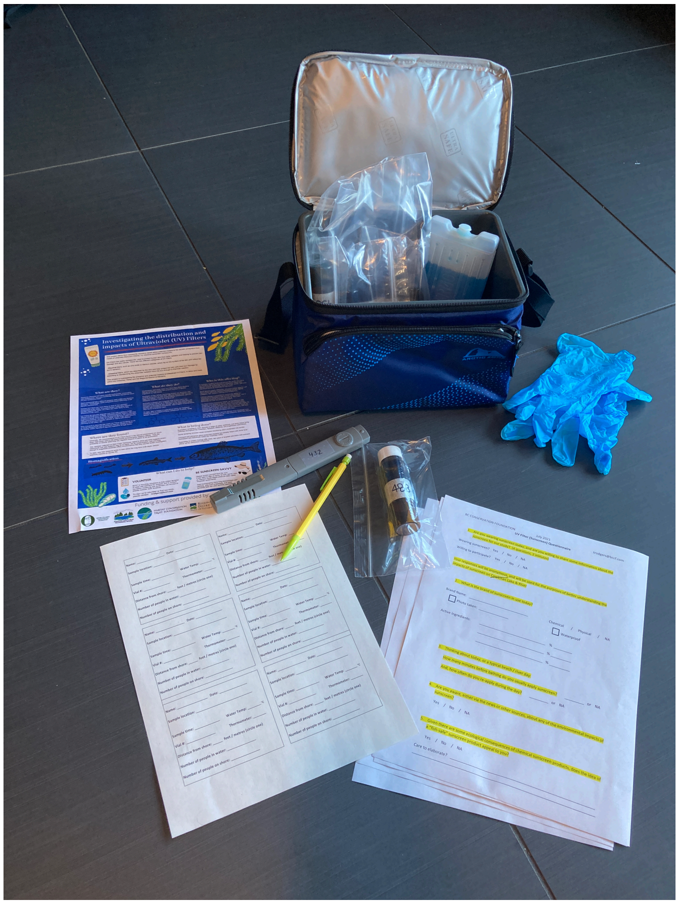

Figure D3. Example sampling kit provided to volunteers: background information, data sheets, sample vials, ice packs, gloves, thermometer, pencils, public questionnaires, and a laminated standard operating procedure handbook containing site directions, sampling instruction, & safety information (not pictured).

Figure D4. Rebekah Aplin collects a sediment sample from Gordon Bay Provincial Park beach.

Figure D5. Example brown trout sampled in Year 2. (Photo courtesy: Marley Bates).

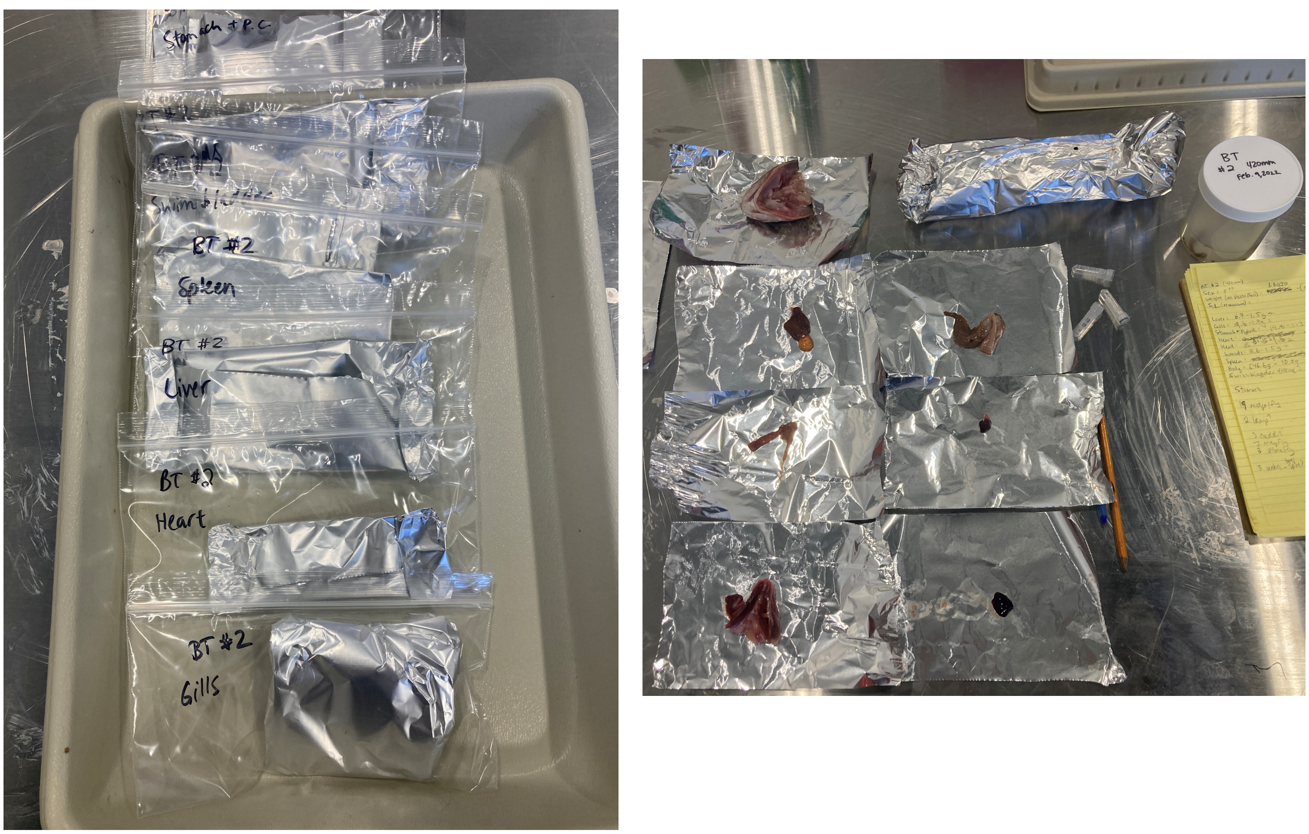

Figure D6. Fish sample processing; sorted by organs, stomach content examined and otoliths extracted.