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Oxybenzone contamination from sunscreen pollution and its ecological threat to Hanauma Bay, Oahu, Hawaii, U.S.A.

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Abstract

Hanauma Bay is a 101-acre bay created by the partial collapse of a volcanic cone and once supported a vibrant coral reef system. It is the most popular swimming area in the Hawaiian Islands and has been reported to have averaged between 2.8 and 3.5 million visitors a year between the 1980s and the 2010s, with visitors averaging between 3000–4000 a day and peaking around 10,000–13,000 per day. Concentrations of oxybenzone and other common UV filters were measured in subsurface water samples and in sands from the beach-shower areas in Hanauma Bay. Results demonstrate that beach showers also can be a source of sunscreen environmental contamination. Hydrodynamic modeling indicates that oxybenzone contamination within Hanauma Bay's waters could be retained between 14 and 50 h from a single release event period. Focusing on only oxybenzone, two different Hazard and Risk Assessment analyses were conducted to determine the danger of oxybenzone to Hanauma Bay's coral reef system. Results indicate that oxybenzone contamination poses a significant threat to the wildlife of Hanauma Bay. To recover Hanauma Bay's natural resources to a healthy condition and to satisfactorily conserve its coral reef and sea grass habitats, effective tourism management policies need to be implemented that mitigate the threat of sunscreen pollution.

Keywords:

Hanauma bay, Coral, Sunscreen, Oxybenzone, Risk assessment, Hydrodynamic modelling

1 Introduction

Hanauma Bay is a 101-acre bay created by the partial collapse of a volcanic cone and once supported a vibrant coral reef system. In 1967, it was designated as Hawaii's first Marine Life Conservation District and is located within the U.S. Hawaiian Islands Humpback Whale National Marine Sanctuary. It is critical coral reef habitat, as well as habitat for Hawaiian Monk Seals and Green Sea Turtles. Hawaii's Clean Water Act (Chapter 11-54-3(c)(1) Hawaii Administrative Rules) classifies Hanauma Bay's water as Class AA waters, which needs to "... remain in their natural pristine state as nearly as possible with an absolute minimum of pollution or alteration of water quality from any human-caused source or actions."

Hanauma Bay is the most popular swimming area in the Hawaiian Islands and was reported to have averaged between 2.8 and 3.5 million visitors a year in the 1980s to the early part of 2010, with an average of between 3000 and 4000 visitors a day and peaking around 10,000–13,000 per day (Mariani, 1989; Wagner, 1993; TenBruggencate, 1994; Mak and Moncur, 1995). In the era of COVID-19, the bay had been closed to all visitors from March 2020 to December 2020. In the Post-COVID shutdown in 2021, the City of Honolulu is permitting no more than 1000 visitors per day. Most of these visitors use over-the-counter sunscreen products for ultraviolet light (UV) sun-protection. As recommend by the U.S. Food & Drug Administration, these products should be applied to an average sized, relatively bare-torso swimmer in amounts of about 36 g every 90 min (U.S. FDA, 2019). This is predominantly because swimming and sweat-inducing activities causes the discharge of the sunscreen product from the skin into the environment, reducing the Sun-Protection Factor efficacy of the product (U.S. FDA, 1978).

Sunscreen products predominantly use UV-filter drugs such as oxybenzone, avobenzone, octocrylene, octinoxate, octisalate and homosalate, as well as zinc oxide and titanium dioxide (Ross, 2021). The increasing use of sunscreen UV-filter drugs has triggered concern about their emissions into the environment (Molins-Delgado et al., 2016; O'Malley et al., 2021). As a consequence of their constant use and uninterrupted release, these chemicals are classified as pseudo-persistent environmental pollutants, and nowadays constitute one of the most important chemical families designated as contaminants of emerging concern by the U.S. EPA (Blitz and Norton, 2008; Diaz-Cruz and Barceló, 2015).

Before 2019, oxybenzone (benzophenone-3; 2-hydroxy-4-methoxyphenyl phenylmethanone; CAS No. 131-57-7) was one of the dominant UV-filters used in sunscreen products (Ross, 2021). Levels of oxybenzone utilization in sunscreen products has significantly decreased after 2019 as a result of global response in consumer awareness of the dangers of this chemical to marine resources and from laws banning their sale or use in places such as Hawaii, Palau, The Marshall Islands, Thailand, the Northern Mariana Islands and a number of Caribbean islands such as Aruba, Bonaire, and the U.S. Virgin Islands. In the U.S. and E.U., oxybenzone concentrations are regulated at 6% in sunscreen and cosmetic products (U.S. FDA, 2019; European Union, 2019). A recent revision amendment introduced by the Opinion of the European Commission's Scientific Committee on Consumer Safety recommended the reduction of the current maximum concentration of oxybenzone in a consumer product from 6% to 2.2% because of reproductive, developmental, and carcinogenic concerns (SCCS, 2021). In Australia, oxybenzone concentrations have a maximum concentration of 10%, while Japan allows for no more than 5% (Australian Government, 2013; Japan, 2000).

The danger of UV-filters to wildlife receptors has been known for at least the past 20 years, starting with the works of Schlumpf, Fent, and others, which demonstrated that UV-filters such as oxybenzone can cause endocrine disruption in vertebrate systems and developmental toxicity in fish (Schlumpf et al., 2001, 2004; Schreurs et al., 2002; Ma et al., 2003; Schlumpf et al., 2004; Kunz et al., 2006; Kunz and Fent, 2006). A number of studies show that oxybenzone poses a realistic hazard by either inducing acute toxicity or acting as an endocrine/developmental disruptor to aquatic and marine invertebrates, including sea urchins, bivalves, and arthropods (Li, 2012; Bošnjak et al., 2013; Ozaez et al., 2014; Paredes et al., 2014; Chaves Lopes et al., 2020; O'Donovan et al., 2020; Thorel et al., 2020). Oxybenzone is also toxic to a wide number of algae and plants, including marine phytoplankton and macroalgae (Mao et al., 2017; Zhong et al., 2019a, 2019b, 2020; Teoh et al., 2020). Oxybenzone can be detrimental to corals, ranging from mortality to inducing pathologies such as bleaching, and gross planula deformations, as well as causing genotoxicity, endocrine disruption and significant shifts in its metabolome (Danovaro et al., 2008; Downs et al., 2016; He et al., 2019a, 2019b; Stien et al., 2020; Wijgerde et al., 2020). Sunscreen pollution can also threaten endangered species, such as sea turtles and marine mammals (Alonso et al., 2015; Cocci et al., 2020). Finally, emerging science has demonstrated a detrimental interaction between oxybenzone toxicity and climate change factors, such as elevated temperatures and ocean acidification (Chaves Lopes et al., 2020; Wijgerde et al., 2020).

A 2016 investigation into the oxybenzone contamination of Hanauma Bay saw water-column oxybenzone concentrations of more than 1600 ng/L (Booth and Manning, 2017). The amount of daily-swimming activity, estimated swimmer discharge rates, and the preliminary UV-filter survey makes pertinent the dilemma of whether swimmer and beach activity pose a potential threat to the sustainability of Hanauma Bay's coral reef ecosystem and marine mammal and sea turtle habitats. To determine if these contaminating sources pose a danger to the coral reef ecosystem of Hanauma Bay a hazard identification and risk assessment of oxybenzone concentrations need to be conducted.

The Hazard Identification and Risk Assessment are critical elements of the investigative and management process for addressing the issue of environmental contamination by individual ingredients of sunscreen. The goal of a Hazard Identification is to allow for the construction of a scientifically defensible argument regarding exposure of specific wildlife receptors to a discrete contaminant which can cause a toxicological/pathological response (NRC, 1983; U.S. EPA, 1992; 1998, 2004; NRC, 2009; European Commission, 2019). Hazard Identification analysis also helps to recognize the specific pathologies a chemical contaminant may cause to relevant ecological receptors during a field investigation. For example, are the levels of a sunscreen contaminant (e.g., oxybenzone) in an area high enough to induce mortality, genotoxicity, coral bleaching, decreases in photosynthetic conditions, or developmental deformities? One of the applications of the results of a Hazard Assessment is for Hanauma Bay resource managers to identify and implement effective mitigation measures to reduce contamination levels to ensure some level of protection for Hanauma's ecological integrity.

In this study, we measured concentrations of oxybenzone, other benzophenone chemical species, and a group of benzotriazoles in subsurface water samples that were collected in

the back reef zone most utilized by swimmers in Hanauma Bay. We measured the levels of oxybenzone and other common UV filters in sand samples in the beachshower area in Hanauma Bay, to determine if this could be a second source of sunscreen contamination. Exposure duration is a key consideration in toxicity investigation; thus, we conducted a preliminary examination of the retention time of oxybenzone (and other sunscreen compounds) within Hanauma Bay. In the peer-reviewed, publicly accessible literature, oxybenzone is predominantly the organic (petrochemical) UV filter organic compound with any substantive LC50 and EC50 “effect characterization” data. Thus, we focus only on oxybenzone for a risk assessment, combining environmental contamination data with the ecotoxicological data from the published literature, and then applied to a Hazard and a Risk Assessment to determine the danger of oxybenzone to Hanauma Bay's coral reef system.

In 2018, the State of Hawaii's legislature passed and enacted a regulatory prohibition for the sale and distribution of sunscreen products containing oxybenzone and octinoxate (2018 Act 104). This regulatory action would not go into effect until January 1, 2021. This study provides a “snapshot” baseline for oxybenzone levels before the implementation of the ban, as well as before the 2020 COVID shutdown where Hanauma Bay was closed to all visitors for almost six months. Surveys of sample collected in 2020 and 2021 will be contrasted with oxybenzone samples taken before the passing of the legislation, the implementation of the regulatory prohibition, and the banning of all visitors during the COVID shutdown.

2 Materials and methods

2.1 Sample collection

Seawater samples from Hanauma Bay were collected using precleaned 1-L amber glass bottles with Teflon lined lids (I-Chem, 300 series, VWR). Sample locations are indicated in Fig. 1A. Samples were collected approximately 30 cm below the surface of the water on November 17, 2017, between 16:00 and 17:30 Hawaii Standard Time. Water samples were extracted as described below within an hour after sampling. To minimize cross-contamination, samples were collected by single individuals which did not wear any sunscreen products within 72 h prior to the sampling period or during the sampling period. Samplers washed themselves in liquid nox soap to remove potential oxybenzone residue from themselves before going into the water to sample. Sampling method followed a modified method as described by U.S. NOAA for coral reef sediment collection methods for toxicology (https://cdhc.noaa.gov/education/fi_eld_health.aspx).

A Hanauma Bay, Oahu Island, Hawaii, U.S.A.
Oxybenzone Survey, November 17, 2017 4:00-5:30 pm PST

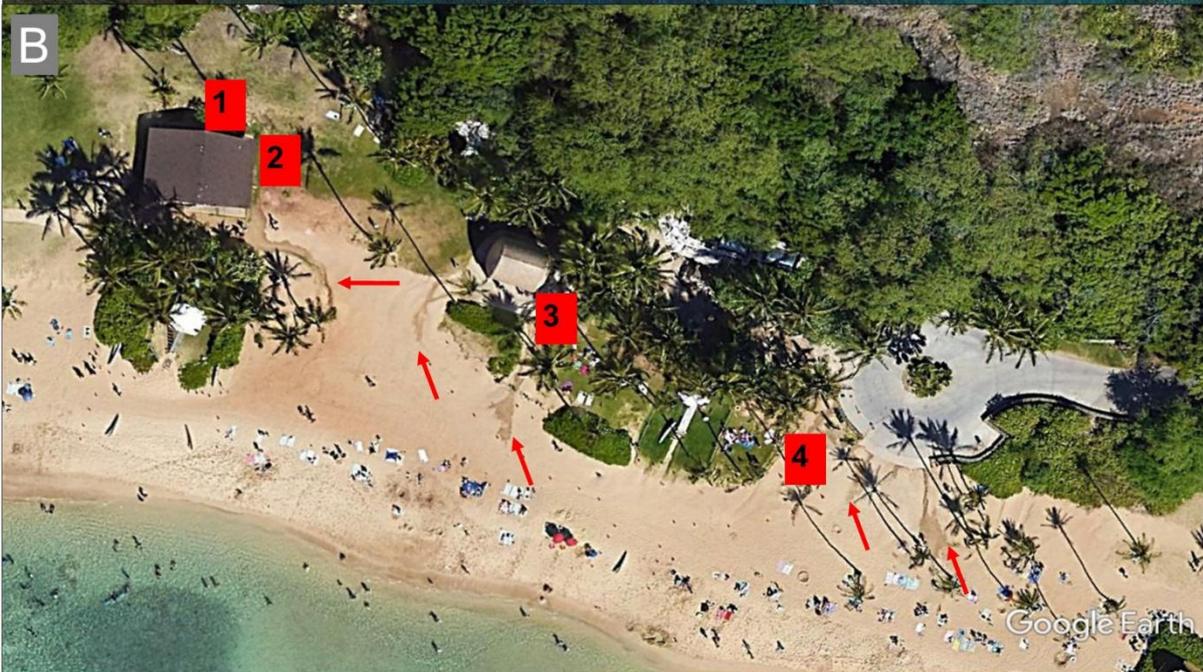
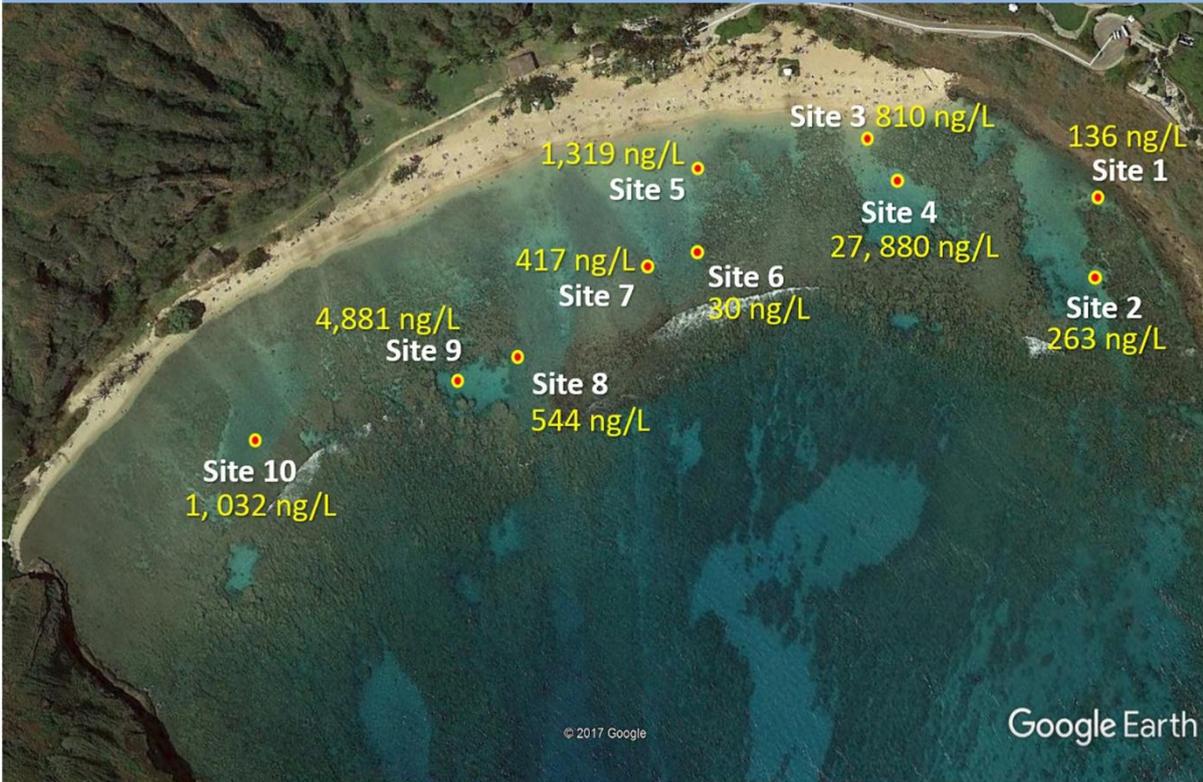


Fig. 1

Panel A - Sampling locations and oxybenzone concentration within Hanauma Bay, Oahu, State of Hawaii, U.S.A. Water samples were collected approximately 30 cm below the water surface. Panel B - Beach sand sampling locations within the run-off plume of the two beach showers in Hanauma Bay, City of Honolulu, State of Hawaii, U.S.A. Sand samples (indicated by numbers in red squares) consisted of collecting the top 5 cm of surface sand within a ~10 cm × 10 cm sampling square and were collected on January 27, 2020. Numbers designating

sample sites are the same designations in Table 1. Arrows indicate observable rivulets from shower runoff.

2.2 Water sample extraction

Sample volumes of 100–200 mL were loaded onto Strata™-X 33 µm polymeric reversed phase C18 cartridges (500 mg/12 mL; Phenomenex) to extract, purify and concentrate the target analytes by solid phase extraction (SPE). After loading, the cartridges were washed with 3 mL of HPLC-grade water and dried under a gentle current of nitrogen. Then, the analytes were eluted with (a) 7.5 mL of a solution of ethyl acetate and dichloromethane 1:1 v/v (EtAc:DCM (1:1)) and (b) 2 mL of DCM. The extracts were joined and evaporated with nitrogen until near dryness and then transferred into a LC-vial prior to full evaporation. Reconstitution was performed with 0.5 mL of HPLC-grade water containing the isotopically labelled internal standards. Finally, 20 µl of the extracts were analyzed by HPLC-MS/MS.

2.3 Water sample extract analysis by HPLC-MS/MS

The list of target analytes surveyed in the water samples can be found in Supplemental Table 1. Oxybenzone was the primary target analyte, but the extraction and analytical methodology screened for a range of other hydrocarbon UV filters (Supplemental Table 1). The determination of the target compounds was accomplished by high performance liquid chromatography-tandem mass spectrometry in a Symbiosis Pico chromatograph from Spark Holland (Emmen, The Netherlands) operated in off-line mode, and coupled to a 4000 Q TRAP™ mass spectrometer from Applied Biosystems-Sciex (Foster City, CA, USA). The chromatographic separation was achieved on a LiChorCART® Purospher® STAR® RP-18 EC (125 mm × 2.0 mm, 5 µm) from Merck (Darmstadt, Germany), preceded by a guard column LiChorCART® 4-4 Purospher® STAR® RP-18 ec (5 µm). The mobile phase consisted of water and acetonitrile (ACN) both HPLC grade with a 0.1% formic acid. The chromatographic gradient was as follows: the initial conditions of 5% ACN, increasing to 75% in 7 min, and to 100% in the next 3 min. Pure organic conditions were kept constant for 5 min and in the next 2 min until initial conditions were reached. The analytes were determined using electrospray ionization (ESI+) under positive mode and selected reaction monitoring (SRM) mode for improved sensitivity and selectivity. Two transitions per compound were registered, the more intense for quantification and the second one for confirmation.

2.4 Quality control/quality assurance for LC-MS analysis of water samples

To ensure the reliability of the results, several preventive measures were taken i.e. the use of gloves during the whole analytical process to avoid cross contamination, the use of solvents and reagents exclusively employed for these analyses, and the extensive cleaning treatment of all laboratory glass material with organic solvents of different polarity (MeOH, DCM, and acetone) and further heating at 400 °C of non-volumetric glass material for 15 h. Different methodological blanks were included (reagent blanks were run by processing HPLC water in the same way as samples. and procedural blank without matrix or sample). A surrogate standard (BP-¹³C) as evaluation of the extraction performance and isotopically labelled standards for the quantification of the target analytes (isotopic dilution quantification approach was used. As quality control, several mixture standard solutions at

known concentrations of the target analytes were included in the analysis' sequences randomly. MeOH solutions were systematically (every ten samples) run within the analysis' sequences to prevent memory effects and flux potential retained compounds in the columns and connections.

All analyses were carried out following the EU normative (Commission Decision, 2002/657/EC) in which compounds were identified with the chromatographic retention time (t_R) comparing with those of the standards at a maximum tolerance of 2.5% and that ion intensities between the selected SRM transitions were below 15%.

Quantification was conducted through internal-standard calibration using isotopically labelled standards (Supplemental Table 2). The total run time for each sample was 23 min. The method performance is shown in (Supplemental Table 2).

The limits of detection (LOD) and quantitation (LOQ) are provided in Supplemental Table 3. The recovery rates were calculated from spiked sand samples, which were extracted with MeOH using the same protocol as for extraction of natural sand samples (Rodrigues et al., 2021). The recovery rates were 85% for homosalate, 88% for benzophenone and 100% for Tinosorb M, avobenzene, oxybenzone, butyloctyl salicylate, octinoxate, octisalate, Tinosorb S, and octocrylene (Supplemental Table 3).

2.5 Sand sample extraction analysis by HPLC-MS/MS

Initial analysis of samples from beach sites 1, 2, and 3 exhibited extremely high concentrations of several of the UV filter analytes; the initial analytical run demonstrated that the sample concentrations for some of the target analytes were above the maximum concentration calibrant (Fig. 1B). For beach samples sites 1, 2, and 3, 0.2 g of sand were used for extraction. For beach sand sample 4, 2 g of sand was used. For each sand sample, 7 replicates were extracted and analyzed. In all cases, sands were not dried before being added into the extraction tubes. If need be, excess water was removed by spreading the sand on a filter paper. Sands were extracted with MeOH (2 mL) and the concentration of UV filters in the supernatant was measured by direct injection in UHPLC-HRMS following the protocol previously described (Rodrigues et al., 2021). The concentration in the supernatant was calculated by comparison of peak areas with those from an external calibration curve. After analysis, the solvent and the sand water were removed by evaporation with a GeneVac HT-4X. The exact mass of dry sand gave the initial mass of water in the sand and the total volume of supernatant (2-mL MeOH + sand water), allowing for correction of the concentrations in supernatant and in sand.

2.6 Target analytes and standards for sand sample analysis

The list of target analytes for sand samples can be found in Supplemental Table 2. Benzophenone (BP, CAS# 119-61-9), Tinosorb M (methylene bis-benzotriazolyl tetramethylbutylphenol; CAS# 103597-45-1), and oxybenzone (CAS# 131-57-7) were purchased from Sigma-Aldrich (Lyon, France), while Tinosorb S (bis-ethylhexyloxyphenol methoxyphenyl triazine; CAS# 187393-00-6), avobenzene (butyl methoxydibenzoylmethane; CAS# 70356-09-1), homosalate (CAS# 118-56-9), octisalate (ethylhexyl salicylate; CAS# 118-

60-5) and octocrylene (CAS# 6197-30-4) were kindly provided by Pierre Fabre Laboratories. Butyloctyl salicylate (CAS# 190085-41-7) was obtained from Innospec Active Chemicals. Octinoxate (ethylhexyl methoxycinnamate; CAS# 5466-77-3) was obtained from Accustandard (Cat# ALR144 N).

Quality control/quality assurance for LC-MS analysis of water samples

3.1 Pollution retention modeling

Both the particle pathway and retention will affect how released pollutants influence local water quality and ecosystem health (Du et al., 2019). A 2DH hydrodynamic and particle tracking model was implemented to estimate the mixing and dispersion of sunscreen in the water of Hanauma Bay (Tsanis et al., 2006; Jiang et al., 2017). The model developed for this study solves the Navier-Stokes equations for shallow water with the hydrostatic pressure assumption. The computational grid is shown in Supplemental Fig. 1. An ocean model should provide a realistic large-scale circulation while also resolving small-scale flow features down to the scale of individual reefs. Unstructured-mesh ocean-models offer a potential solution to this resolution issue by locally increasing the model resolution close to reefs and islands (Lambrechts et al., 2008; Thomas et al., 2014, 2015). Bathymetry data were acquired from U.S. NOAA's National Centers for Environmental Information (NCEI, <https://www.ncei.noaa.gov/>). For deeper areas, we used the General Bathymetric Chart of the Oceans database (<https://www.gebco.net/>). The sunscreen contaminant release data used in the model were from water sample sites #1, #4, #6, #8, and #10 (Fig. 1A).

The dispersion model was run for an arbitrary one-month period in 2018, including periods of tide-dominant and oceanic-current-dominant conditions. The data for 2017 in Hawaii is still not available (as of October 29, 2021). During periods of oceanic-current domination, a strong shoreward tendency can be observed in current behavior for this area. To illustrate this, three hydrodynamic models were run to determine oxybenzone retention within Hanauma Bay to tidal forcing (Model scenario #1), a combination of tidal and (southward) oceanic current forcing (adopted from global HYCOM data) (Model scenario #2), and a combination of tidal and (southward) oceanic current forcing (adopted from global HYCOM data), but during periods of relatively strong shoreward (northward) current presence (Model scenario #3).

3.2 Hazard and risk methods

For a hazard assessment, there are a number of diverse approaches in calculating a hazard (or risk) quotient (Environment Canada 2013; European Commission 2003a, 2003b, 2003c; European Medicines Agency, 2006; Dussault et al., 2008; Hernando et al., 2006; USEPA 2004, 2020). In this paper, we compare two different hazard/riskquotient assessment methods to determine the threat to wildlife integrity for Hanauma Bay.

The definition of a hazard or risk quotient is “the ratio of the potential exposure to a substance and the level at which no adverse effects are expected” (U.S. EPA, 1998; U.S. EPA, 2018). Hazard quotient equations require at least two parameters: (a) measured environmental concentration (MEC) and (b) a toxicity endpoint (e.g., No observed effect

concentration, lethal concentration for 50% of the population (LC50)). Measured environmental concentrations used for these calculations are found in Fig. 1A.

The first method employed a Hazard Quotient (HQ) calculation following a protocol specified by the U.S. Environmental Protection Agency (U.S. EPA) guidance for pesticides and other chemicals (U.S. EPA, 2004). This guidance also makes provisions for determination of effects for Endangered and Threatened species (U.S. EPA, 2004). This method compares the MEC to an acute toxicity endpoint (e.g., LC50 is the concentration of a chemical where 50% of the organisms die) or EC50 concentration of a chemical (adverse effect observed in 50% of the population for a sublethal endpoint). Toxicity reference values were obtained from the published literature (Table 2). Thus, a HQ is a screening tool that generates measures of levels of concern, though this method does not provide probability-based information of risk nor does it account for chronic exposure effects, only acute effects (Tannenbaum et al., 2003).

Table 2. Oxybenzone hazard quotient for Acute Toxicity in Hanauma Bay, Oahu, Hawaii using US EPA method. Color chart: Red = Severe condition for a potential toxic effect, ≥ 0.5 ; Yellow – Moderate threat condition for a potential toxic effect, 0.1–0.5; Green = Low risk of acute toxicity, 0.05–0; Gold = ≥ 0.05 Acute Risk for endangered animal species.

				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	
				0.1362	0.2627	0.8103	27.8800	1.3190	0.0303	0.4165	0.5443	4.8810	1.0320	Environ. Concen. µg/L
SPECIES			Toxicity Reference Value µg/L											References for Toxicity Reference Values
Coral cells in vitro														
<i>Acropora cervicornis</i>	coral cells 4hr light	LC ₅₀	9.00	0.02	0.03	0.09	3.10	0.15	0.00	0.05	0.06	0.54	0.11	Downs et al. 2016
<i>Montastraea cavernosa</i>	coral cells 4hr light	LC ₅₀	52.00	0.00	0.01	0.02	0.54	0.03	0.00	0.01	0.01	0.09	0.02	Downs et al. 2016
<i>Orbicella annularis</i>	coral cells 4hr light	LC ₅₀	74.00	0.00	0.00	0.01	0.38	0.02	0.00	0.01	0.01	0.07	0.01	Downs et al. 2016
<i>Pocillopora damicornis</i>	coral cells 4hr light	LC ₅₀	8.00	0.02	0.03	0.10	3.49	0.16	0.00	0.05	0.07	0.61	0.13	Downs et al. 2016
<i>Porites astreoides</i>	coral cells 4hr dark	LC ₅₀	340.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.01	0.00	Downs et al. 2016
<i>Porites divaricata</i>	coral cells 4hr light	LC ₅₀	36.00	0.00	0.01	0.02	0.77	0.04	0.00	0.01	0.02	0.14	0.03	Downs et al. 2016
<i>Stylophora pistillata</i>	coral cells 4hr light	LC ₅₀	679.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	coral cells 4hr light	LC ₅₀	42.00	0.00	0.01	0.02	0.66	0.03	0.00	0.01	0.01	0.12	0.02	Downs et al. 2016
Coral Planula (early life stage)														
<i>Stylophora pistillata</i>	Planula 8h dark	LC ₅₀	12800.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula 8h light	LC ₅₀	2900.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula 24h dark	LC ₅₀	799.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula 24h light	LC ₅₀	139.00	0.00	0.00	0.01	0.20	0.01	0.00	0.00	0.00	0.04	0.01	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 8h dark	EC ₅₀	737000.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 8h light	EC ₅₀	133000.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 24h dark	EC ₅₀	137.00	0.00	0.00	0.01	0.20	0.01	0.00	0.00	0.00	0.04	0.01	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 24h light	EC ₅₀	49.00	0.00	0.01	0.02	0.57	0.03	0.00	0.01	0.01	0.10	0.02	Downs et al. 2016
Invertebrates														
<i>Daphnia magna</i>	neonate crustacean 24h mortality	LC ₅₀	7630.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Sun et al. 2016
<i>Daphnia magna</i>	neonate crustacean 48h mortality	LC ₅₀	1090.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	Du et al. 2017
<i>Daphnia magna</i>	neonate crustacean 24h immobility	EC ₅₀	2700.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Molins-Delgado et al. 2016
<i>Daphnia magna</i>	neonate crustacean 48h immobility	EC ₅₀	1670.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	Sieratowicz et al. 2011
<i>Daphnia magna</i>	neonate crustacean 72h immobility	EC ₅₀	1600.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	Molins-Delgado et al. 2016
<i>Paracentrotus lividus</i>	sea urchin - 48 h larval growth	EC ₅₀	3280.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Paredes et al. 2014
<i>Mytilus galloprovincialis</i>	mussel embryos 48h % normal D-larvae	EC ₅₀	3472.59	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Paredes et al. 2014
<i>Siriella armata</i>	mysid, crustacean 24h neonates 96h mortality	LC ₅₀	710.76	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.00	Paredes et al. 2014
Algae														
<i>Desmodesmus subspicatus</i>	Green algae 72h growth inhibition	EC ₅₀	960.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.00	Sieratowicz et al. 2011
<i>Chlamydomonas reinhardtii</i>	green microalgae 10d growth inhibition	EC ₅₀	1850.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	Mao et al. 2017
<i>Chlorella vulgaris</i>	green microalgae 72h growth inhibition	EC ₅₀	22400.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Pablos et al. 2015
<i>Chlorella vulgaris</i>	green microalgae 96h growth inhibition	EC ₅₀	6860.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Sun et al. 2016
<i>Chlorella vulgaris</i>	green microalgae growth inhibition 96h	EC ₅₀	2980.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Du et al. 2017
<i>Isochrysis galbana</i>	microalgae 72h growth rate	EC ₅₀	13.87	0.01	0.02	0.06	2.01	0.10	0.00	0.03	0.04	0.35	0.07	Paredes et al. 2014
<i>Microcystis aeruginosa</i>	cyanobacterium 10d inhibition of Chla production	EC ₅₀	2460.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Mao et al. 2017
<i>Skeletonema pseudocostatum</i>	algal diatom 72h growth inhibition	EC ₅₀	251.00	0.00	0.00	0.00	0.11	0.01	0.00	0.00	0.00	0.02	0.00	Petersen et al. 2014
Fish														
<i>Brachydanio rerio</i>	96h	LC ₅₀	3890.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Du et al. 2017
<i>Danio rerio</i>	Embryos Lethality 96h	LC ₅₀	4153	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	Meng et al. 2017

The equation for the acute hazard quotient is $HQ = (MEC)/(\text{organism's } EC_{50} \text{ or } LC_{50} \text{ with 96-h or less of exposure to the toxicant})$. An Effect Factor or Uncertainty Factor were not included in this equation. This quotient was derived for each of the 10 samples and compared to U.S. EPA's Level of Concern of 0.5 for aquatic animals (U.S. EPA, 2004; Gwinn et al., 2020), which were highlighted in red (Table 2).

A second method was used to calculate risk quotients that was based on the European Commission guidance regarding risk quotient (RQ) determination. The European Commission methodology has been adopted in the development of several ecological risk assessment guidelines (ECHA, 2008; European Commission 1996, 2003a, 2003b, 2003c; Environment Canada 2012; European Medicines Agency, 2006; Dussault et al., 2008; Hernando et al., 2006). With this method, the actual or predicted environmental concentration (MEC) is compared to a derived known or Predicted No-Effect Concentration (PNEC) which is derived by dividing the LC_{50} , EC_{50} , or NOEC by an uncertainty (or assessment) factor (UF). Thus, the $RQ = (MEC)/(PNEC)(UF)$. For this RQ determination, an UF of 1000 was selected for the extrapolation of the EC_{50} , LC_{50} or No Observable Effect Concentration (NOEC) values to estimate no-effect values (PNEC) (Chapman et al., 1998; Dussault et al., 2008; Environment Canada, 2012). In cases where the NOEC was not known, but the Lowest Observable Effect Concentration (LOEC) was known, the LOEC was divided by two to calculate a predicted NOEC (ECHA, 2008). Toxicity reference values were obtained from the published literature (Table 3).

Table 3. Risk Quotient for Acute Toxicity in Hanauma Bay, Oahu Hawaii using European Union method for Cnidarian species, invertebrate (non-Cnidarian) species, plant and algae species, and fish species. Color chart: RED = Severe condition for a potential toxic effect ≥ 1 ; Orange = Moderate threat condition for a potential toxic effect = 0.5 to 1.0; Yellow = Condition of concern 0.5 to 0.1.

				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Environ. Concen. µg/L
SPECIES			Toxicity Reference Value µg/L	0.1362	0.2627	0.8103	27.8800	1.3190	0.0303	0.4165	0.5443	4.8810	1.0320	References for Toxicity Reference Values
				<i>Coral cells in vitro</i>										
<i>Acropora cervicornis</i>	coral cells 4hr light	LC ₅₀	9.00	15.13	29.19	90.03	3097.78	146.56	3.37	46.28	60.48	542.33	114.67	Downs et al. 2016
<i>Montastraea cavernosa</i>	coral cells 4hr light	LC ₅₀	52.00	2.62	5.05	15.58	536.15	25.37	0.58	8.01	10.47	93.87	19.85	Downs et al. 2016
<i>Orbicella annularis</i>	coral cells 4hr light	LC ₅₀	74.00	1.84	3.55	10.95	376.76	17.82	0.41	5.63	7.36	65.96	13.95	Downs et al. 2016
<i>Pocillopora damicornis</i>	coral cells 4hr light	LC ₅₀	8.00	17.03	32.84	101.29	3485.00	164.88	3.79	52.06	68.04	610.13	129.00	Downs et al. 2016
<i>Porites astreoides</i>	coral cells 4hr light	LC ₅₀	340.00	0.40	0.77	2.38	82.00	3.88	0.09	1.23	1.60	14.36	3.04	Downs et al. 2016
<i>Porites divaricata</i>	coral cells 4hr light	LC ₅₀	36.00	3.78	7.30	22.51	774.44	36.64	0.84	11.57	15.12	135.58	28.67	Downs et al. 2016
<i>Stylophora pistillata</i>	coral cells 4hr dark	LC ₅₀	679.00	0.20	0.39	1.19	41.06	1.94	0.04	0.61	0.80	7.19	1.52	Downs et al. 2016
<i>Stylophora pistillata</i>	coral cells 4hr light	LC ₅₀	42.00	3.24	6.25	19.29	663.81	31.40	0.72	9.92	12.96	116.21	24.57	Downs et al. 2016
<i>Coral & Cassiopeia Planula (early life stage)</i>														
<i>Cassiopea xamachana</i>	Planula Lethality	NOEC	2.28	59.74	115.22	355.39	12228.07	578.51	13.30	182.68	238.73	2140.79	452.63	Fitt & Hoffman 2020
<i>Cassiopea xamachana</i>	Planula swimming 48 hr	NOEC	114.00	1.19	2.30	7.11	244.56	11.57	0.27	3.65	4.77	42.82	9.05	Fitt & Hoffman 2020
<i>Cassiopea xamachana</i>	Planula Swim-speed 48 hr	NOEC	114.00	1.19	2.30	7.11	244.56	11.57	0.27	3.65	4.77	42.82	9.05	Fitt & Hoffman 2020
<i>Cassiopea frondosa</i>	Planula Lethality	NOEC	2.28	59.74	115.22	355.39	12228.07	578.51	13.30	182.68	238.73	2140.79	452.63	Fitt & Hoffman 2020
<i>Cassiopea frondosa</i>	Planula swimming 48 hr	NOEC	114.00	1.19	2.30	7.11	244.56	11.57	0.27	3.65	4.77	42.82	9.05	Fitt & Hoffman 2020
<i>Cassiopea frondosa</i>	Planula Swim-speed 24 hr	NOEC	114.00	1.19	2.30	7.11	244.56	11.57	0.27	3.65	4.77	42.82	9.05	Fitt & Hoffman 2020
<i>Stylophora pistillata</i>	Planula 8h dark	LC ₅₀	12800.00	0.01	0.02	0.06	2.18	0.10	0.00	0.03	0.04	0.38	0.08	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula 8h light	LC ₅₀	2900.00	0.05	0.09	0.28	9.61	0.45	0.01	0.14	0.19	1.68	0.36	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula 24h dark	LC ₅₀	799.00	0.17	0.33	1.01	34.89	1.65	0.04	0.52	0.68	6.11	1.29	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula 24h light	LC ₅₀	139.00	0.98	1.89	5.83	200.58	9.49	0.22	3.00	3.92	35.12	7.42	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 8h dark	EC ₅₀	737000.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.00	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 8h light	EC ₅₀	133000.00	0.00	0.00	0.01	0.21	0.01	0.00	0.00	0.00	0.04	0.01	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 24h dark	EC ₅₀	137.00	0.99	1.92	5.91	203.50	9.63	0.22	3.04	3.97	35.63	7.53	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula deformity 24h light	EC ₅₀	49.00	2.78	5.36	16.54	568.98	26.92	0.62	8.50	11.11	99.61	21.06	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula DNA Damage 8h light	NOEC	11.40	11.95	23.04	71.08	2445.61	115.70	2.66	36.54	47.75	428.16	90.53	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula DNA Damage 8h dark	NOEC	22.80	5.97	11.52	35.54	1222.81	57.85	1.33	18.27	23.87	214.08	45.26	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula bleaching 8h light	NOEC	1.14	119.47	230.44	710.79	24456.14	1157.02	26.61	365.35	477.46	4281.58	905.26	Downs et al. 2016
<i>Stylophora pistillata</i>	Planula bleaching 8h dark	NOEC	1.14	119.47	230.44	710.79	24456.14	1157.02	26.61	365.35	477.46	4281.58	905.26	Downs et al. 2016

				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Environ. Concen. µg/L
				0.1362	0.2627	0.8103	27.8800	1.3190	0.0303	0.4165	0.5443	4.8810	1.0320	
SPECIES			Toxicity Reference Value µg/L											References for Toxicity Reference Values
Invertebrates														
<i>Corbicula fluminea</i>	clams 96h filter feeding inhibition	NOEC	315.00	0.43	0.83	2.57	88.51	4.19	0.10	1.32	1.73	15.50	3.28	Seoane et al. 2020
<i>Corbicula fluminea</i>	clams 96h increased CAT activity	NOEC	625.00	0.22	0.42	1.30	44.61	2.11	0.05	0.67	0.87	7.81	1.65	Seoane et al. 2020
<i>Corbicula fluminea</i>	clams 96h increased glutathione reductase activity	NOEC	1250.00	0.11	0.21	0.65	22.30	1.06	0.02	0.33	0.44	3.90	0.83	Seoane et al. 2020
<i>Corbicula fluminea</i>	clams 96h increased lipid peroxidation	NOEC	315.00	0.43	0.83	2.57	88.51	4.19	0.10	1.32	1.73	15.50	3.28	Seoane et al. 2020
<i>Daphnia magna</i>	neonate crustacean 48h mortality	LC ₅₀	1700.00	0.08	0.15	0.48	16.40	0.78	0.02	0.25	0.32	2.87	0.61	Boyd et al. 2021
<i>Dugesia japonica</i>	adult planaria 24h mortality	LC ₅₀	2200.00	0.06	0.12	0.37	12.67	0.60	0.01	0.19	0.25	2.22	0.47	Li et al. 2012
<i>Dugesia japonica</i>	adult planaria 48h mortality	LC ₅₀	900.00	0.15	0.29	0.90	30.98	1.47	0.03	0.46	0.60	5.42	1.15	Li et al. 2012
<i>Dugesia japonica</i>	adult planaria 72h mortality	LC ₅₀	700.00	0.19	0.38	1.16	39.83	1.88	0.04	0.60	0.78	6.97	1.47	Li et al. 2012
<i>Dugesia japonica</i>	planaria 96h mortality	LC ₅₀	500.00	0.27	0.53	1.62	55.76	2.64	0.06	0.83	1.09	9.76	2.06	Li et al. 2012
<i>Daphnia magna</i>	neonate crustacean 24h mortality	LC ₅₀	7630.00	0.02	0.03	0.11	3.65	0.17	0.00	0.05	0.07	0.64	0.14	Sun et al. 2016
<i>Daphnia magna</i>	neonate crustacean 48h mortality	LC ₅₀	1090.00	0.12	0.24	0.74	25.58	1.21	0.03	0.38	0.50	4.48	0.95	Du et al. 2017
<i>Daphnia magna</i>	neonate crustacean 24h immobility	EC ₅₀	2700.00	0.05	0.10	0.30	10.33	0.49	0.01	0.15	0.20	1.81	0.38	Molins-Delgado 2016
<i>Daphnia magna</i>	neonate crustacean 48h immobility	EC ₅₀	1670.00	0.08	0.16	0.49	16.69	0.79	0.02	0.25	0.33	2.92	0.62	Sieratowicz et al. 2011
<i>Daphnia magna</i>	neonate crustacean 72h immobility	EC ₅₀	1600.00	0.09	0.16	0.51	17.43	0.82	0.02	0.26	0.34	3.05	0.65	Molins-Delgado 2016
<i>Daphnia magna</i>	neonate crustacean 48h mortality	EC ₅₀	1900.00	0.07	0.14	0.43	14.67	0.69	0.02	0.22	0.29	2.57	0.54	Fent et al. 2010
<i>Daphnia magna</i>	neonate crustacean 48h immobility	EC ₅₀	3030.00	0.04	0.09	0.27	9.20	0.44	0.01	0.14	0.18	1.61	0.34	Jang et al. 2016
<i>Daphnia magna</i>	neonate crustacean 48h immobility	EC ₅₀	1200.00	0.11	0.22	0.68	23.23	1.10	0.03	0.35	0.45	4.07	0.86	Boyd et al. 2021
<i>Daphnia magna</i>	juvenile crustacean 48h immobility	EC ₅₀	2900.00	0.05	0.09	0.28	9.61	0.45	0.01	0.14	0.19	1.68	0.36	Na et al. 2021
<i>Daphnia magna</i>	juvenile crustacean 48h ROS production	NOEC	25.00	5.45	10.51	32.41	1115.20	52.76	1.21	16.66	21.77	195.24	41.28	Na et al. 2021
<i>Daphnia magna</i>	juvenile crustacean 48h total antioxidant capacity	NOEC	25.00	5.45	10.51	32.41	1115.20	52.76	1.21	16.66	21.77	195.24	41.28	Na et al. 2021
<i>Daphnia magna</i>	juvenile crustacean 48h lipid peroxidation	NOEC	25.00	5.45	10.51	32.41	1115.20	52.76	1.21	16.66	21.77	195.24	41.28	Na et al. 2021
<i>Paracentrotus lividus</i>	sea urchin 48 h larval growth 4-arm-pluteus	EC ₅₀	3280.00	0.04	0.08	0.25	8.50	0.40	0.01	0.13	0.17	1.49	0.31	Paredes et al. 2014
<i>Mytilus galloprovincialis</i>	mussel embryos 48h % normal D-larvae	EC ₅₀	3472.59	0.04	0.08	0.23	8.03	0.38	0.01	0.12	0.16	1.41	0.30	Paredes et al. 2014
<i>Siriella armata</i>	mysid, crustacean 24h neonates 96h mortality	EC ₅₀	710.76	0.19	0.37	1.14	39.23	1.86	0.04	0.59	0.77	6.87	1.45	Paredes et al. 2014

				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Environ. Concen. µg/L
SPECIES			Toxicity Reference Value µg/L	0.1362	0.2627	0.8103	27.8800	1.3190	0.0303	0.4165	0.5443	4.8810	1.0320	References for Toxicity Reference Values
				<i>Algae and vascular plants</i>										
Arthrospira sp.	cyanobacteria 7d Net photosynthesis	NOEC	0.23	597.37	1152.19	3553.95	122280.70	5785.09	133.03	1826.75	2387.28	21407.89	4526.32	Zhong et al. 2019
Arthrospira sp.	cyanobacteria 20d growth inhibition	NOEC	0.23	597.37	1152.19	3553.95	122280.70	5785.09	133.03	1826.75	2387.28	21407.89	4526.32	Zhong et al. 2019
Arthrospira sp.	cyanobacteria 20d Respiration rate	NOEC	0.23	597.37	1152.19	3553.95	122280.70	5785.09	133.03	1826.75	2387.28	21407.89	4526.32	Zhong et al. 2019
Chlamydomonas reinhardtii	green microalgae 10d growth inhibition	EC ₅₀	1850.00	0.07	0.14	0.44	15.07	0.71	0.02	0.23	0.29	2.64	0.56	Mao et al. 2017
Chlorella vulgaris	green microalgae 72h growth inhibition	EC ₅₀	22400.00	0.01	0.01	0.04	1.24	0.06	0.00	0.02	0.02	0.22	0.05	Pablos et al. 2015
Chlorella vulgaris	green microalgae 96h growth inhibition	EC ₅₀	6860.00	0.02	0.04	0.12	4.06	0.19	0.00	0.06	0.08	0.71	0.15	Sun et al. 2016
Chlorella vulgaris	green microalgae growth inhibition 96h	EC ₅₀	2980.00	0.05	0.09	0.27	9.36	0.44	0.01	0.14	0.18	1.64	0.35	Du et al. 2017
Chlorella sp.	green microalgae 7d Net respiration rate	NOEC	0.11	1194.74	2304.39	7107.89	244561.40	11570.18	266.05	3653.51	4774.56	42815.79	9052.63	Zhong et al. 2019
Chlorella sp.	green microalgae 7d Net photosynthesis	NOEC	0.11	1194.74	2304.39	7107.89	244561.40	11570.18	266.05	3653.51	4774.56	42815.79	9052.63	Zhong et al. 2019
Chlorella sp.	green microalgae growth inhibition 20d	NOEC	0.02	5973.68	11521.93	35539.47	1222807.02	57850.88	1330.26	18267.54	23872.81	214078.95	45263.16	Zhong et al. 2019
Cucumis sativus	vascular plant 24h photosynthesis	NOEC	570.00	0.24	0.46	1.42	48.91	2.31	0.05	0.73	0.95	8.56	1.81	Zhong et al. 2020
Cucumis sativus	vascular plant 24h mitochondrial respiration	NOEC	570.00	0.24	0.46	1.42	48.91	2.31	0.05	0.73	0.95	8.56	1.81	Zhong et al. 2020
Isochrysis galbana	Haptophyta algae 72h growth rate	EC ₅₀	13.87	9.82	18.94	58.42	2010.09	95.10	2.19	30.03	39.24	351.91	74.41	Paredes et al. 2014
Microcystis aeruginosa	cyanobacterium 10d inhibition Chla production	EC ₅₀	2460.00	0.06	0.11	0.33	11.33	0.54	0.01	0.17	0.22	1.98	0.42	Mao et al. 2017
Microcystis aeruginosa	cyanobacterium 96h Chla content	NOEC	0.005	27240.00	52540.00	162060.00	5576000.00	263800.00	6066.00	83300.00	108860.00	976200.00	206400.00	Mao et al. 2020
Microcystis aeruginosa	cyanobacterium 96h Carotenoid content	NOEC	0.01	27240.00	52540.00	162060.00	5576000.00	263800.00	6066.00	83300.00	108860.00	976200.00	206400.00	Mao et al. 2020
Microcystis aeruginosa	cyanobacterium 96h Oxidative damage	NOEC	0.005	27240.00	52540.00	162060.00	5576000.00	263800.00	6066.00	83300.00	108860.00	976200.00	206400.00	Mao et al. 2020
Microcystis aeruginosa	cyanobacterium 96h Catalase/SOD	NOEC	0.005	27240.00	52540.00	162060.00	5576000.00	263800.00	6066.00	83300.00	108860.00	976200.00	206400.00	Mao et al. 2020
Skeletonema pseudocostatum	algal diatom 72h growth inhibition	EC ₅₀	251.00	0.54	1.05	3.23	111.08	5.25	0.12	1.66	2.17	19.45	4.11	Petersen et al. 2014
Scenedesmus obliquus	Green algae 4d growth rate	NOEC	250.00	0.54	1.05	3.24	111.52	5.28	0.12	1.67	2.18	19.52	4.13	Lee et al. 2020
Scenedesmus obliquus	Green algae 4d decrease in dry cell weight	NOEC	500.00	0.27	0.53	1.62	55.76	2.64	0.06	0.83	1.09	9.76	2.06	Lee et al. 2020
Tetraselmis sp.	Marine green algae 7d Growth Inhibition	NOEC	1.00	136.20	262.70	810.30	27880.00	1319.00	30.33	416.50	544.30	4881.00	1032.00	Thorel et al. 2020

				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Environ. Concen. µg/L
				0.1362	0.2627	0.8103	27.8800	1.3190	0.0303	0.4165	0.5443	4.8810	1.0320	
SPECIES			Toxicity Reference Value µg/L											References for Toxicity Reference Values
<i>Fish</i>														
<i>Betta splendens</i>	Adult 28d aggressive swimming velocity	NOEC	100	1.36	2.63	8.10	278.80	13.19	0.30	4.17	5.44	48.81	10.32	Chen et al. 2018
<i>Betta splendens</i>	Adult 28d aggressive opercular display	NOEC	5	27.24	52.54	162.06	5576.00	263.80	6.07	83.30	108.86	976.20	206.40	Chen et al. 2018
<i>Brachydanio rerio</i>	96h	LC ₅₀	3890.00	0.04	0.07	0.21	7.17	0.34	0.01	0.11	0.14	1.25	0.27	Du et al. 2017
<i>Carassius auratus</i>	Adult 28d change in gut microbiota	NOEC	1	136.20	262.70	810.30	27880.00	1319.00	30.33	416.50	544.30	4881.00	1032.00	Zhang et al. 2020
<i>Carassius auratus</i>	Adult 28d decreased body weight	NOEC	1	136.20	262.70	810.30	27880.00	1319.00	30.33	416.50	544.30	4881.00	1032.00	Zhang et al. 2020
<i>Carassius auratus</i>	Adult 7d increase in hepatic oxidative damage	NOEC	2	68.10	131.35	405.15	13940.00	659.50	15.17	208.25	272.15	2440.50	516.00	Zhang et al. 2020
<i>Carassius auratus</i>	Adult 7d Suppression of	NOEC	2	68.10	131.35	405.15	13940.00	659.50	15.17	208.25	272.15	2440.50	516.00	Zhang et al. 2020
<i>Carassius auratus</i>	Adult 7d Suppression of IgM	NOEC	1	136.20	262.70	810.30	27880.00	1319.00	30.33	416.50	544.30	4881.00	1032.00	Zhang et al. 2020
<i>Danio rerio</i>	Embryos Lethality 96h	LC ₅₀	4153	0.03	0.06	0.20	6.71	0.32	0.01	0.10	0.13	1.18	0.25	Meng et al. 2020
<i>Danio rerio</i>	Embryos Vitellogenin 1 48h	NOEC	500	0.27	0.53	1.62	55.76	2.64	0.06	0.83	1.09	9.76	2.06	Rodriguez-Fuentes 2015
<i>Danio rerio</i>	Embryos 24h CYP3A65 Induction	NOEC	104	1.31	2.53	7.79	268.08	12.68	0.29	4.00	5.23	46.93	9.92	Meng et al. 2020
<i>Danio rerio</i>	Embryos 24h CYP1A Induction	NOEC	104	1.31	2.53	7.79	268.08	12.68	0.29	4.00	5.23	46.93	9.92	Meng et al. 2020
<i>Danio rerio</i>	Embryos 24h CYP1B Induction	NOEC	104	1.31	2.53	7.79	268.08	12.68	0.29	4.00	5.23	46.93	9.92	Meng et al. 2020
<i>Danio rerio</i>	Embryos 24h CYP1B Induction	NOEC	104	1.31	2.53	7.79	268.08	12.68	0.29	4.00	5.23	46.93	9.92	Meng et al. 2020
<i>Danio rerio</i>	Embryos 24h Estrogen Receptorα Induction	NOEC	104	1.31	2.53	7.79	268.08	12.68	0.29	4.00	5.23	46.93	9.92	Meng et al. 2020
<i>Danio rerio</i>	Embryos 24h Vitellogenin1 Induction	NOEC	104	1.31	2.53	7.79	268.08	12.68	0.29	4.00	5.23	46.93	9.92	Meng et al. 2020
<i>Danio rerio</i>	Phenotypic sex ratio Fewer males more females	NOEC	191	0.71	1.38	4.24	145.97	6.91	0.16	2.18	2.85	25.55	5.40	Kinnberg et al. 2015
<i>Danio rerio</i>	Gonad maturation in females	NOEC	191	0.71	1.38	4.24	145.97	6.91	0.16	2.18	2.85	25.55	5.40	Kinnberg et al. 2015
<i>Danio rerio</i>	Gonad maturation in males	NOEC	388	0.35	0.68	2.09	71.86	3.40	0.08	1.07	1.40	12.58	2.66	Kinnberg et al. 2015
<i>Danio rerio</i>	Adult male 12 day increase	NOEC	63	2.16	4.17	12.86	442.54	20.94	0.48	6.61	8.64	77.48	16.38	Kinnberg et al. 2015
<i>Danio rerio (Transgenic line)</i>	14 dpf embryo 120 hr Obesogen Disruptor	NOEC	57	2.39	4.61	14.22	489.12	23.14	0.53	7.31	9.55	85.63	18.11	Kopp et al. 2017
<i>Danio rerio (Transgenic line)</i>	14 dpf embryo 120 hr Circadian Disruptor	NOEC	57	2.39	4.61	14.22	489.12	23.14	0.53	7.31	9.55	85.63	18.11	Kopp et al. 2017
<i>Danio rerio</i>	Embryo ~24h Neurotoxic development	NOEC	5	27.24	52.54	162.06	5576.00	263.80	6.07	83.30	108.86	976.20	206.40	Tao et al. 2020
<i>Danio rerio</i>	Embryo ~24h Locomotor disruption	NOEC	5	27.24	52.54	162.06	5576.00	263.80	6.07	83.30	108.86	976.20	206.40	Tao et al. 2020
<i>Danio rerio</i>	Adult 14d Gene suppression of esr1, ar, cyp19b	NOEC	2.4	56.75	109.46	337.63	11616.67	549.58	12.64	173.54	226.79	2033.75	430.00	Bluthgen et al.2012
<i>Danio rerio</i>	Adult 14d Antiandrogenic gene suppression	NOEC	8.2	16.61	32.04	98.82	3400.00	160.85	3.70	50.79	66.38	595.24	125.85	Bluthgen et al.2012
<i>Oncorhynchus mykiss</i>	14d - vitellogenin in males	NOEC	374.50	0.36	0.70	2.16	74.45	3.52	0.08	1.11	1.45	13.03	2.76	Coronado et al. 2008
<i>Oryzias latipes</i>	21d - vitellogenin in males	NOEC	61.00	2.23	4.31	13.28	457.05	21.62	0.50	6.83	8.92	80.02	16.92	Coronado et al. 2008
<i>Oryzias latipes</i>	21d % eggs hatching	NOEC	310.00	0.44	0.85	2.61	89.94	4.25	0.10	1.34	1.76	15.75	3.33	Coronado et al. 2008
<i>Poecilia reticulata</i>	DNA Damage COMET Tail Length 96h	NOEC	0.05	2724.00	5254.00	16206.00	557600.00	26380.00	606.60	8330.00	10886.00	97620.00	20640.00	Almeida et al. 2019
<i>Poecilia reticulata</i>	DNA Damage Micronucleus 96h	NOEC	0.5	272.40	525.40	1620.60	55760.00	2638.00	60.66	833.00	1088.60	9762.00	2064.00	Almeida et al. 2019

A number of endpoints not commonly used as regulatory toxicological endpoints are included in column 2 of Table 3. However, all of these toxicity endpoints can be argued to reflect aspects necessary for population-level survival and reproductive fitness in real world

situations (Goulson and Kleijn, 2013; Moore et al., 2004; Ruel and Ayres, 1999; Schafer et al., 1994).

The criteria for Levels of Concern for organisms in ecosystems for interpreting the RQ is based on a four-tier ranking system (European Commission 1996; Sanchez-Bayo et al., 2002; Hernando et al., 2006). Based on the American National Standards Institute recommendations for Hazard Communications, a color scheme is used for ease of visualization of the Levels of Concern for this methodology (Table 3). Red boxes indicate RQ values greater than 1, indicating an unacceptable risk requiring immediate action, and is the standard criteria for the Level of Concern within the European Commission framework. Orange boxes represent values between 0.5 and 1.0; a moderate concern of an acute impact. Yellow boxes represent values between 0.1 and 0.49, indicating a lower risk of impact. White boxes indicate no concern of danger.

4. Results

4.1. Measured environmental levels

Oxybenzone was measured from water column samples at each of 10 sampling locations within Hanauma Bay. Concentrations ranged from 136 to 27,880 ng/L as depicted in Fig. 1A (nanograms per liter is equivalent to parts per trillion). Other benzophenones or benzotriazoles (UV filters) also were measured in the bay. Benzophenone-1 was detected at a concentration of 21.94 ng/L from Site 9. Benzotriazole was detected in waters from Site 7 (16.48 ng/L), Site 8 (18.6 ng/L) and Site 9 (18.78 ng/L).

Beach sand collected from four beach-shower sites (Fig. 1B) was screened for the presence of 10 UV filters (Table 1). Nine UV filters were detectable at each site, although several were below the limit of quantitation, depending on the site. Butylloctyl salicylate was not detected at any of the sites. The highest concentrations were found at Site 1 and the lowest at Site 4 with only four of the UV filters within quantitation limits. Octisalate was the highest measured UV filter among Sites 1–3, followed by homosalate. The concentrations of UV filters in Table 1 are shown as uncorrected for recovery rates.

Table 1. Concentration of UV filters in sand samples expressed in micrograms of UV filter per gram of sand. BS = butyloctyl salicylate < LOQ = Below limit of quantitation, but detectable. <LOD = Below limit of detection.

Concentration of UV-Filters in Sand ($\mu\text{g/g}$)				
Site 1	Site 2	Site 3	Site 4	
Tinosorb S	7.5	<LOQ	<LOQ	<LOQ
Avobenzone	28.0	10.9	23.7	<LOQ
Benzophenone	1.6	1.3	1.5	0.16
Oxybenzone	35.0	9.8	27.3	0.39
BS	<LOD	<LOD	<LOD	<LOD
Octinoxate	33.0	9.3	26.8	0.40
Octisalate	133.1	18.7	78.2	<LOD
Homosalate	101.8	26.2	67.0	<LOQ
Tinosorb M	<LOD	<LOD	<LOD	<LOD
Octocrylene	50.0	20.0	40.8	1.26

4.1.1. Sunscreen retention time in Hanauma Bay

Modelling scenarios for the retention time of sunscreen contaminants in Hanauma Bay are:

- Model scenario #1, Tidal Forcing = ~ 50 h (Fig. 2)

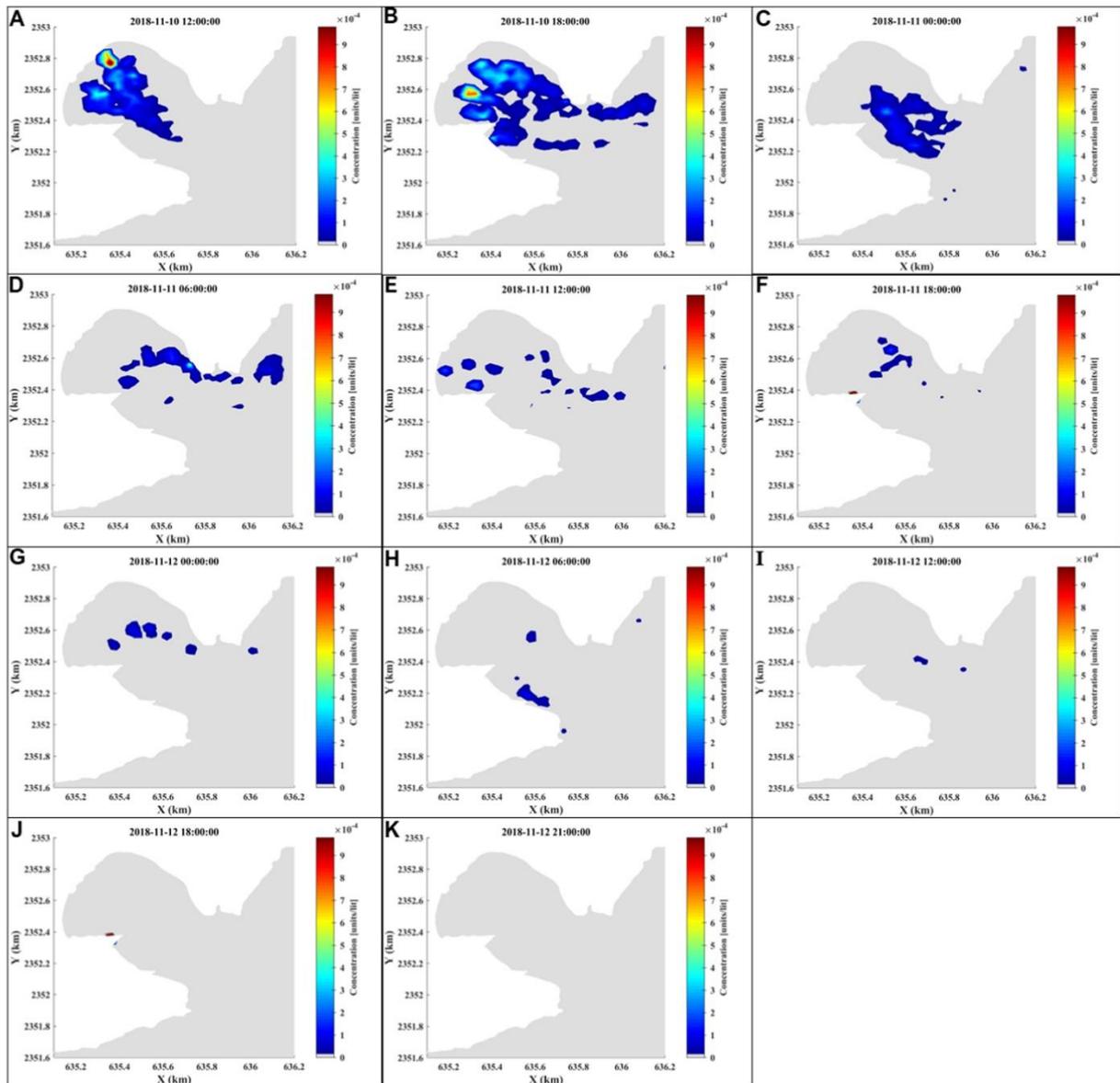


Fig. 2. Model results for Scenario #1 (assuming the presence of pure tidal currents) – the concentration releases at a unit rate at five locations inside the bay during the period of 9 a.m. to 4 p.m. on November 10th, 2018, and the concentration distribution is shown after (A) 3 h, (B) 9 h, (C) 15 h, (D) 21 h, (E) 27 h, (F) 33 h, (G) 39 h, (H) 45 h, (I) 51 h, (J) 57 h and (K) 63 h. The retention time is the longest under these conditions.

- Model scenario #2, Tidal and Oceanic (southward) Current Forcing = ~14 h (Fig. 3)

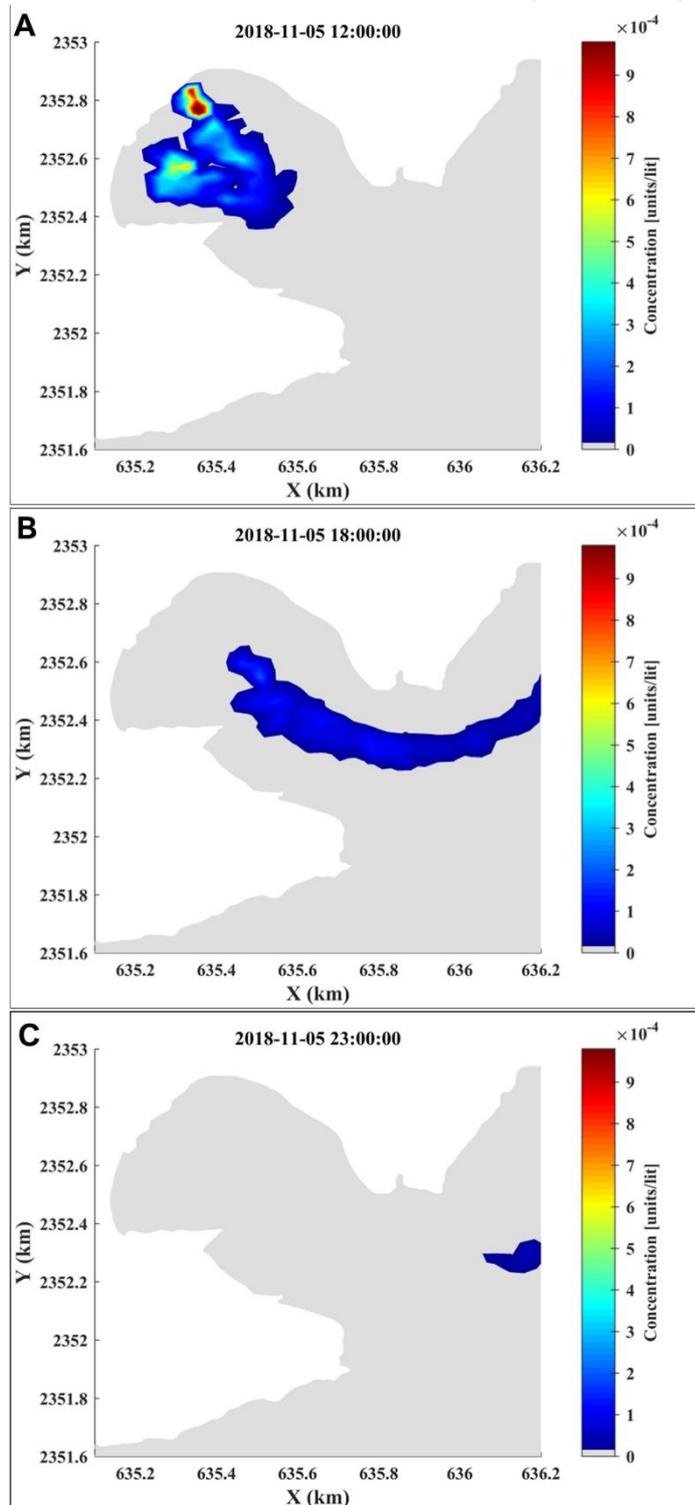


Fig. 4. Model results for Scenario #3 (in the presence of northward oceanic currents) – the concentration releases at a unit rate at five locations inside the bay during the period of 9 a.m. to 4 p.m. on November 7th, 2018, and the concentration distribution is shown after (A) 3 h, (B) 9 h, (C) 15 h, (D) 21 h, (E) 27 h, (F) 33 h, (G) 39 h, and (H) 41 h. The retention time is much longer under these conditions.

Under conditions that lack a southern swell or storms approaching from any direction, and when currents run to the south, contamination is flushed out of the Bay quickly - within 24 h.

When currents run to the north (about 30% of the time), retention is much longer, assuming that there is no further interference from increasing number of bathers.

4.2. Hazard/risk analysis

Hazard and risk quotients are meant as a proactive means to determine if a chemical contaminant in a system is at a concentration that may pose a threat to a specific species population at that locality, or to the ecological integrity in that locality (U.S. EPA, 2004; Gwinn et al., 2020). It is also used to help define whether a system is polluted by a chemical; pollution being defined as causing or potentially causing a harmful effect (Connell and Miller, 1984). Calculated HQs for an acute exposure for each species and toxicity reference value are shown in Table 2. Based on the guidance by U.S. EPA (U.S. EPA, 2004; Gwinn et al., 2020), the most sensitive receptor represented in this dataset is *Pocillopora damicornis* coral cells exposed to oxybenzone during a 4 h exposure involving light ($LC_{50} = 8.0 \mu\text{g/L}$) (Table 2). Based on the coral *in vitro* data, Sites 4 and 9 exhibited high risk to an acute exposure (Table 2). Based on the toxicity reference value for *Stylophora pistillata* planula exposed for 24 h with a day/night circadian cycle, Site 4 exhibited a high risk to acute exposure to oxybenzone at the measured concentration.

The RQ calculated using the European Commission (1996, 2003a, 2003b, 2003c) method is argued to be a more rigorous and realistic estimation for risks than the U.S. EPA method for identifying threats to ecological integrity (Crane and Giddings, 2004).

Comparing coral cells exposed to oxybenzone in the light, the most threatened of the species was *P. damicornis*, while the relatively least-at-risk was *Porites astreoides*, and then *Orbicella annularis* (Table 3). Calculated risk quotients indicate acute risk for all sites (Table 3). Based on these data (Fig. 1A), Sites 4 and 9 exhibited severe acute danger, while sites 3, 5, 7, 8 and 10 exhibited unacceptable danger for endangered animals and sites 3, 5, and 10 exhibited increased risk for toxicity for non-endangered organisms.

For non-cnidarian invertebrate species, risks were less pronounced though Sites 4 and 9 again contained concentrations of oxybenzone that posed the highest risks (Table 3). Sites 1, 2, and particularly 6 showed the least risk of adverse effects from oxybenzone exposure, although all sites including these three exhibited some level of risk based on one or more endpoints.

Sites 4 and 9 posed the most threatening concentrations to every species of microalgae, as well as for all of the biomarker parameters (Table 3).

Due to the relatively high sensitivity of fish toxicological parameters to oxybenzone, fish exhibited some of the highest levels of concern across most of the sampled area within Hanauma Bay (Table 3). *Brachydanio rerio* (96 h LC_{50}) and *Danio rerio* (96 h embryo LC_{50}) were the least at risk across the various sites. Markers for DNA damage were calculated to have enormously high-risk quotient values (e.g., *Poecilia reticulata*, Table 3).

5. Discussion

The over-arching question is whether sunscreen contamination poses a threat to the coral reef and seagrass ecosystems within Hanauma Bay, and whether anthropogenic activities such as sunbathing, swimming and snorkeling should be seen as a source of pollution. The second question we pose is whether beach showers can be a potential source of sunscreen contamination, since these waste waters are not collected by a municipal sewage system (untreated waste), but runs freely back into the bay. The third question we pose is, what is the potential retention time of a single day of contamination within Hanauma Bay?

Recognition of sunscreen pollution means that resource managers and policy makers can confidently consider this a putative threat and consider options for mitigation. To begin to address the concern by managers of this “sunscreen sheen,” it must first be determined if the composition in its entirety, or as individual components of a sunscreen mixture, pose a hazard to Hanauma's ecological integrity. One such probable singular chemical component of sunscreen pollution is oxybenzone.

Oxybenzone was detected in 100% of the water samples within Hanauma Bay. Concentration ranges were considerable, and were most likely a result of eddy formations between the reef and the shoreline, as well as tidal forcing (James, 2002; Wan et al., 2013; Weeks et al., 2015).

This is the first study to demonstrate that beach showers near reefs may be a significant source of sunscreen chemical contamination into the environment. Sunscreen residue that is not released by swimmers during swimming can still contaminate Hanauma Bay via shower and rainfall run-off. Under the U.S. Clean Water Act, beach showers that are not connected to a municipal waste-water system can be classified as a “point source” of pollution for not just navigable waters, but for both State and U.S. federally protected waters (U.S. Clean Water Act, 2019).

Hawaii experiences a monsoonal-like climate, exhibiting a dry season and a wet season. During the dry season, sunscreen contaminants may accumulate to high concentrations in the sand within the shower plumes. The first rains of the rainy season may result in a high concentration pulse of sunscreen contaminants entering the receiving waters of Hanauma Bay. This pulse may pose an extreme and lethal hazard to the wildlife within Hanauma Bay. The dry season (May–August) sees less than 0.5 cm of precipitation per month within Hanauma. September is the beginning of the rainy season; November can see more than 1.25 cm of rain. This suggests that the highest concentrations of sunscreen contamination may occur in September, which is also the warmest time of the year and where coral bleaching events become observable. The period of sampling in November may exhibit the lowest level of sunscreen contamination, both as a condition of lower tourism intensity and increased flushing from peak precipitation.

Understanding the retention time of a pollutant allows for rigorous design and assessment of mitigation policies. Bay closures are becoming a resource management tool to relieve tourism pressures on targeted natural resources (County of Hawai'i, 2020; Koh and Fakfare, 2020; County of Hawai'i, 2021; Fox, 2021). How long should a bay be closed to ensure rapid dissipation of the pollution so as not to exceed hazardous action levels? How many days

should a bay be opened per week, and under what schedule that it may ensure a pollutant build-up does not occur (e.g., open for one day, then closed the next two days)? Or should bays under high retention rates only be opened when oceanic flushing exceeds a minimal level of water exchange? Design and creation of marine protected areas might also require this information; reefs in bodies of water that have high retention rates may need more protection than more coastally exposed reefs. Finally, environmental impact assessments/statement regarding residential and resort development need to consider the impact of increased visitor density from their businesses on the bay that are adjacent to their properties (U.S. EPA, 2011; Gross, 2018).

Coastal modeling of pollutants may also be necessary to determine other reef communities that are under risk of exposure. After exiting Hanauma Bay, dispersion models of pollutants were directly influenced by shelf circulation, ocean currents, and the interaction between the shallow shelf and deep ocean (Du et al., 2019). In addition, mesoscale eddies in the ocean can be influential in altering the water exchange between ocean and shelf as they move and dissipate (James, 2002; Wan et al., 2013). Could hydrophobic sunscreen pollutants be re-introduced into Hanauma Bay from these mesoscale behaviors? This complex situation needs more attention, as well as more comprehensive and sophisticated modeling and measurement attempts, along with a detailed understanding of oceanic current components in the area, which can be achieved from a data analysis of available global modelling over a longer period of time.

In this study, we compared the two major methodological approaches to risk assessment for contaminant data of oxybenzone collected in Hanauma Bay, Hawaii. The U.S. EPA Hazard quotient method is analogous to describing a chemical weapon detonation in a given area where it rapidly kills 50% of the population in the dispersion zone within a very short amount of time (e.g., 48–96 h). It is the most simplistic of the deterministic models, and it inherently does not tell you about the consequence of the population that survives after an acute exposure, how long that population would survive after the initial exposure, its long-term impacts on the health of the acute-exposed population, or the impact of the exposure event when exposed to low concentrations to the toxicant (Somani and Romano, 2000; Volans and Karalliedde, 2002; Tannenbaum et al., 2003). The U.S. EPA method also says nothing about the threat of persistent, daily exposures to the toxicant at lower concentrations. Nor does it accommodate more recent social and scientific concerns of relying solely on animal-sacrifice toxicological models. But given these limitations, this hazard assessment model demonstrated that some areas within Hanauma Bay, especially site 4, posed a serious threat to coral short-term survivability.

Site 4 of Hanauma Bay exhibited the greatest threat estimation (Table 2) using the US EPA method. It was the only site for coral planula, when exposed in the light, to have a hazard quotient value above 0.5. For the microalgae, *Isochrysis galbana*, the hazard quotient value was above 2, which is consistent with the fact the oxybenzone is particularly noxious to photosynthetic organisms. Oxybenzone is especially toxic to the electron transport chain of photosynthesis and oxidative phosphorylation in algae and plants, resulting in a reduction of growth and biomass (Mao et al., 2017; Zhong et al., 2019a; Zhong et al., 2019b). These high concentrations of oxybenzone and its effect on photosynthetic integrity may explain why there is an increased sensitivity of heat stress to induce coral bleaching. Oxybenzone can

induce a bleaching pathology, and increase a coral's sensitivity to temperature stress, resulting in a bleaching response below the common bleaching temperature of 2 weeks at 30.3 °C (Downs et al., 2009, 2013, 2016; Wijgerde et al., 2020). Bleaching was observed in Hanauma Bay in 2015, even though temperatures never exceeded 29.8 °C (Rodgers et al., 2017; <https://www.coralreefwatch.noaa.gov/>). Bleaching was observed on reefs immediately adjacent to tourism-dense coastlines in 2019, but were relatively absent in more remote reefs or lightly visited reefs. These observations are consistent with the findings of Wijgerde et al. (2020) that oxybenzone exposure coupled with elevated temperatures increases the risk of coral to bleaching pathologies.

The model adopted by the European Commission is seen as the more relevant and accurate model that may explain the current condition of a geographic habitat that is contaminated with a personal care product chemical or pharmaceutical (Blasco et al., 2020). It uses uncertainty or assessment factors to calculate a more realistic account of a receptor's toxicological sensitivities at different stages of its life history. Like the U.S. EPA method, it is limited by the temporal nature of the effect concentration data (e.g., 8 or 48 h vs. 10 days), and thus can only calculate a risk assessment for the temporal time frame associated with the effect concentration data. Nonetheless, it provides a more relevant determination for the “danger” threshold of a contaminated site.

For *Stylophora* coral, all the sites had a risk quotient above 1, except Site 6, indicating that coral planulae are explicitly threatened with deformation (Table 3). For both deformity and mortality, light was an exacerbating factor that increased the threat of oxybenzone. This is consistent with oxybenzone and other benzophenone species acting as a photo-toxicant across a range of species (Downs et al., 2014, 2016; Zhang et al., 2021). Sites 4 and 9 exhibited risk quotient values above 1 for all the species included in this assessment. It is noted that *P. damicornis* is a species that should be found in abundance in the near-shore fringing reef area of Hanauma Bay. Impromptu and formal surveys looking for *P. damicornis* indicate that this species may be extinct within Hanauma Bay (Severino et al., 2020).

U.S. and European Union government regulatory agencies are advocating for the discontinuation of whole organism toxicity testing and shifting to New Approach Methods that utilize *in vitro* cell-toxicity testing for chemicals (Gwinn et al., 2020). Coral cells have been used to ascertain the toxicity of chemicals for over 10 years (Downs et al., 2010; Roger et al., 2021). Coral husbandry for ecotoxicological work is technically challenging and resource intensive. Coral cell toxicity testing shows remarkably similar exposure-concentration/response behavior as the intact colonial coral fragment (Downs et al., 2016). This is reflected in the pattern of risk quotients exhibited among the 10 sites between *Stylophora* coral cells in the light and dark versus *Stylophora* planula in the light and the dark (Table 3). *Pocillopora damicornis* is supposed to be an endemic species in Hanauma Bay, but is now possibly extinct (Dave Gulko, Hawaii Dept. Land & Natural Resources, personal communication). It is a much more stress-sensitive species compared to more massive-morphological coral species. The risk quotient for *Pocillopora* was above one for all 10 sites, indicating that it would be unlikely to survive for long in this type of daily polluted environment. Comparing coral cells exposed to oxybenzone in the light, the most threatened of the species was *P. damicornis*, while the relatively least-at-risk was *Porites astreoides*, and

then *Orbicella annularis* (Table 3). This is consistent with the predicted Species Sensitivity distribution that branching morphologies are less stress tolerant than the “boulder” morphologies (Downs et al., 2016).

For coral and jellyfish planula, all the sites except for Site 6 posed a serious risk to planula viability based on the majority of the endpoints (Table 3). Planula deformity LC₅₀s and EC₅₀s (8 h dark or 8 h light) showed the least risk across most sites. For coral planula, DNA damage, zooxanthellae loss or damage (bleaching) precedes planula deformation – coral experienced significant shifts in these biomarkers at the 8 h of exposure mark when compared to coral deformity. Coral morphological deformation occurs later in the pathological timeline, and the risk assessment reflects this. Furthermore, it can be argued that these cellular pathologies may predict the occurrence of the gross-morphological planula deformity (Moore et al., 2004).

In Table 3, Sites 4 and 9 posed the most threatening concentrations to every species of microalgae, as well as for all of the biomarker parameters. Seagrass beds should populate sandy patches in the center and mouth of the bay, though they have been conspicuously absent or denuded for at least the past 20 years. There are no ecotoxicological studies for marine vascular plants for oxybenzone. The closest surrogate for a vascular plant is cucumber (*Cucumis sativus*), the work of Zhong et al. (2020) demonstrated that oxybenzone could adversely affect above-ground productivity by poisoning both the photosynthetic pathway, as well as the mitochondrial electron transport chain. Except for Site 6, all the sites posed a threat to photosynthetic and mitochondrial function, which can have an impact to above ground productivity and reproductive effort (Table 3). The toxicological sensitivity of microalgae to oxybenzone, and the risk condition for Hanauma Bay, calls into question the potential adverse effects on the phytoplankton community structure.

Coral reef natural resources are under threat from intensive tourism. The most famous example is Maya Bay, Thailand. In 2018, Maya Bay saw more than 2.6 million visitors. The progression of ecological decay over the past 15 years was becoming impossible to disregard. In response, the Thai authorities shutdown Maya Bay for an indefinite amount of time to allow the habitats in Maya Bay to naturally recover, and to devise and implement a tourism management plan that would allow the sustainable interaction between tourists and biodiverse and ecological conservation (Koh and Fakfare, 2020). After six months without tourism, the recovery of wildlife was astonishingly observed (Dr. Thron Thamrongnawasawat, personal communication).

Hanauma Bay's waters are renowned for being grayishly turbid even on doldrum days, and swimmers exiting the water complain that they smell like a hodgepodge of “sunscreen fragrances” (<https://www.hawaii.edu/news/2020/07/20/hanauma-bay-water-clarity/>; Bourlin, 2020) During the COVID-19 lockdown in 2020, no tourists or recreational visitors were allowed within Hanauma Bay for nine months (Caldwell, 2020). Reports indicate that water clarified to a point where the water returned to having a blue hue, and a significant increase in fish and invertebrate abundance were observed (Cruz, 2020). This expulsion of biodiversity during the tourism-visitation period of the past 30 years may be directly tied to sunscreen pollution within the bay. A recent study demonstrated that commercial sunscreens not only induced lethality at high concentrations in shrimp, but

lower concentrations repelled the organism from the contaminated area, resulting in a *population immediate decline* phenomenon (Araujo et al., 2020). Maintenance of biodiversity and ecological community demographics may require banning all sunscreen products in the area until proven that the products do not result in repellence. The focus of this study was on oxybenzone, but the presence of other sunscreen contaminants should be noted. The water analysis method focused almost exclusively on oxybenzone, but other contaminants were also observed, including benzophenone-1 (a metabolite of oxybenzone, and a cosmetic ingredient) and benzotriazole. Both compounds are renowned endocrine disruptors. Benzophenone-1 is much more toxic than the parent compound; it is 200-fold more estrogenic than oxybenzone (Gago-Ferrero et al., 2012). Regarding benzotriazoles' ecotoxicity, evidence indicates that these chemicals have endocrine disrupting properties, induces oxidative stress, hepatotoxicity and neurotoxicity in both freshwater and marine fish (Tangtian et al., 2012; Liang et al. 2014, 2016, 2017). Further studies should be conducted that focus on the distribution and impact of sunscreen compounds, not only avobenzone, octocrylene, octinoxate, octisalate and homosalate, but also other sunscreen ingredients such as butyltolyl salicylate, ethylhexyl methoxycrylene, parabens and phenoxyethanol. All of these UV filter compounds, including the carcinogen benzophenone, impart some level of endocrine disruption to animals, and begs the question of the endocrine disrupting potential of Hanauma Bay receiving waters, and how they may be impacting the Hanauma Bay's proximate habitats and endangered species.

By logical progression, a pertinent question is whether the sullied waters of Hanauma Bay during intensive tourism periods poses a threat to public health, especially to pregnant persons and children (DiNardo and Downs, 2019a; DiNardo and Downs, 2019b). Several recent studies by the U.S. FDA and a host of literature dating back to the 1990s demonstrates that oxybenzone and other benzophenones can be readily absorbed through the skin and into body, being retained in blood for up to two weeks above 0.5 ng/mL (FDA action level), and contaminate tissues such as breastmilk, liver, kidney and brain tissues (Hayden et al., 1997; Sarveiya et al., 2004; Gonzalez et al. 2005; Janjua et al., 2008; Matta et al. 2019, 2020). It is recommended by the U.S. FDA and the American Academy of Pediatrics that infants under the age of six months not be exposed to oxybenzone and that exposure to oxybenzone in the first trimester of pregnancy may pose a risk to the gestating fetus (American Academy of Pediatrics, 2012; Huo et al., 2016; Wnuk et al., 2018a, 2018b; Wnuk et al. 2019; DiNardo and Downs, 2019a; Khalid and Abdollahi, 2021; U.S. FDA, 2021). If oxybenzone can pose a teratogenic threat to embryonic and larval development in fish, it begs the question of Hanauma Bay's oxybenzone contamination and the potential danger to public health, especially for pregnant persons that choose to avoid oxybenzone exposure (Wang et al., 2021). We call upon the U.S. EPA to generate the experimental data for exposure characterization, the U.S. FDA to conduct reproductive and developmental toxicological characterization on oxybenzone and other UV filter compounds so as to conduct a sound human risk assessment that can be utilized by jurisdictional agencies.

In Table 2, Table 3, it is obvious that different working groups found different values for the same parameters (e.g., *Daphnia* immobility, *Chlorella* growth inhibition); some values for a single parameter had a 20-fold difference (*Chlorella* EC₅₀ 72 h growth inhibition). One obvious source of variation is the fidelity to a standard methodology, such as the difference between U.S. EPA methods and OECD methods. Another significant source for these variations could be from how the exposure-solutions are made. Serial dilutions of polyaromatic hydrocarbons (PAH) are not a valid method for making dilutions because the

PAH partitioning is not homogenous in the water-column (Blenkisopp et al., 1996). Organizations that set standardizations for methodologies (e.g., OECD, U.S. EPA, etc) need to provide guidance documentation to accommodate nuances for both solubilization of the target xenobiotics, as well as biological availability of the target xenobiotic. In the field of drug *in vitro* toxicology, specific exposure-media are designed and generated that will allow for measurable solubilization and biological availability of a hydrophobic or partially hydrophobic compound (Gura, 2008; Downs et al., 2010). Oxybenzone is rather insoluble in seawater or in pure water, and requires an organic matrix for solution solubilization, not unsimilar to “naturally” occurring sea dissolved organic matter (Xu and Guo, 2017). In order to generate high-confidence risk assessments, ecotoxicologists need to standardize their exposure-response methods.

In this study, we showed that oxybenzone posed a serious threat to Hanauma Bay's coral reef system. Furthermore, we demonstrated that swimmers in the water are one of the principal sources of contamination for oxybenzone, and that other sources, such as beach showers, may also contribute to the bay's contamination. Currently, the City of Honolulu has closed the bay to visitors on Monday and Tuesday of each week. This policy is meant to reduce the impacts visitors have on the coral reef community. Knowledge of the retention time of oxybenzone from a single day event may prompt discussions in creating a bay-accessibility schedule that would have a lower impact to Hanauma Bay. In 2021, the City of Honolulu has limited the number of visitors to no more than 1000 persons per open day. This management policy may have a positive impact in reducing contaminant loads in the bay, and a follow-up contaminant survey may provide data not only to assess this possibility, but to also determine a more effective carrying capacity program for Hanauma Bay.

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Declaration of competing interest

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

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