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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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Corresponding Author:	Craig Downs, Ph.D. Haereticus Environmental Laboratory clifford, UNITED STATES
First Author:	Craig Downs, Ph.D.
Order of Authors:	Craig Downs, Ph.D.
	C.A. Downs
	Elizabeth Bishop
	Silvia Diaz Cruz, PHD
	Abbas Haghshenas, PHD
	Didier Stien
	Alice M.S. Rodrigues
	Cheryl M. Woodley
	Adrià Sunyer-Caldú
	Shadan Nasseri Doust, PHD
	William Espero
	Gene Ward
	Aref Farhangmehr
	S. Maryam Tabatabaee Samimi
	Michael J. Risk, PHD
	Philippe Lebaron, PHD
	Joseph N. DiNardo
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Oxybenzone contamination from sunscreen pollution and its ecological threat to Hanauma Bay, Oahu, Hawaii, U.S.A.

C.A. Downs[1,5*], Elizabeth Bishop[2], M. Silvia Diaz Cruz[3], S. Abbas Haghshenas[4], Didier Stien[5], Alice M.S. Rodrigues[5], Cheryl M. Woodley[6], Adrià Sunyer-Caldú[3], Shadan Nasseri Doust[4], William Espero[7,8], Gene Ward[9], Aref Farhangmehr[4], S. Maryam Tabatabaee Samimi[4], Michael J. Risk[10], Philippe Lebaron[5], Joseph N. DiNardo[11]

[1]Haereticus Environmental Laboratory, P.O. Box 92, Clifford, VA 2453, USA

[2]Friends of Hanauma Bay, P.O. Box 25761, Honolulu, HI 96825-07610, USA

[3] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Severo Ochoa Excellence Center. Spanish Council for Scientific Research (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain.

[4]Institute of Geophysics, University of Tehran, Tehran, Iran

[5]Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes, USR3579, Observatoire Océanologique, 66650 Banyuls-sur-mer, France

[6]U.S. National Oceanic & Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean Science, Coral Disease & Health Program, Hollings Marine Laboratory, 331 Ft. Johnson Rd. Charleston, SC 29412, USA

[7]Hawaii State Senate, Senate District 19, Hawaii State Capitol, 415 S. Beretania St. Honolulu, HI 96813, USA

[8]Presently retired

[9]Hawaii State Legislature, House District 17, Hawaii State Capitol, 415 S. Beretania St. Honolulu, HI 96813, USA

[10]Department of Earth Sciences, McMaster University, Hamilton, Ontario, L8S 4M1 Canada

[11]Vesuvius, Virginia, USA

*Corresponding author: cadowns@haereticus-lab.org

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. Hanauma Bay is the most popular swimming area in the Hawaiian Islands and has been reported to have averaged between 2.8-3.5 million visitors a year in the late 1980s and 1990s. In the 2010s, visitors averaged between 3,000-4,000 a day and peaked 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 hours 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.

Key Words: Hanauma Bay, coral, sunscreen, oxybenzone, risk assessment, hydrodynamic modelling

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 2.8-3.5 million visitors a year in the late 1980s and 1990s (Mak & Moncur, 1995). In the era of the late 2010s, accurate visitor statistics are difficult to access, but most sources report at least an average of between 3,000 to 4,000 visitors a day and peaking around 10,000-13,000 per day. In the era of COVID-19, the bay had been closed to all visitors from March, 2020 to December, 2020. 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 grams every 90 minutes (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. The increasing use of sunscreen UV-filter drugs has triggered concern about their emissions into the environment (Molins-Delgado et al., 2016). 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 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-methoxphenyl phenylmethanone; CAS No. 131-57-7) was one of the dominant UV-filters used in sunscreen products. In the U.S. and E.U., oxybenzone concentrations are regulated at 6% in sunscreen and cosmetic products (European Union, 2019). In Australia, oxybenzone concentrations have a maximum concentration of 10%, while Japan allows for no more than 5% (Australian Government, 2013; Japan, 2000). A 2016 investigation into the contamination of Hanauma Bay saw water-column oxybenzone concentrations of more than 1,600 ng/L, and avobenzone concentrations were above 1,500 ng/L (Booth & 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.

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; Schreurs et al., 2002; Ma et al., 2003, Schlumpf et al., 2004; Kunz et al., 2006; Kunz & 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; Ozáez et al., 2014; Paredes et al., 2014; 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; Zhong et al., 2019b; Zhong et al., 2020; Teoh et al., 2020). Oxybenzone can be detrimental to corals, ranging from mortality, bleaching, and gross planula deformations to genotoxicity, endocrine disruption and significant shifts in its metabolome (Donavaro et al., 2008; Downs et al., 2016; He et al., 2019a; He et al., 2019b; Stien et al., 2020; Wijgerde et al., 2020). Sunscreen pollution can also impact 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).

The Hazard Identification is a critical element of the investigative and management process in addressing the issue of environmental contamination by sunscreen products. 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 safety 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 beach-shower area in Hanauma Bay, to determine if this could be a second source of sunscreen contamination. We also conducted a preliminary examination of the retention time of oxybenzone (and other sunscreen compounds) within Hanauma Bay. Focusing only on oxybenzone, environmental contamination data were combined 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.

Materials and Methods

Sample Collection

Seawater samples from Hawaii were collected using precleaned one-liter amber glass bottles with Teflon lined lids (I-Chem, 300 series, VWR). Sample locations are indicated in **Figure 1**. Samples were collected approximately 30 cm below the surface of the water on November 17, 2017, between 16:00 and 17:30 Pacific Standard Time.

Wate 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 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.

Water sample extract analysis by HPLC-MS/MS

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 x 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 follow: 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 five minutes and in the next two minutes 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. Quantification was conducted through internal-standard calibration using isotopically labelled standards (**Table 1**). The total run time for each sample was 23 minutes. The method performance is shown in (**Table 2**).

Sand sample extraction analysis by HPLC-MS/MS

Benzophenone (BP), 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), avobenzone (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# ALR144N).

Initial analysis of samples from beach sites 1, 2, and 3 exhibited extremely high concentrations of several of the UV filter analytes that were above the highest concentration calibrant (**Figure 2**). For beach samples sites #1, #2, and #3, 0.2 grams of sand were used for extraction. For beach sand sample #4, two grams 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. in review). 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. The limits of detection (LOD) and quantitation (LOQ) are provided in 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. in press). The recovery rates were 85 % for homosalate, 88 % for benzophenone and 100 % for Tinosorb M, avobenzone, oxybenzone, butyloctyl salicylate, octinoxate, octisalate, Tinosorb S, and octocrylene.

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., 2007; 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 **Figure 3**. 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 (**Figure 1**).

The dispersion model was run for an arbitrary one-month period in 2018, including periods of tide-dominant and oceanic-current-dominant conditions. 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).

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 2003; European Medicines Agency 2006; Dussault et al. 2008; Hernando et al. 2006; USEPA 2004, 2020). In this paper, we compare two different hazard/risk-quotient 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, 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 (LC_{50}). Measured environmental concentrations used for these calculations are found in **Figure 1**.

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., LC_{50} is the concentration of a chemical where 50% of the organisms die) or EC_{50} concentration of a chemical (adverse effect observed in 50% of the population for a sub-lethal endpoint). Toxicity reference values were obtained from the published literature (**Table 5**). 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 (Tannenbaum et al., 2003).

The equation for the acute hazard quotient is HQ = (MEC)/ (organism's EC_{50} or LC_{50} with 96-hours or less of exposure to the toxicant). An Affect 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 5**).

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, 2003; Environment Canada 2013; 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 LC50, EC50, 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., 2009; Dussault et al., 2008; Means et al., 1993; Environment Canada, 2013). 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 6**).

A number of endpoints not commonly used as regulatory toxicological endpoints are included in **Table 6**. 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, 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 6**). 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.

Results

Measured Environmental Levels

Oxybenzone was measured from water column samples at each of 10 sampling locations within Hanauma Bay. Concentrations ranged from 136-27,880 ng/L as depicted in **Figure 1** (nanograms per liter is equivalent to parts per trillion). Other benzophenones or benzotriazoles (UV blockers) also were measured in the bay. Benzophenone-1 was detected at a concentration of 21.94 ng/L from Site 9. Benzotriazole was detect 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 (Figure 2) was analyzed for the presence of 10 UV filters (**Table 4**). All 10 UV filters were detectable at each site, although several were below the limit of quantitation, depending on the site. 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 4** are shown as uncorrected for recovery rates.

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 hours (**Figure 4**)

• Model scenario #2, Tidal and Oceanic (southward) Current Forcing = ~14 hours (**Figure 5**)

• Model scenario #3: Tidal and Shoreward Oceanic Current Forcing = ~ 41 hours (**Figure 6**)

Under normal conditions, when currents run to the south, contamination is flushed out of the Bay quickly - within 24 hrs. 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.

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 & Miller, 1984). Calculated HQs for an acute exposure for each species and toxicity reference value are shown in **Table 5**. 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 hour exposure involving light (LC50 = 8.0 µg/L) (**Table 5**). Based on the coral *in vitro* data, Sites 4 and 9 exhibited high risk to an acute exposure (**Table 5**). 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, 2003) 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 6**). This is consistent with the predicted Species Sensitivity distribution that branching morphologies are less stress tolerant than the "boulder" morphologies (Downs et al., 2016). Calculated risk quotients indicate acute risk for all sites (**Table 6**). Based on these data (**Figure 1**), 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. It should be 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.

For coral and jellyfish planula, all the sites with the exception of Site 6 posed a serious risk to planula viability based on the majority of the endpoints (**Table 6**). Planula deformity LC_{50}s and EC_{50}s (8 hrs dark or 8 hrs light) showed the least risks 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 hour 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 that the occurrence of the gross-morphological planula deformity (Moore et al., 2004).

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 7**). Sites 1,2, and particularly 6 showed the least risks 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 8**). 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, the closest surrogate for a vascular plant is cucumber (*Cucumis sativus*). 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 8**). The toxicological sensitivity of microalgae to oxybenzone, and the risk condition for Hanauma Bay, calls into question the potential effects on the phytoplankton community structure.

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 6**). Brachydanio rerio (96 hr LC_{50}) and Danio rerio (96 hr 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 9**).

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 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 the 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.

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 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 hazard 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 of the pollutants was 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. 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 hours). 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 & Romano, 2000; Volans & Karalliedde, 2002). 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 5**) 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; Zhang et al., 2019a; Zhang 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 two weeks at 30.3ºC (Downs et al., 2009; Downs et al., 2013; Downs et al., 2016; Wijgerde et al. 2020). Bleaching was observed in Hanauma Bay in 2015, even though temperatures never exceeded 29.8°C (Rodger 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 US EPA method, it is limited by the temporal nature of the effect concentration data (e.g., 8 or 48 hours 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 6**). 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; Downs et al., 2016; Zhang et al., 2021). Sites 4 and 9 exhibited risk quotient vales above 1 for all the species included in this assessment.

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 6**). *Pocillopora damicornis* is supposed to be an endemic species in Hanauma Bay, but is now possibly extinct (Dave Gulko, 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.

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." 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; Liang et al. 2016; Liang et al. 2017).

The survey of sunscreens in beach sand was based on a validated methodology that attains more than 85% recovery of each of the surveyed analytes, meaning that these concentrations are accurate, and that 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 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).

DECLARATIONS

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent for publication: Not applicable.

U.S. National Oceanic and Atmospheric Administration Disclaimer: The scientific results and conclusions, as well as any opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of NOAA or the Department of Commerce. The mention of any commercial product is not meant as an endorsement by the Agency or Department.

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Availability of Data and Materials

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Figure and Table Legends

Figure 1. 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.

Figure 2. 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 x 10 cm sampling square and were collected on January 27, 2020. Numbers designating sample sites are the same designations in Table 4. Arrows indicate observable rivulets from shower run-off.

Figure 3. The unstructured computational grid developed for the dispersion model.

Figure 4. 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 am to 4 pm 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.

Figure 5. Model results for Scenario #2 (in the presence of southward oceanic currents) – the concentration releases at a unit rate at five locations inside the bay during the period of 9 am to 4 pm on November 5th, 2018, and the concentration distribution is shown after (A) 3 h, (B) 9 h and (C) 14 h. Most of the contamination is gone in less than a day.

Figure 6. 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 am to 4 pm 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.

Table 1. Name, acronym and CAS Number of the organic UV filters and UV stabilizers investigated in the water extracts. Internal standards used for quantification were also included.

Table 2. Performance of the HPLC-(ESI+)-MS/MS method applied.

Table 3. Instrumental limit of detection in solution; limits of quantitation in solution and in sand samples. BS = butyloctyl salicylate. LOD = Limit of detection. LOQ = Limit of quantitation.

Table 4. 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.

Table 5. 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

Table 6. Risk Quotient for Acute Toxicity in Hanauma Bay, Oahu Hawaii using European Union method for Cnidarian species. Color chart: RED= Severe condition for a potential toxic effect ≥1; Yellow= Moderate threat condition for a potential toxic effect = 0.5 to 1.0; Green= Condition of concern 0.5 to 0.1.

Table 7. Risk Quotient for Acute Toxicity in Hanauma Bay, Oahu Hawaii using European Union method for invertebrate species. Color chart: RED= Severe condition for a potential toxic effect ≥1; Yellow= Moderate threat condition for a potential toxic effect = 0.5 to 1.0; Green= Condition of concern 0.5 to 0.1.

Table 8. Risk Quotient for Acute Toxicity in Hanauma Bay, Oahu Hawaii using European Union method for plant and algal species. Color chart: RED= Severe condition for a potential toxic effect ≥1; Yellow= Moderate threat condition for a potential toxic effect = 0.5 to 1.0; Green= Condition of concern 0.5 to 0.1.

Table 9. Risk Quotient for Acute Toxicity in Hanauma Bay, Oahu Hawaii using European Union method for fish species. Color chart: RED= Severe condition for a potential toxic effect ≥1; Yellow= Moderate threat condition for a potential toxic effect = 0.5 to 1.0; Green= Condition of concern 0.5 to 0.1.

COMPOUND	ACRONYM	CAS Nº
Benzophenones		
Benzophenone 1	BP1	131-55-6
Benzophenone 2	BP2	131-55-5
Benzophenone 3 (oxybenzone)	BP3	131-57-7
4-Hydroxybenzophenone (p-benzoylphenol)	4HB	1137-42-4
4,4-Dihydroxybenzophenone	4DHB	611-99- 4
2,2'-Dihydroxy-4-methoxybenzophenone (Benzophenone 8, dioxybenzone)	DHMB, BP8	131-53-3
2,4,4'-Trihydroxybenzophenone	THB	1470-79-7
Benzotriazoles (UV Blockers)		
1,2,3-Benzotriazole	BZT	95-14-7
5-Methyl-1-H-benzotriazole	MeBZT	136-85-6
5,6-Dimethyl-1-H-benzotriazole	DMeBZT	4184-79-6
Isotopically labeled internal standards		
2-Hydroxy-4-methoxybenzophenone-d5 (for benzophenones)	BP3-d5	1219798-54-5
1H-Benzotriazole-d4 (for MeBZT and DMeBZT)	BZT-d4	1185072-03-0

Table 1. Name, acronym and CAS Number of the organic UV filters and UV stabilizers investigated in the water extracts. Internal standards used for quantification were also included.

Compound	Calibration range (ng/ml)	r^2	Instrumental		Method		Recovery Rate (%)
			ILOD (pg)	ILOQ (pg)	LOD (ng/ml)	LOQ (ng/ml)	
BP1	0.1-1000	0.999	0.5	1.8	0.253	0.845	103
BP2	0.1-1000	0.999	1.4	4.6	0.262	0.872	97
BP3	0.1-1000	0.999	0.6	2.0	0.030	0.101	96
4HB	0.1-1000	0.999	0.8	2.7	0.297	0.991	103
4DHB	0.1-1000	0.999	0.4	1.3	0.341	1.137	65
DHMB	0.1-1000	0.999	0.2	0.7	0.177	0.589	109
EtPABA	0.1-1000	0.999	0.4	1.4	0.494	1.648	114
4MBC	0.1-1000	0.997	0.3	0.9	0.060	0.199	82
BZT	0.1-1000	0.999	0.6	2.1	0.031	0.104	83
MeBZT	0.1-1000	0.999	0.3	1.1	0.016	0.053	78
DMeBZT	0.1-1000	0.999	0.2	0.7	0.010	0.034	72

Table 2. Performance of the HPLC-(ESI+)-MS/MS method applied.

	In solution		In sands Q-T	In sand U
	LOD (ng/mL)	LOQ (ng/mL)	LOQ (µg/g)	LOQ (µg/g)
Tinosorb S	22	216	2.2	0.22
Avobenzone	34	342	3.4	0.34
Benzophenone	7	72	0.7	0.07
Oxybenzone	9	89	0.9	0.09
BS	94	936	9.4	0.94
Octinoxate	9	89	0.9	0.09
Octisalate	93	932	9.3	0.93
Homosalate	72	720	7.2	0.72
Tinosorb M	35	349	3.5	0.35
Octocrylene	23	232	2.3	0.23

Table 3. Instrumental limit of detection in solution; limits of quantitation in solution and in sand samples. BS = butyloctyl salicylate. LOD = Limit of detection. LOQ = Limit of quantitation.

	Concentration of UV-Filters in Sand (µg/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

Table 4. Concentration of UV filters in sand samples expressed in micrograms of UV filter in gram of sand. BS = butyloctyl salicylate < LOQ = Below limit of quantitation, but detectable. <LOD = Below limit of detection.

				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														
Acropora cervicornis	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
Montastraea cavernosa	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
Orbicella annularis	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
Pocillopora damicornis	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
Porites astreoides	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
Porites divaricata	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
Stylophora pistillata	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
Stylophora pistillata	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)														
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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														
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Paracentrotus lividus	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
Mytilus galloprovincialis	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
Siriella armata	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														
Desmodesmus subspicatus	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
Chlamydomonas reinhardtii	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
Chlorella vulgaris	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
Chlorella vulgaris	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
Chlorella vulgaris	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
Isochrysis galbana	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
Microcystis aeruginosa	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
Skeletonema pseudocostatum	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														
Brachydanio rerio	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
Danio rerio	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

Table 5. EPA HQ

Table 6.

				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														
Acropora cervicornis	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
Montastraea cavernosa	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
Orbicella annularis	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
Pocillopora damicornis	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
Porites astreoides	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
Porites divaricata	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
Stylophora pistillata	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
Stylophora pistillata	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
Coral & Cassopeia Planula (early life stage)														
Cassiopea xamachana	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
Cassiopea xamachana	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
Cassiopea xamachana	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
Cassiopea frondosa	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
Cassiopea frondosa	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
Cassiopea frondosa	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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
Stylophora pistillata	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	
				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
Invertebrates														
Corbicula fluminea	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
Corbicula fluminea	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
Corbicula fluminea	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
Corbicula fluminea	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
Daphnia magna	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
Dugesia japonica	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
Dugesia japonica	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
Dugesia japonica	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
Dugesia japonica	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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 1016
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Daphnia magna	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
Paracentrotus lividus	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
Mytilus galloprovincialis	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
Siriella armata	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

Table 7. EU RQ Invertebrates

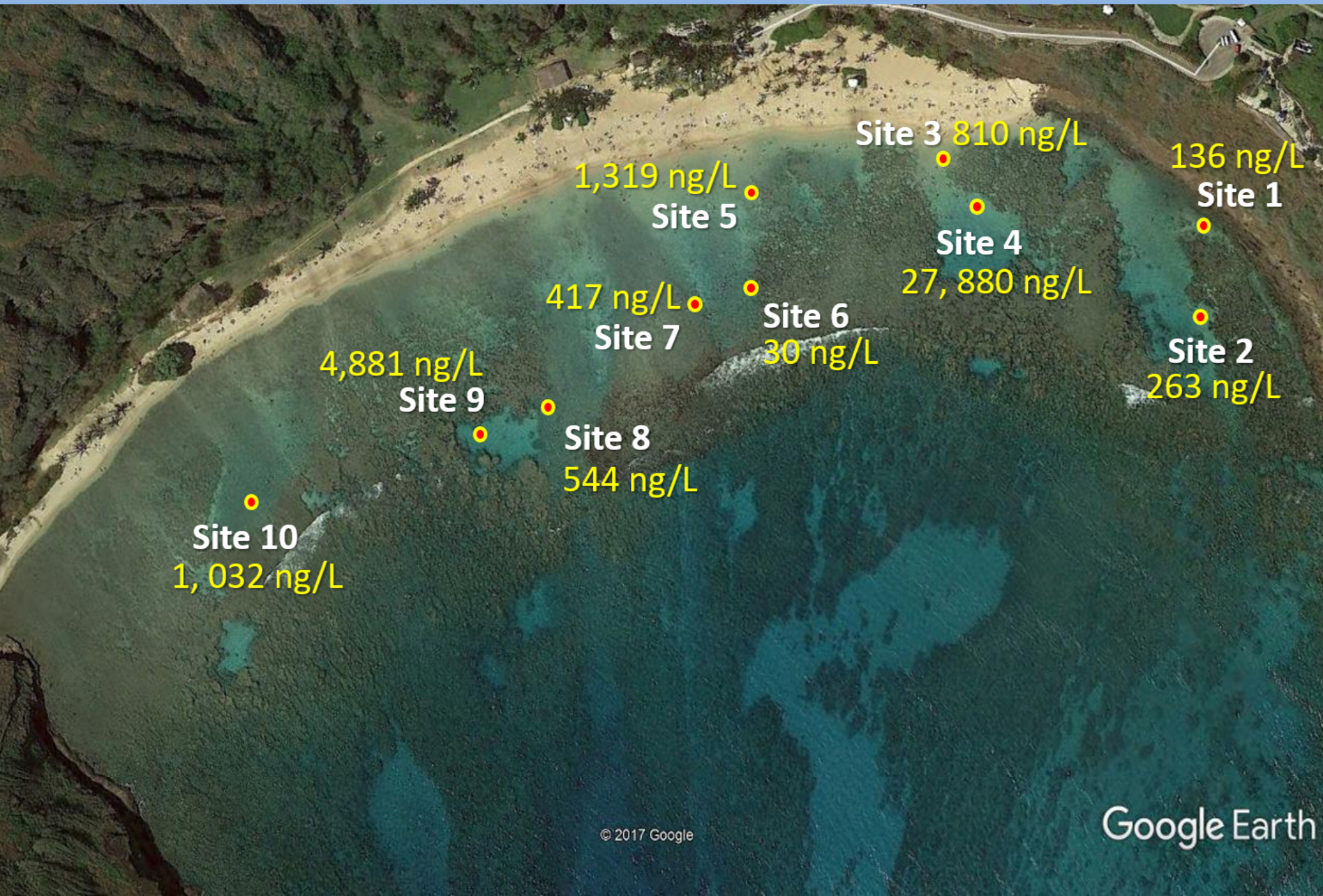
Table 8. EU RQ Plants and Algae

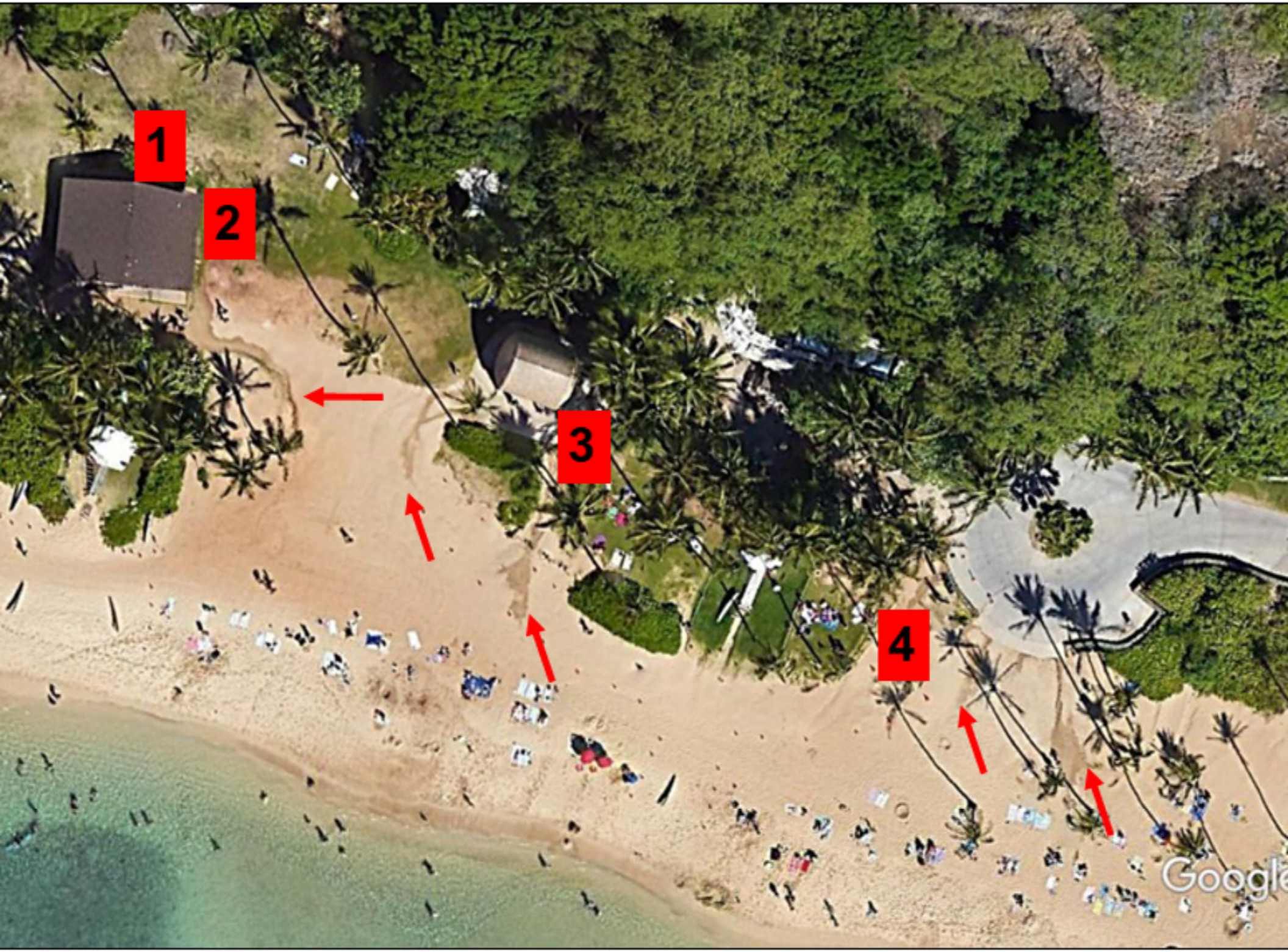
				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	
				0.1362	0.2627	0.8103	27.8800	1.3190	0.0303	0.4165	0.5443	4.8810	1.0320	Environ. Concen. µg/L
SPECIES			Toxicity Reference Value µg/L											References for Toxicity Reference Values
Algae and vascular plants														
Arthrospira 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

Table 9. EU RQ Fish

				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
Fish														
Betta splendens	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
Betta splendens	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
Brachydanio rerio	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
Carassius auratus	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
Carassius auratus	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
Carassius auratus	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
Carassius auratus	Adult 7d Suppresion 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
Carassius auratus	Adult 7d Suppresion 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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio	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
Danio rerio (Transgenic line)	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
Danio rerio (Transgenic line)	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
Danio rerio	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
Danio rerio	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
Danio rerio	Adult 14d Gene supression 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
Danio rerio	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
Oncorhynchus mykiss	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
Oryzias latipes	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
Oryzias latipes	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
Poecilia reticulata	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
Poecilia reticulata	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

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

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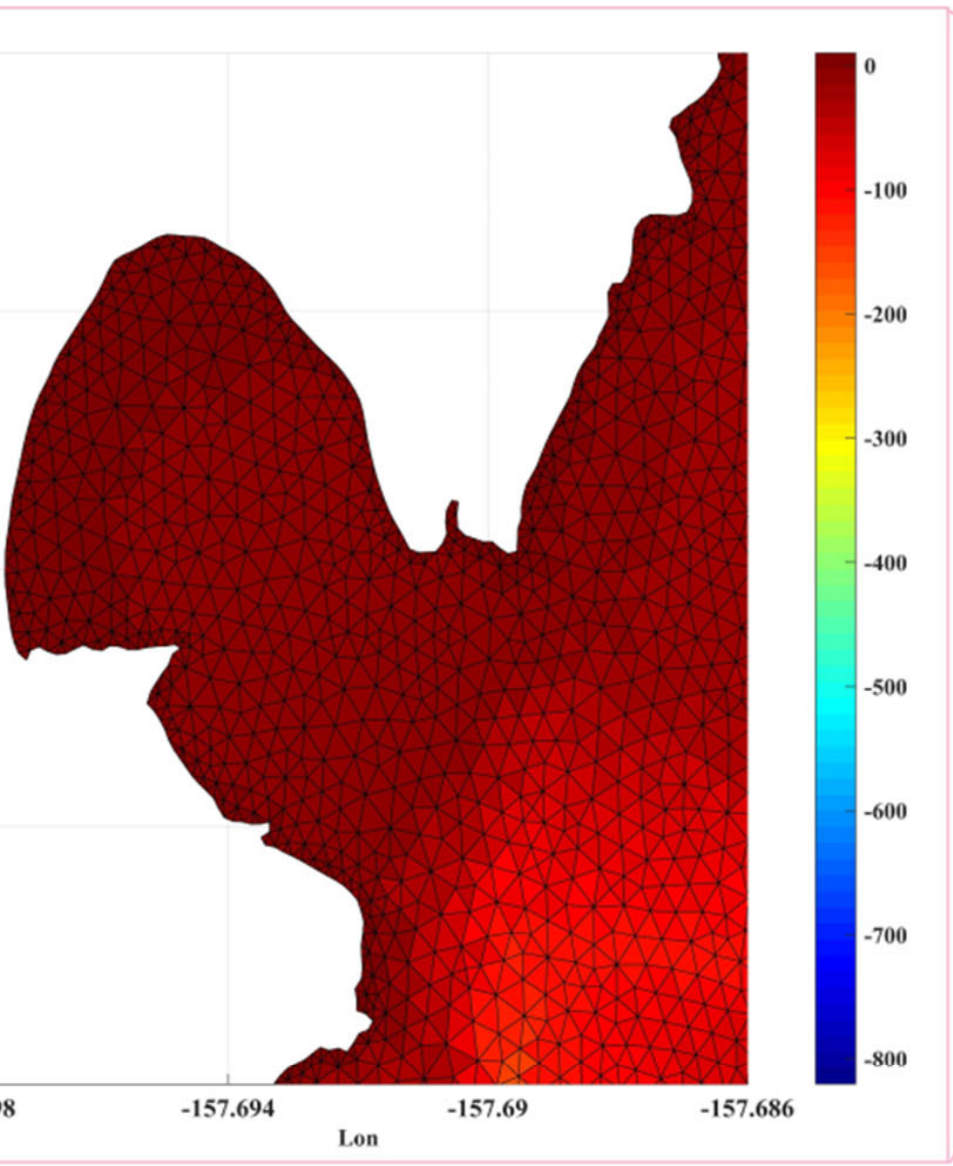

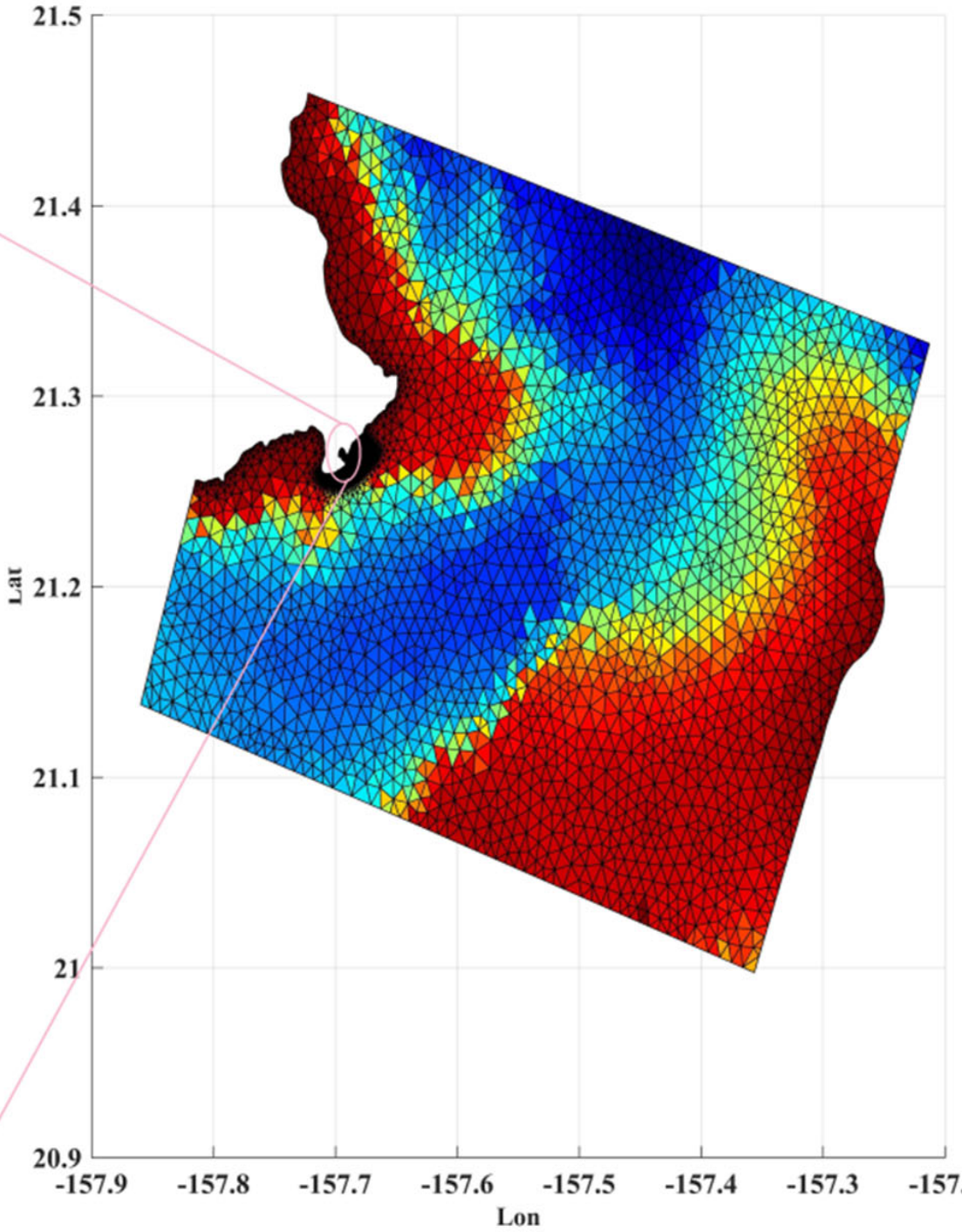

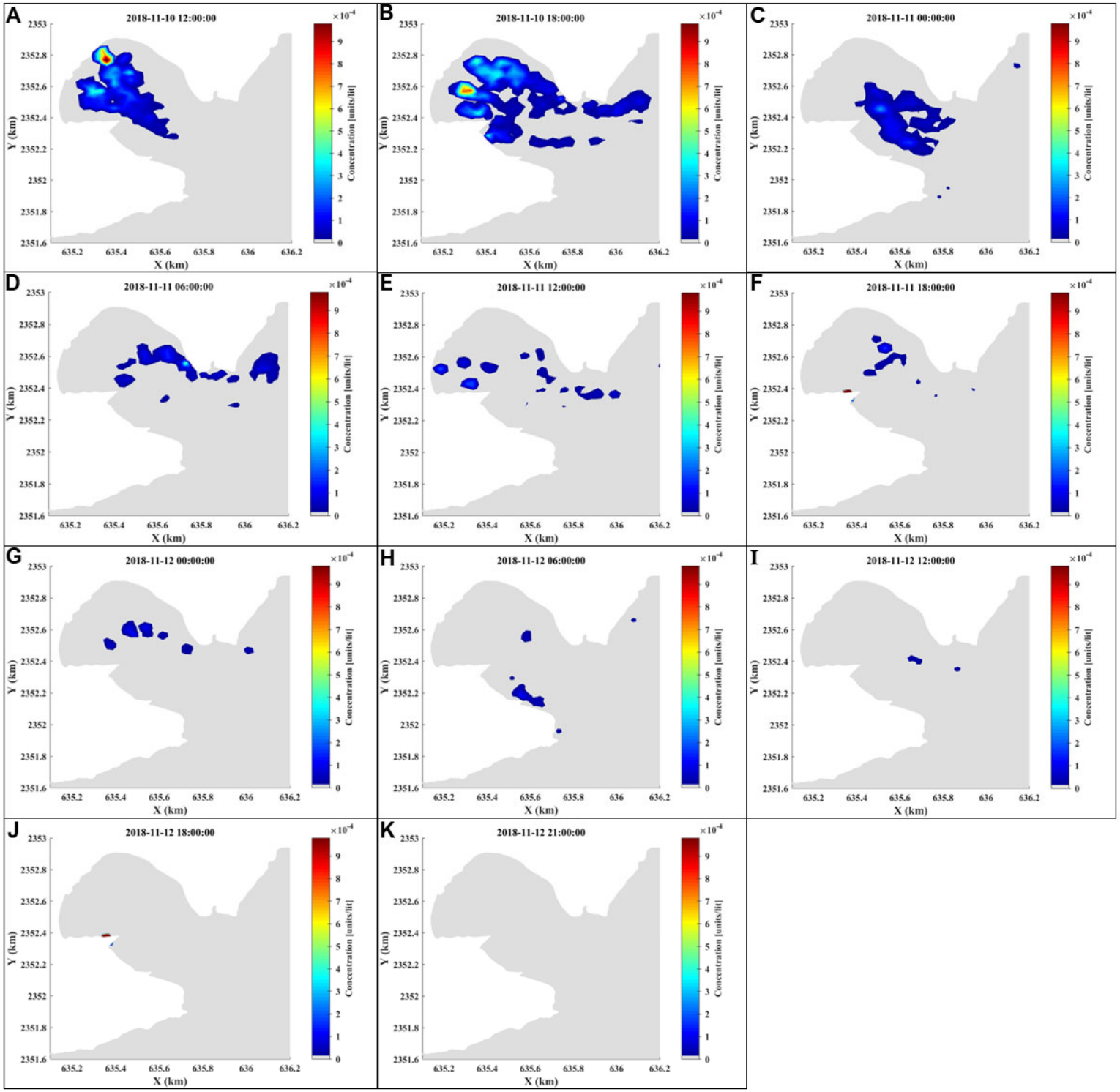
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B
2018-11-10 18:00:00
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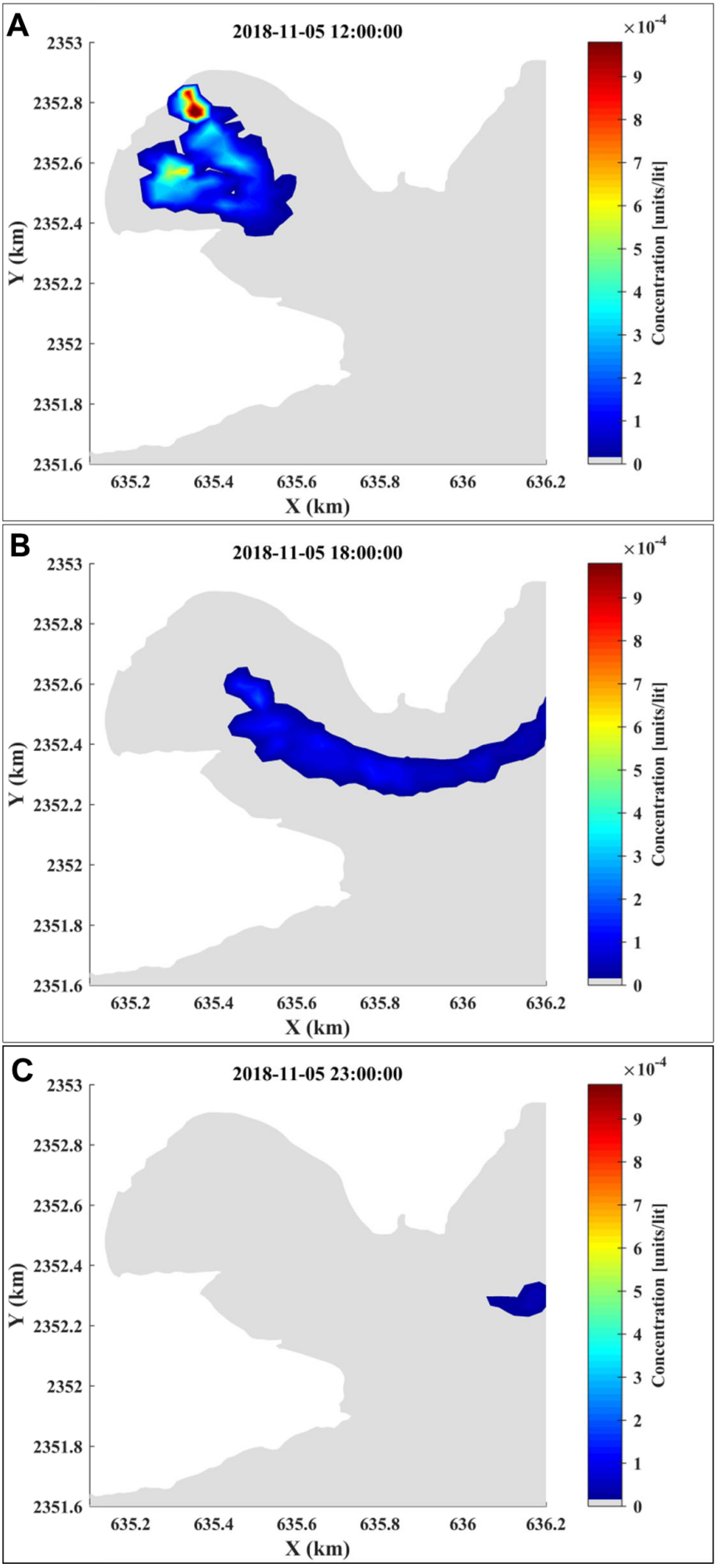
D
2018-11-11 06:00:00
E
2018-11-11 12:00:00
F
2018-11-11 18:00:00
G
2018-11-12 00:00:00
H
2018-11-12 06:00:00
I
2018-11-12 12:00:00
J
2018-11-12 18:00:00
K
2018-11-12 21:00:00
Y (km)
X (km)
Concentration [units/lit]
×10⁻⁴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Y (km)
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Concentration [units/lit]
×10^-4
2353
2352.8
2352.6
2352.4
2352.2
2352
2351.8
2351.6
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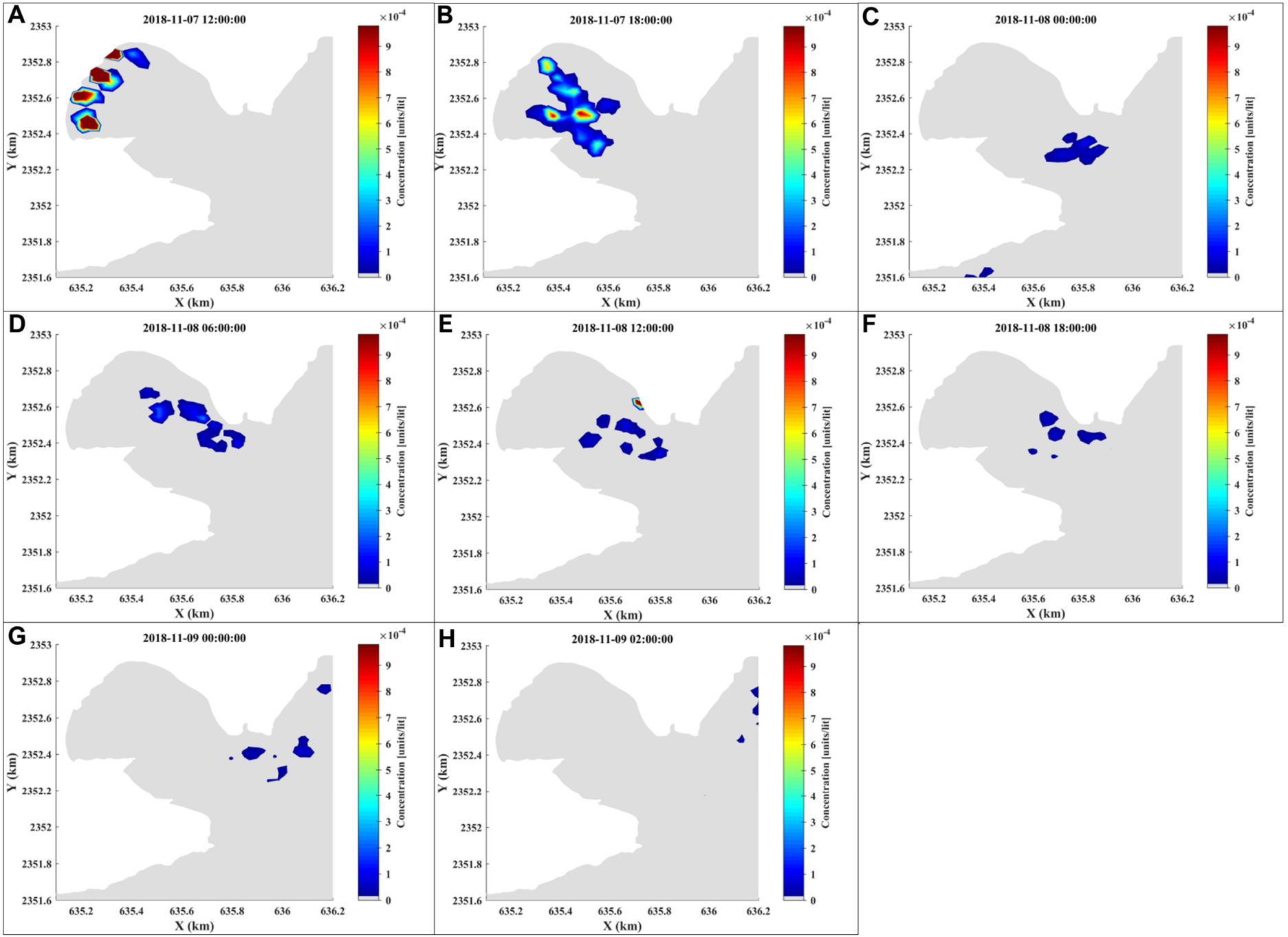
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G
2018-11-09 00:00:00
H
2018-11-09 02:00:00
Y (km)
X (km)
Concentration [units/lit]
×10⁻⁴

Haereticus
Environmental Laboratory

P.O. Box 92, Clifford, VA 24533
Email: cadowns@haereticus-lab.org
www.haereticus-lab.org

July 21, 2021

Editorial Board of Chemosphere

Dear Editor,

We are submitting a manuscript entitled "Oxybenzone contamination from sunscreen pollution and its ecological threat to Hanauma Bay, Oahu, Hawaii, U.S.A." and is authored by me and 15 other co-authors.

Neither this manuscript nor any of the material therein have been published, and it is not under consideration for publication elsewhere.

The purpose of this manuscript is to address the contamination of oxybenzone in Hanauma Bay, and the potential risk this chemical can pose to its coral reef community. This information has implications to the mitigation and regulatory policies regarding sunscreen pollution in Hawaii, and in many coastal and island nations that are dealing with intense tourism, sunscreen pollution, and conservation of their threatened and declining biodiversity.

We respectfully request that this manuscript not be sent to a personal care product industry scientist for review. There is a strong financial conflict-of-interest between our work and their funders, and many are currently paid as consultants or receive funding to promote a specific product-protection narrative. If you feel the need to send it to an industry-financed reviewer, please contact me to discuss this issue.

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11455 Saskatchewan Drive, Edmonton, Alberta, T6G 2E9, Canada
Email: greg.goss@ualberta.ca

Prof. Alex Rogers, PhD
Dept. of Zoology and Conservation Biology
University of Oxford
Email: alex.rogers@revocean.org (preferred)
Email: alex.rogers@zoo.ox.ac.uk

Prof. Pierre Mineau, PhD
Subject Editor, Earth Systems and Environmental Sciences, Elsevier Publishing
Email: pierre.mineau2@gmail.com

Prof. Sarah Montesdeoca-Esponda, PhD
Instituto Universitario de Estudios Ambientales y Recursos Naturales (i-UNAT), Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de Gran Canaria, Spain
Email: sarah.montesdeoca@ulpgc.es

Prof. Aaron Boyd, PhD
University of Alberta, Department of Biological Sciences
Edmonton T6G 2E9, Canada
Email: boyd2@ualberta.ca

Prof. Xiaonan Wang, PhD
State Key Laboratory of Environmental Criteria and Risk Assessment, State Environmental Protection Key Laboratory of Ecological Effects and Risk Assessment of Chemicals, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
Email: wangxn@craes.org.cn

Thank you for you consideration.

Craig A. Downs, PhD

Executive Director, Haereticus Environmental Laboratory
Invited Professor, Sorbonne University

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

C.A. Downs[1,5*], Elizabeth Bishop[2], M. Silvia Diaz Cruz[3], S. Abbas Haghshenas[4], Didier Stien[5], Alice M.S. Rodrigues[5], Cheryl M. Woodley[6], Adrià Sunyer-Caldú[3], Shadan Nasseri Doust[4], William Espero[7,8], Gene Ward[9], Aref Farhangmehr[4], S. Maryam Tabatabaee Samimi[4], Michael J. Risk[10], Philippe Lebaron[5], Joseph N. DiNardo[11]

[1]Haereticus Environmental Laboratory, P.O. Box 92, Clifford, VA 2453, USA

[2]Friends of Hanauma Bay, P.O. Box 25761, Honolulu, HI 96825-07610, USA

[3] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Severo Ochoa Excellence Center. Spanish Council for Scientific Research (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain.

[4]Institute of Geophysics, University of Tehran, Tehran, Iran

[5]Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes, USR3579, Observatoire Océanologique, 66650 Banyuls-sur-mer, France

[6]U.S. National Oceanic & Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean Science, Coral Disease & Health Program, Hollings Marine Laboratory, 331 Ft. Johnson Rd. Charleston, SC 29412, USA

[7]Hawaii State Senate, Senate District 19, Hawaii State Capitol, 415 S. Beretania St. Honolulu, HI 96813, USA

[8]Presently retired

[9]Hawaii State Legislature, House District 17, Hawaii State Capitol, 415 S. Beretania St. Honolulu, HI 96813, USA

[10]Department of Earth Sciences, McMaster University, Hamilton, Ontario, L8S 4M1 Canada

[11]Vesuvius, Virginia, USA

*Corresponding author: cadowns@haereticus-lab.org

Highlights (3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

Highlights

- Oxybenzone concentrations ranged between 30 nanograms/L to 27,880 nanograms/L in subsurface, near-shore waters in Hanauma Bay, Hawaii.
- Beach showers are a point-source of contamination, having sand contamination of UV sunscreens between 0.39 μg/g to 35 μg/g of oxybenzone, 1.26 μg/g to 50 μg/g of octocrylene, 0.4 μg/g to 33 μg/g of octinoxate, below the limit of detection to 133 μg/g of octisalate, and 0.16 μg/g to 1.6 μg/g of the carcinogen, benzophenone.
- Hydrodynamic modeling indicated that oxybenzone contamination could be retained within Hanauma Bay between 14 and 50 hours from a single release event.
- Risk assessment of oxybenzone in Hanauma Bay demonstrated a threat to the ecological integrity to Hanauma Bay's coral reef.

Declaration of interests

☐ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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. For transparency, we make the following declaration:

Declaration of competing financial interests – Haereticus Environmental Laboratory has received funding from the U.S. Environmental Protection Agency and the U.S. Department of Interior, but this funding did not contribute and is no way associated with this study. One Laboratoire de Biodiversité et Biotechnologies Microbiennes project is financed in the context of the Pierre Fabre Skin Protect Ocean Respect action. The work reported in this manuscript was not supported by Pierre Fabre Laboratories. The interpretation and views expressed in this manuscript are not those of the company.