Assessing Occupational Ozone Exposure at a Marine Mammal Hospital

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Abstract

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Background: Ozone is a popular water sterilization agent utilized in marine mammal husbandry to maintain the pool water in the animal enclosures. The health hazards posed by ozone are well understood, but its role as an occupational hazard, especially in marine mammal husbandry, is understudied and not well characterized. The purpose of this study was to assess occupational exposure to ozone off-gassed from a water treatment system at a marine mammal hospital and to understand how environmental conditions (temperature, relative humidity, wind speed, and wind direction) might modulate the ozone concentrations being off-gassed from the water treatment system.

Methods: We conducted area monitoring of ozone in three zones within the marine mammal hospital facility for five, 8-hour days per zone. Environmental data was collected using a weather station that was mounted in one location for the duration of the data collection. The difference in ozone concentrations in the study zones were evaluated. Correlations between ozone and temperature and ozone and relative humidity were assessed. The effect of wind behavior on

ozone was characterized using Conditional Bivariate Function Plots. The effect of all of the environmental conditions on predicted ozone cocnentrations were further characterized using a predictive Quasi-Poisson model.

Results: Ozone concentrations were highest closest to the water treatment system. Correlations between ozone and temperature and ozone and relative humidity were stronger further away from the source. Wind speed and direction had a stronger influence on ozone concentrations at the site than temperature and relative humidity. The environmental conditions were unable to explain all the variability in ozone concentrations at the study site.

Conclusions: Ozone concentrations were greatest closest to the source and environmental conditions had varying impacts on the exposures. The results of this study found that there were exposures over the Ceiling limit set by WA L&I and the recommended exposure limit set by NIOSH. This data can inform decisions and recommendations in the workplace to reduce over-exposure to ozone. This data also demonstrates that ozone can pose a significant occupational hazard in a marine mammal hospital setting. More research on employee personal exposure to ozone is needed to better understand the impact of ozone exposure in this understudied industry.

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Finally, I'd like to thank my family, my friends, and my cats for their moral support throughout this research project.

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Acronyms and Definitions

ACGIH – American Conference of Governmental Industrial Hygienists
EPA – Environmental Protection Agency
GFG - GFG Model G460 1-6 Gas Multi-gas detector
NAAQS - National Ambient Air Quality Standard
NOAA - National Oceanic and Atmospheric Administration
NOx - Nitrogen oxides
O_3 – Ozone
OSHA – Occupational Safety and Health Administration
$TWA_{8\text{-hour}}$ – 8-hour Time Weighted Average
UV – ultraviolet
VOCs – volatile organic compounds
WA Ecology - Washington State Department of Ecology
WA L&I – Washington Department of Labor and Industry

Introduction

Ozone and its application in a marine mammal hospital

Ozone (O_3) is a colorless to blue gas with a pungent, chlorine-like, odor which is made up of three oxygen atoms that form an unstable bond which rapidly decomposes upon its formation (Batakliev et al., 2014). Ozone is produced when oxygen (O_2) molecules are separated by an energy source into individual oxygen atoms, which then collide to form ozone (O_3) (Batakliev et al., 2014). In our atmosphere, ozone naturally occurs in the upper atmosphere where it forms the ozone layer and absorbs harmful ultra-violet (UV) radiation (Gerhartz, 1991). At Earth's surface, ground level ozone is formed through a photo-chemical reaction involving heat and ultraviolet radiation from the sun, nitrogen oxides (NOx) and volatile organic compounds (VOCs) (Placet, et al., 2000). NOx and VOCs can be produced by anthropogenic activities such as pollution from cars and industrial facilities (Placet, et al., 2000). Ground level ozone is a pollutant and hazardous to human health. Ozone is not only found in the atmosphere and ground-level pollution. Controlled use of ozone as a disinfectant is utilized within a variety of industries. In marine mammal husbandry, ozone is used as a pool water disinfectant to maintain a healthy enclosure for the animals (Gage & Whaley, 2009). When ozone is used in this way, there is a need to control exposures to workers as well as animals while also ensuring that the ozone is sufficiently disinfecting water to remove biohazardous materials and potentially zoonotic pathogens.

Workers engaged in work that involves animals face a unique variety of occupational hazards, which are further complicated by the human-animal interactions that define this type of work. Biological hazards, especially the risk of occupational zoonotic infection, are prominent hazards of concern especially for workers that handle wildlife. Most emerging infectious diseases in humans are zoonotic in origin, with wildlife representing a significant source of recent disease emergence events (Jones et al., 2008). Stringent infection control protocols in a veterinary hospital are a necessary control method to prevent disease transmission in the workplace between humans and animals, as well as transmission between animals (Gage & Whaley, 2009). In marine mammal husbandry, pool water quality is an important aspect of patient care. The National Oceanic and Atmospheric Administration (NOAA) has outlined best practices in water quality guidelines in marine mammal enclosures because access to adequately clean water with species-appropriate water quality parameters is vital, especially for potentially immunocompromised patients (Gage & Whaley, 2009). The use of ozone is an approved method to keep micro-organisms in the water below harmful levels (Gage & Whaley, 2009).

Ozone can destroy bacteria, viruses, and helminths utilizing mechanisms such as direct oxidation and destruction of the cell wall, reactions with radical by-products of ozone decomposition, and damage to the constituents of nucleic acids to disinfect (Boner & Lau, 1999). Ozone has a short half-life and reacts rapidly, therefore when ozone is used in a water treatment system it must be generated onsite through an ozone generator (Spotte, 1991). Air passes through the ozone generator where oxygen is exposed to an energy source that dissociates the oxygen molecules into atoms which go onto form ozone. This ozone is immediately mixed with system water in large contact chambers, or it is injected into the water. The ozone rapidly reacts with the wastewater with very little leftover oxidant because of its minute half-life (Coakley & Crawford

1998). A schematic of how ozone is generally used in a water treatment system is shown in Figure 1.

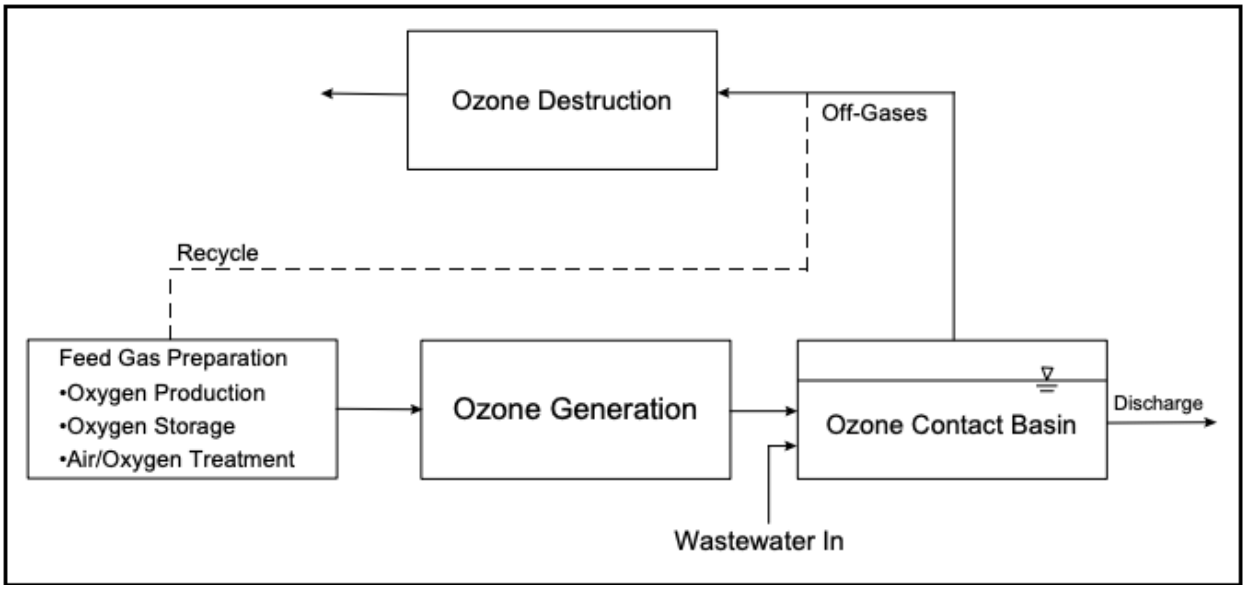

Figure 1. Air is treated and Oxygen is stored or prepared as a feed gas which is then injected into the ozone generation chamber. In this chamber, the oxygen is exposed to an energy source where the oxygen (O_2) molecules are split apart by the radiation and subsequently the free atoms (O) collide with adjacent O_2 to form ozone (O_3). After this, the ozone is sent into a contact basin where it comes into contact with and treats the wastewater. Finally, excess ozone is off gassed from the system into the surrounding area or it is sent into an ozone destruction component where it is reverted back to oxygen. Build-up of this off-gassed ozone may provide a route of exposure to workers in the surrounding area. Source: EPA 1986

Ozone as a Health Hazard

The primary route of exposure to ozone is inhalation, secondary routes are absorption through the skin or the eyes. What makes ozone an effective disinfectant, namely its ability to act as a strong, highly reactive oxidizer, also makes it a powerful respiratory irritant. Inhaled ozone rapidly reacts with human airways, causing respiratory inflammation and functional decrements in airway flow (Bromberg, 2016). Individual response to ozone is highly variable but in general, people with pre-existing respiratory diseases such as asthma experience more intense respiratory symptoms following exposure (Hazucha et al., 2003). In a study comparing lung function decrement to similar ozone exposure in individuals with moderate asthma and healthy individuals, the cohort with asthma had a larger decrement, as determined by a lower forced expiratory volume in 1 second (FEV1) response, and more evidence of bronchoconstriction than those without asthma. (Horstman et al., 1995). There are two possible mechanisms that may cause this increased susceptibility. First, those with asthma might be more sensitive to ozone due to preexisting airway inflammation and reactivity, and therefore experience the respiratory effects seen in those without asthma but at lower ozone concentrations or with a greater severity of symptoms. The exact mechanism behind what causes individual variability in ozone sensitivity is largely unknown however (Bromberg, 2016). The other suggested mechanism is

that ozone exposure causes further airway injury, inflammation, and resultant reactivity (Kehrl et al., 1999).

At low levels, the acute effects of ozone exposure are typically reversible, with the physiological effects limited to the respiratory tract. Recovery to baseline can vary from a few hours up to 48 hours after an elevated ozone exposure (Bromberg, 2016). Ozone is highly reactive, and its reactive intermediates create a cascade effect that may play an additional role in causing injury to the airway (Bromberg, 2016). The major acute respiratory symptoms after exposure to ozone inhalation of 100-600 ppb for 1 – 4 hours include decreased lung capacity, inflammation of the lower airways, increased bronchial reactivity to inhaled bronchoconstrictors such as allergens, and mild to moderate bronchoconstriction (Bromberg, 2016). In the general population, increases in emergency room visits for asthma related complications associated with acute exposure to temporary increases in ambient ozone has been well documented within cities throughout the world. (Fauroux et al., 2000; Mar & Koenig, 2009; Burnett et al., 1997).

The effects to the lung resulting from short-term exposure to ozone are primarily restrictive, with mild obstructive effects. Restrictive lung conditions are conditions that hinder one's ability to fully expand their lungs with air, while obstructive conditions make it difficult to exhale all the air in the lungs (Martinez-Pitre et al., 2022) The restrictive impact of acute ozone exposure on lung function is demonstrated by a variable decrease in lung vital capacity on spirometry testing. This change is due to a functional decrease in inspiratory capacity which is caused by involuntary, neural inhibition of inhalation at high lung volumes. This is typically accompanied by coughing, pain, burning, or discomfort that prevents a person from taking a deep breath, resulting in shortness of breath (Hazucha et al., 1989). These symptoms and changes in lung function are thought to be caused by a stimulation of airway neural receptors and transmission to the central nervous system (Krishna et al., 1996). The result is a decrease in measured Force Vital Capacity (FVC) and overall ability to inhale deeply in acutely overexposed individuals. Obstructive effects are reflected by decreases in FEV1 (Hazucha, 1989). In healthy individuals, these decreases in lung function have been observed in response to ozone concentrations as low as 60-70 ppb following 6.6-hour exposures in a protocol involving quasi-continuous exercise (Adams, 2006). After acute exposure, the ozone-induced inflammatory response develops over a longer period than the changes in lung function. This inflammatory response may be a result of secondary oxidation products that activate pro-inflammatory pathways in airway epithelial cells (Mudway & Kelly, 2000). After repeated acute exposures, this inflammatory response may be responsible for the development of chronic lung inflammation and chronic airway hyperreactivity (AHR) (Mudway & Kelly, 2000).

Chronic exposure to ground level ozone has been associated with increased mortality at a population level. Multiple epidemiologic studies of populations have found that a 10-ppb daily increase in ozone concentration is associated with an increase in daily mortality, specifically cardiovascular and respiratory mortality (Bell et al., 2004; Di et al., 2017; O'Neill et al., 2004). At an individual level, chronic exposure to ozone induces oxidative stress in tandem with the acute effects described above (Bromberg, 2016). This sustained oxidative stress may be a mechanism by which ozone causes permanent damage to the respiratory system. Researchers studying chronic obstructive pulmonary disease (COPD) use chronic ozone exposure to create a model of this disease in mice (Triantaphyllopoulos et al., 2011). Chronic, high level, exposure

amplifies the acute effects of ozone and can result in chronic inflammation and cellular damage consistent with pulmonary emphysema (Triantaphyllopoulos et al., 2011). Chronic inflammation, alveolar damage, and emphysema are characteristics of COPD (Triantaphyllopoulos et al., 2011). In humans, chronic exposure between 2-4 weeks has been demonstrated to contribute to sustained oxidative stress which increases lipid peroxidation (Chen et al., 2007). Oxidative stress plays an important role in the pathogenesis of COPD (Wiegman et al., 2020).

Occupational exposure to ozone can be both chronic and acute. Ambient ozone is ubiquitous as a component of air pollution and therefore outdoor worker populations are frequently exposed. Some industries utilize ozone in their processes such as wastewater treatment, aquaria water treatment, and treatment of paper in paper mills (Rice 1996; Kingsley 2008; Freire, 2011). Ozone is a recognized hazard in aquaculture, which is a closely related field to marine mammal husbandry (Fry et al., 2019). Occupational exposure can also occur while performing job tasks that generate ozone such as during arc-welding (Azari et al., 2011). Outdoor workers can be chronically exposed to ambient ozone, and the magnitude of these exposures can be intensified by higher levels of physical exertion due to increased respiration causing individuals to inhale more ozone which may result in a greater response (Hu et al., 1994). Multiple studies on outdoor worker cohorts have shown that respiratory effects have been induced by exposures well below the federal regulatory limit (OSHA PEL) of 100 ppb over an 8-hour time-weighted average ($TWA_{8\text{-hour}}$). In mail carriers, an outdoor worker population, studies have found that acute decreases in lung function occur in those exposed to O_3 concentrations below current ambient air quality standards and occupational exposure limits (Chan & Wu, 2005). Another study which assessed the effect of ambient ozone exposure on the lung function of a group of outdoor farm workers found that decreased lung function was observed at mean exposures of 40 ppb over an 8-hour shift (Brauer et al., 1996).

In industries where ozone is utilized, workers can experience chronic low-level exposures and a unique exposure scenario, referred to as peak exposures. Peak exposures are exposures to high concentrations of ozone for a brief period of time. In both circumstances, there have been records of workers developing ozone-induced occupational asthma. Peak exposures of 300 ppb and greater have been associated with asthma and wheezing in pulp mill workers (Henneberger et al., 2005). Chronic exposure to ozone concentrations of 40 ppb at a workplace that generated ozone through high-voltage electrical discharge has been associated with work-related asthma in a worker (Lee et al., 1989). The worker had a personal history of asthma, but the occupational exposure to ozone increased the frequency and severity of asthmatic attacks (i.e.: work exacerbated asthma) and his peak expiratory flow rates were significantly reduced. When assigned to a different job task with no ozone exposure, his condition improved. This worker was the only one in his position that experienced acute exposure-related symptoms which is most likely due to his history of having asthma making him more sensitive to ozone and more likely to have more severe responses to lower concentrations than those without asthma (Lee et al., 1989).

As mentioned above, workplaces involving animals may need to consider the effects of workplace exposures on animal health as well as worker health. Outside of laboratory animals used in research, research on the effect of ozone on animal health is minimal. Laboratory animal models used to model human response to ozone exposure have demonstrated that ozone can cause deleterious acute and chronic health effects in animals. Ozone-induced health or

behavioral effects in mammalian wildlife, especially marine mammal wildlife, represent an under studied area of investigation. There is evidence suggesting that exposure to ozone in reptiles and amphibians can induce defensive physiological and behavioral reactions (Dohm et al., 2008; Mautz & Dohm, 2004). In captive marine mammals, premature ocular disease is common in pinnipeds compared to their wild counterparts. These injuries tend to manifest after spending an extended period of time in captivity (6+ years) and have been linked to pool water quality and use of oxidants such as ozone in water disinfection (Latson, 2009). Exposure to residual ozone in pool water is suspected to cause excessive watering of the eye and uncontrollable twitching of the eye in captive seals and sea lions (Gage & Aczm, 2011). These symptoms have been documented based on anecdotal observations by veterinarians and there is a need for more formal studies of ozone exposure-effect relationships in different species.

Regulations and recommendations

Marine mammal hospitals tend to be a combination of indoor and outdoor facilities. To determine allowable ozone exposure in such settings, there are multiple relevant acceptable outdoor ozone exposure limits because ozone can be both an outdoor air pollutant and a workplace exposure. For the general population, the Environmental Protection Agency has established a National Ambient Air Quality Standard (NAAQS) for ozone of 70 ppb as an 8-hour Time Weighted Average ($TWA_{8\text{-hour}}$) (OAR US EPA, 2020). Another way that the EPA presents acceptable air quality levels is through the Air Quality Index (AQI), a unitless measurement that helps visualize this information for the general public. Using the airnow.gov AQI calculator, we can extrapolate AQI to concentrations of ozone levels over an 8-hour TWA (Table 1).

Table 1. *AQI category and values with the equivalent ozone concentration in ppb as an 8-hour TWA.*

AQI category	**AQI Value**	**Ozone concentration (ppb, $TWA_{8\text{-hour}}$)**
Good	0 – 50	0 – 54
Moderate	51 – 100	55 – 70
Unhealthy for sensitive individuals	101 – 150	71 – 85
Unhealthy	151 – 200	86 – 105
Very Unhealthy	201 - 300	106 – 200

Relevant occupational regulations for Washington state include exposure limits set by the Occupational Safety and Health Administration (OSHA) and the Washington State Department of Labor and Industry (WA L&I). OSHA uses a Permissible Exposure Limit (PEL) of 100 ppb $TWA_{8\text{-hour}}$ and 300 ppb as a 15-minute Short Term Exposure Limit (STEL) (OSHA, 2017). WA L&I has a PEL of 100 ppb $TWA_{8\text{-hour}}$ and a Ceiling limit of 300 ppb. Ceiling limits are limits that are not to be exceeded at any point in the workplace.

Recommended exposure limits (REL) are limits that are recommended by non-regulatory agencies and considered to be best practices. Agencies that produce such limits include the National Institute of Occupational Safety and Health (NIOSH), The American Conference of Governmental Industrial Hygienists (ACGIH), and the American National Standards Institute

(ANSI). NIOSH recommended exposure limits (RELs) include a Ceiling limit of 100 ppb and an Immediate Danger to Life and Health (IDLH) of 5 ppm. ACGIH recommends a Threshold Limit Value (TLV) $TWA_{8\text{-hour}}$ with varying ozone limits, stratified by workload. These TLVs are $TWA_{8\text{-hour}}$ of 50 ppb for heavy work, 80 ppb for moderate work, and 100 ppb for light work. Table 2 summarizes these regulatory and recommended exposure limits.

Inhaled ozone is distributed throughout the lungs, 10% is absorbed in the upper airway, 65% in the lower airways, and 25% more distally (Hu et al., 1994). This distribution and uptake of ozone in the lungs is dependent on the inspiratory flow rate, increased flow rates result in more ozone uptake (Hu et al., 1994). This is quite relevant to occupational exposure scenarios because workers performing more intensive tasks will receive a higher ozone dose. Currently, ACGIH is the only agency that has taken this into account when creating their recommended exposure limits, which are considered best practices and are not enforceable by law.

Table 2. *Summary table of the regulatory and recommended exposure limits for ozone relevant to the general population and to workers.*

	Standards	**Concentration of Ozone**	**Limit type**
Regulatory Limits	EPA	70 ppb	$TWA_{8\text{-hour}}$
	OSHA PEL	100 ppb	$TWA_{8\text{-hour}}$
	WA L&I	100 ppb	$TWA_{8\text{-hour}}$
		300 ppb	Ceiling
Recommended Limits	NIOSH REL	100 ppb	Ceiling
	ACGIH TLV	100 ppb	$TWA_{8\text{-hour}}$, light work
		80 ppb	$TWA_{8\text{-hour}}$, moderate work
		50 ppb	$TWA_{8\text{-hour}}$, heavy work
	ANSI	100 ppb	$TWA_{8\text{-hour}}$
		300 ppb	15-min STEL

The effect of environmental parameters on ground level ozone

Ground level ozone found in air pollution is formed through a photochemical reaction involving two classes of common air pollutants: nitrogen oxides (NOx) and volatile organic chemicals (VOC) (Placet, et al., 2000). Initiation and propagation of these reactions are dependent on the level of sunlight present, which results in a seasonal and diurnal pattern in ozone concentrations. Ambient air Ozone concentrations are generally highest in the afternoon and during the fall, spring, and summer months because that is when the influence of direct sunlight is greatest (US

EPA, 2020). In combination with precursors, environmental parameters such as temperature, wind speed, relative relative humidity, and precipitation are important variables in ground level ozone formation (US EPA 2020).

For the marine animal rehabilitation workers and volunteers at the study site, their busiest season is during the summer months which correlates with the harbor seal pupping season in the Pacific Northwest (Seal Sitters, 2015). There is an influx of seal pup patients during this time, which means that there is a simultaneous influx of staff and volunteers onsite caring for these animals, creating an elevated potential incidence of exposure to increased ambient ozone concentrations in outdoor air in addition to the ozone being produced on site by the water treatment system.

Specific Aims

While the hazard posed and mechanism by which exposure to ozone results in adverse health outcomes is relatively well understood, research focusing on occupational exposure to ozone through water treatment systems is limited, particularly in animal centered settings where ozone is used to disinfect water such as in marine wildlife hospitals, aquaria, and marine mammal exhibits. Our study seeks to provide insight into the magnitude of ozone concentrations off gassed by the water treatment system of a marine mammal rehabilitation facility and whether environmental conditions might modulate the ozone exposure of facility workers.

The primary objective of this study was to perform area monitoring and to characterize occupational ozone concentrations in specific areas (zones) of the facility that represent different potential worker exposure scenarios. In this study, the ozone exposure of primary focus was the ozone being emitted from the water treatment system. The secondary objective was to understand how the environmental parameters temperature, relative humidity, wind speed, and wind direction mediate the magnitude of the ozone exposure scenario in the study zones. Understanding the environmental conditions that are associated with high area concentrations of ozone will be beneficial in understanding the behavior of ozone and providing guidance on how to safely work around water treatment systems that use ozone in these types of workplace settings. The third objective was to characterize peak ozone exposures in the marine mammal facility.

Aim 1. Determine the differences in ozone concentrations among three different zones within the marine mammal hospital facility.

Aim 2. Characterize the effect of temperature, relative humidity, wind speed, and wind direction on ozone concentrations.

> ***Aim 2a***. Evaluate whether there is a relationship between temperature, relative humidity and ozone, and compare correlations between each parameter among the three study zones.
>
> ***Aim 2b.*** Evaluate whether there is a relationship between wind speed, wind direction, and ozone, characterize how wind behavior modulates ozone, and compare these relationships among each zone.

Aim 3. Characterize peak ozone concentrations and describe the environmental conditions associated with those peaks.

The outcomes of these aims will assist us in evaluating the magnitude of ozone concentrations being off gassed by this system, the potential for workers to experience hazardous ozone exposures due to the water treatment system, and the role of environmental conditions on the area concentrations of ozone. Ozonation is a common disinfectant in water treatment systems in marine mammal husbandry but there is very little research focusing on how this application may have worker health implications and the potential for over-exposure. The resulting data may provide beneficial insight into the health risks of a currently under-studied workplace exposure and provide insight into possible control strategies.

Methods

Study Design

This project is an observational study that examines the ozone concentrations and potential occupational exposures to ozone in workers and volunteers who work at a West coast marine mammal hospital. This study utilized area sampling with a direct reading device (electrochemical sensor) and a mounted weather station at the study site to monitor ozone concentrations and the hyper-local environmental conditions that may influence ozone concentrations at this site. Three zones were selected to conduct ozone monitoring with respect to distance from the ozone source, the ozone contact-basins in the water treatment system. The study site, measurement methods, and data analysis methods are further described in the following sections.

Research study setting

The hospital has an enclosure outside with two pools for patients and a tent with small pools where more fragile patients are kept in isolation before they are moved to the bigger outdoor pools and finally released back into the wild (Image 1). The water treatment system servicing the two outdoor pools is located next to the pool deck (Image 2).

The water treatment system

The marine mammal hospital uses a custom water treatment system made up of components from different companies. To filter out debris from the pool water, they use foam fractionation and a sand filter. The foam fractionators are RK2 (RK2 Systems, San Marcos CA). To disinfect the water, they use ozone and UV. The ozone components are made by MicroPlasma Ozone (MicroPlasma Ozone, Champaigne, IL). The two towers are the site of UV sterilization, foam fractionation, and ozone interaction with contaminated pool water (Image 2).

There are two pools on the pool deck, one 8-foot pool and one 12-foot pool. Each tower separately services each pool. The 8-foot pool's system is comprised of 1 heater, 1 UV sterilizer, one sand filter, a small fractionator, and an ozone generation system. The 12-foot pool is serviced by a larger fractionator and separate ozone generator, UV, heater, and two sand filters. The pool water supplies are completely isolated from each other to avoid cross contamination between pools. The water treatment system is essentially made up of two systems that work independently of each other to service their respective pool. The systems are on as long as their respective pool is running and were on for the duration of the data collection.

Both ozone generation systems are made up of multiple components. As described previously (Figure 1), there are multiple phases to generate and utilize ozone in a water treatment system. While both systems operate independently, they function in a similar manner. This process starts with ambient air being drawn into a dehumidifier. The 8' pool uses a Microplasma Kistler (MicroPlasma Ozone, Champaigne, IL) which has a solid-state electronic dehumidifier that regulates the humidity of the ambient air. The 12' pool uses an Airsep Topaz Oxygen Concentrator (MicroPlasma Ozone, Champaigne, IL), which concentrates oxygen from the ambient air, compared to the smaller system that solely uses the ambient air. Concentrating the oxygen allows this system to generate more ozone to service the larger pool. Next, the ozone generator uses electricity to initiate the ozone-forming reaction and generates a mixture of ozone and air or oxygen. The ozone mixture is injected into the towers via venturi injection where it interacts with contaminated pool water. The ozone mixture is maintained at a constant generation of 2 grams per hour in the 8' pool and 5 grams per hour in the 12' pool (MicroPlasma Operation Manual, 2022).

Data collection & Field work

Data collection took place over the course of 18 days in October and November 2021. Ozone monitoring was conducted using a direct reading device, the GFG Model G460 1-6 Gas Multi-gas detector (GFG Instrumentation Inc. Ann Arbor, MI). Environmental conditions were monitored using a weather station. Ozone monitoring occurred in three zones within the facility. These three zones were determined with respect to distance from the ozone source and were selected to represent different exposure scenarios (Figure 1). Images 1 and 2 show actual images of the study site. Five, 8-hour days of ozone monitoring was conducted for each zone. Three extra days were determined to be necessary due to three days of data being corrupted due to a malfunction with the GFG. The data collection occurred in blocks where each zone was monitored 1-week at a time. This was necessary because the busy season was ending soon and when there are no patients expected to be present for an extended period of time, the hospital shuts down part of the water treatment system, which would shut down the ozone towers. With this time constraint, we decided to do the monitoring a week at a time, prioritizing the zones close to the ozone towers in the first two weeks, and the zone used for referent background ozone levels at the site in the third week. Week one sampling occurred during October 12 – 16, week two sampling occurred during October 19 – 23, and week three sampling occurred during October 26 – 30. Three days of data from zone 2 (October 12 – 14) were not included and three more days of data in zone 2 were collected from October 31 – November 2.

All samples were collected over the course of a standard workday, when most volunteers and workers were on site, from 9 am until 5 pm. At the start of each sampling day, the GFG was set up in the respective zone. Zone 1 was closest to the source and selected to represent a worst-case scenario exposure. Here, the GFG was placed close to the shorter of the ozone contact basins, just above it where ozone is off gassed. This location is close to where cleaning tools, boards, and enrichment items for the animals are stored and on occasion workers must be near this ozone tower to access these items or to perform maintenance on the water treatment system. Zone 2 was on the pool deck, where workers are constantly performing tasks, such as feeding the animals and cleaning. This zone represented a typical exposure scenario as it is a highly trafficked area that is near the water treatment system. The monitor was placed on the fence on

the side of the pool deck closest to the ozone towers and in a location that was approximately 5.5 feet from the deck floor and equidistant between the two ozone contact-basins. Zone 3 was selected to represent the background ozone levels at the study site. We determined that the tented area was ideal for this because it had enough distance from the contact basins that ozone concentrations from the water treatment system would not influence potential ozone concentrations in this tent and only ambient ozone would remain if there was any.

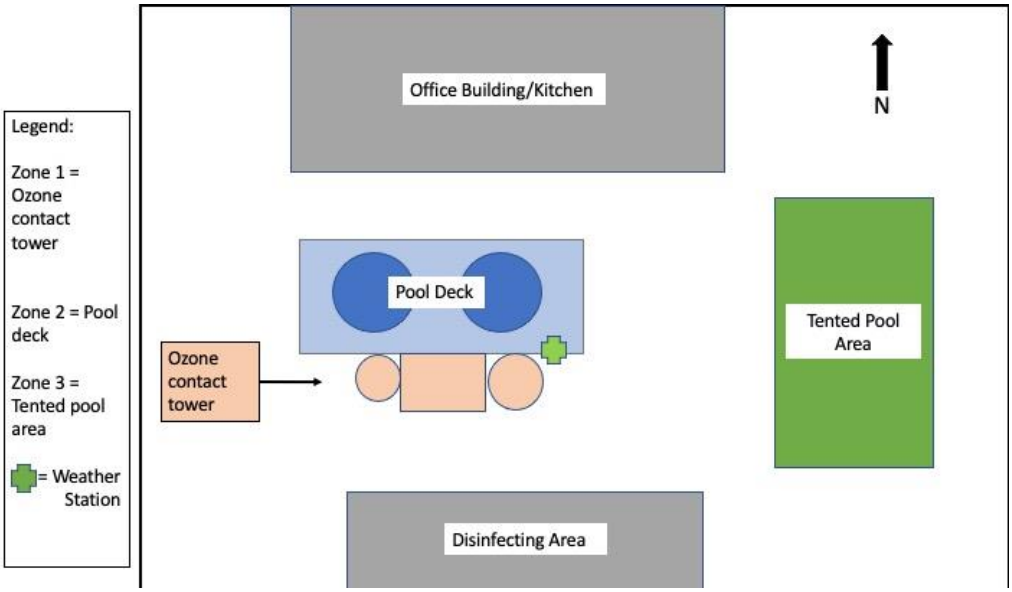

Figure 1. *General layout of the marine mammal hospital and the three study zones. Zone 1 is next to the ozone contact basin, zone 2 is the pool deck, and zone 3 is in the tented pool area. The weather station (denoted by the green cross) was mounted above the pool deck and remained there for the entirety of the study.*

Image 1. *Panoramic image of the study site from the roof of the office building. The white tent (zone 3) is to the left and the pool deck (zone 2) is in the middle. In this image, the 12' pool is on the left and the 8' pool is on the right. The water treatment system (zone 1) is on the opposite side of the pool deck and not shown here. Source: Greg Frankfurter*

Image 2. *Water treatment system components. The two towers are the ozone contact basins. The weather station was mounted on top right the fence of the pool deck and remained in the same location for the entirety of the study. The zone 1 ozone measurements were collected adjacent to the left (smaller) tower.*

Ozone sampling device and weather station

Ozone sampling was performed using a GFG Model G460 1-6 Gas Multi-gas detector (GfG Instrumentation, Inc., Ann Arbor, MI, USA). This device was used in passive sampling mode to ensure that the battery lasted the full 8 hours each study day. This device uses an electrochemical sensor to detect ozone. In passive sampling mode, air passes over the sensor and the device quantifies the ozone concentration by measuring the current generated from the reduction of ozone in the electrochemical cell. The data is measured continuously and is stored on the device. The GFG ozone sensor has a detection range of 0.01 – 1 ppm (10 – 100 ppb), and a resolution of 0.01ppm (10 ppb). Only one GFG was used in this study due to the lack of multiple instruments. The GFG logged averaged ozone concentration every 60 seconds. Prior to data collection, the GFG was calibrated using a Model 306 Ozone Calibration Source (2B Technologies, Boulder CO USA) and ozone calibration standard. Prior to the study we also performed a colocation validation experiment with the Washington State department of Ecology's reference ozone monitor, a Teledyne API T400 (Teledyne API, San Diego CA, USA), in Beacon Hill, Seattle WA to ensure that the GFG measurements were accurate. Figure 1 in the Appendix shows the results of the colocation experiment. The GFG results were noisier than the Teledyne API but followed the trends of the ozone concentrations at the site.

Environmental data was collected using the Ambient Weather WS-2000 Weather Station (Ambient Weather, Chandler AZ, USA). The weather station was installed in fixed location at the study site for the duration of the data collection. The weather station produced data at 1-minute averages. To ensure accuracy, we compared data from the WS-2000 with data from the NOAA weather station at Sea-Tac Airport. This data is in the Appendix (Figures 2 & 3, Table 1). The WS-2000 weather station was within 10% of the NOAA data for relative humidity and temperature. The wind data was more variable but followed the same general trends as the NOAA data, which is understandable because wind behavior can be highly variable depending on local conditions and nearby objects such as buildings. The weather station measured wind speed, wind direction, temperature, relative humidity, UV radiation, and precipitation.

Data Analysis

Aim 1 of this study determined the differences in ozone concentrations among the different zones within the hospital facility. Our hypothesis for this aim was that the ozone exposures would be different based off their proximity to the ozone contact-basins. Our null hypothesis was that there would be no difference in ozone concentration among the work zones. Summary statistics of ozone concentrations in each zone were calculated, including mean, median, standard deviation, sample sizes, and interquartile range (25^{th} percentile to 75^{th} percentile). To determine whether ozone concentrations were different at each zone, we used the Mann-Whitney U test. The Mann-Whitney U test is used to compare whether there is a difference in the dependent variable for two independent groups. The dependent variable in our study is ozone concentration and the two independent groups are Zone 2 and Zone 1. A Mann-Whitney U test is typically used when the sample size is small and/or when the data is not normally distributed.

Aim 2 of this study assessed the effect of environmental conditions on ozone concentrations. The environmental conditions of interest were temperature, relative humidity, wind speed, and wind direction. Our hypothesis for this aim was that ozone concentrations would vary depending on temperature, wind speed and direction, and relative humidity. Our null hypothesis was that there would be no association between ozone concentration and these environmental conditions. To understand the role of temperature and relative humidity on ozone concentration in each zone, we used the Spearman Rank-Order Correlation. The Spearman correlation coefficient, rho, measures the strength and directionality of the association between two ranked variables. Rho is on a scale of -1 to +1, where a value close to -1 or +1 indicates a stronger relationship and a value close to zero indicates a weak or no relationship. The negative or positive indicates the directionality of the relationship. We assessed the relationship between these separate environmental parameters to the ozone concentration in each of the work zones. To do this, we used the 1-minute average data to create 15-minute averages. Then, we assessed the data for outliers. Outliers were defined as ozone observations above $q_{0.75} + 1.5 \times IQR$ or below $q_{0.25} - 1.5 \times IQR$. Where, $q_{0.25}$ and $q_{0.75}$ represent first and third quantiles, respectively. We ultimately decided to keep outliers in the data for this analysis because they provide information on the high ozone exposures that we are interested in understanding. We used the resulting data to perform this analysis. Scatterplots were produced to visualize the strength and direction of the association as determined by the Spearman Rank-Order Correlation test.

To summarize the wind speed and direction conditions for each study zone, we used wind rose plots. These plots were generated in R using the windRose function in the Openair package

(Carslaw and Ropkins, 2012). The wind direction data were summarized by 30° increments. Wind speed data were summarized using the default settings with intervals of 2 mph and break points of 2, 4, 6, and 8+ mph. Percentages of calm wind conditions are calculated as well, calm winds are defined as when the wind speed is zero.

To visualize the relationship between ozone concentration, wind speed, and wind direction we generated conditional bivariate probability function (CBPF) plots. We generated these plots in R using the polarPlot function in the Openair package (Carslaw and Ropkins, 2012). This function is able to extend the conditional probability function (CPF) to a bivariate analysis to produce a CBPF. CPFs are a predictive tool that model and visualize the wind where a pollutant is statistically likely to reach a certain percentile concentration. These are often used in air pollution studies to triangulate and quantify dominant emission sources (Ashbaugh et al., 1985; Vedantham et al., 2013), but a major drawback of this method is that it does not consider wind speed, which can influence the pollutant concentrations at the monitoring site. The CBPF combines the dispersion characteristic information from the bivariate analysis with directional information regarding pollutant source from the CPF. The former is useful in source characterization while the latter is useful in source detection. This method can identify other major emission sources that are too far from the detector to be clearly sensed or other local minor sources that are difficult to detect by displaying the relationship between wind speed, wind direction, and the pollutant. This is useful in understanding the influence of other emission sources and providing a more complete picture of how the study site is affected by them (Uria-Tellaetxe and Carslaw (2014).

The polarPlot function defaults to generating a bivariate polar plot of pollutant concentrations with wind speed and direction data. This shows how concentrations vary by wind behavior. In the function we were able to specify that the CPF be added to the predictive model to create the CBPF. The bivariate polar plot is shown as a continuous surface, where the surfaces are calculated by modelling using smoothing techniques. These plots are constructed by separating wind speed, wind direction, and O_3 concentration data into wind speed-direction bins and the mean O_3 concentration calculated for each bin. Wind direction intervals of 10 degrees and 30 wind speed intervals adequately display the detail of the concentration distribution. Wind direction data is rounded to the nearest 10 degrees and wind speed data is in intervals of 0 to 20 to 30 mph. The wind components are calculated using mean hourly speed and mean wind direction in degrees to generate surface data. A model is applied to the surface data to reduce noise and portray the O_3 concentration as a function of the wind components. This function uses a Generalized Additive Model (GAM) as the framework to fit this model (Hastie and Tibshirani 1990; Wood 2006). GAMs are useful in predictive models where the relationship between variables are non-linear and variable interactions are important, which is typical in air pollution modeling. Then, we added the CPF statistic to generate the CBPF. The CPF equation is defined as:

$$\mathrm{CPF} = \frac{my}{ny}$$

Equation 1. *The CPF equation used in the GAM to generate the CBPF plot. my is the number of samples in the y bin, which has the wind speed and direction data, and ny is the total number of samples in the same wind vector (Ashbaugh et al., 1985).*

All of the environmental parameters along with the ozone data were used in a predictive Quasi-Poisson regression model to predict ozone concentrations in study zones 1 and 2. We decided upon using a predictive model with the goal of predicting when high, and more hazardous, ozone concentrations would occur based on environmental and ozone data. We used 1-minute data to maximize the amount of data available to the training and test sets. To prepare the data to be used in the model, we turned the continuous wind direction vector data into a categorical variable with three categories: upwind from the ozone source, downwind from the ozone source, and neither upwind nor downwind from the ozone source. These categories encompass ranges of wind direction in degrees that are centered on a specific cardinal wind direction. Upwind is centered around wind coming from the north and has a range of northwest to northeast (281.25° - 56.25°). Downwind is centered around wind coming from the south and has a range of southeast to southwest (101.25° - 236.25°). Neither upwind nor downwind encompasses two wind directions, centered around the east and west, with ranges between northeast to southeast (56.26° - 101.24°) and between southwest to northwest (236.26° - 281.24°), respectively.

Ozone, wind speed, temperature, and relative humidity were kept as continuous variables. Then we checked the data for ozone concentration data outliers in the same way as we did for the Spearman analysis. For both zones, there were outliers that fell within the definition of that parameter. We decided not to remove outliers in the zone 2 because we felt that a guiding purpose of the predictive models was to be able to predict higher and potentially hazardous ozone concentrations and there was only one outlier. Zone 1 had many outliers and we ultimately decided to subset the data into a dataset with ozone concentrations above 50 ppb. The purpose for this was that it was important for us to predict the higher concentrations, as this represented our worst-case scenario. We made the assumption that ozone concentrations above 50 ppb in this study zone would be attributed to the water treatment system and not ambient ozone concentrations, based on regional ozone data during the study period from the WA Ecology Beacon Hill air monitoring location, which did not exceed 42 ppb (0.042 ppm) (Appendix, Figure 4).

To generate and test the predictive model, we created a training set and a test set. The data broke the assumption of independence because the training data was highly correlated to the testing data. The purpose of the training set is to train the model to observe the ozone outcome and the environmental measurements for a set of objects. The test set is used to evaluate the performance of the model's predictions and estimate error rate of the trained model. If the same data is used to test the model that was used to train the model, this would result in a biased predictive model because the test and train data are correlated to themselves. To counteract this, we used a randomized block sampling method to create the training and test set. To employ this method, we created blocks of consecutive data in 5-minute increments. Then, we randomly selected 70% of these blocks for the training set and the remaining blocks for the test set. The R code for this is shown in the Appendix. This reduced but did not completely remove the correlation between the training and test data by ensuring the same time blocks were not in both data sets.

The model provides insight on how the environmental parameters, particularly wind speed and direction, influence predicted ozone concentration and help to understand what types of environmental conditions may result in higher ozone exposures. The Quasi-Poisson regression is a generalization of the Poisson regression and is used when modelling an over-dispersed count

variable. This type of regression also assumes that the variance is a linear function of the mean, and not equal to the mean. To evaluate the fit of the model, we compare R-squared values and residual deviance. The coefficients of the regression models were interpreted by comparing how the estimated log of the mean ozone concentration, λ, changes as the environmental parameters increase by one unit and then we can convert the ozone concentration back to original units. By taking the ratio of the two equations, one for a given value of a predictor variable (x) and one after increasing that variable by one unit ($x + 1$), we can determine that exponentiating the coefficient on the predictor variable provides the multiplicative factor by which the mean ozone concentration changes (J. Legler & Roback, 2021):

$$log(\lambda X) = \beta 0 + \beta 1 X$$
$$log(\lambda X + 1) = \beta 0 + \beta 1 (X + 1)$$
$$log(\lambda X + 1) - log(\lambda X) = \beta 1$$
$$log\left(\frac{\lambda X + 1}{\lambda X}\right) = \beta 1$$
$$\frac{\lambda X + 1}{\lambda X} = e^{\beta 1}$$

Equation 2*. Calculating the multiplicative factor by which ozone concentration changes when a predictor increases by 1 unit from the coefficients of the regression model.*

Aim 3 of this study identified and characterized peak ozone concentrations and the environmental conditions with which they were associated. Understanding the amount of ozone that the water treatment system is capable of off-gassing will provide insight into potential exposure scenarios for the marine mammal workers. Taking the environmental conditions surrounding these peaks into consideration will allow us to make observations and provide recommendations on how to safely work around the ozone source.

Results

After the data collection was complete, we re-tested the GFG's calibration and found it to be within -10% to +20% accuracy of the original calibration concentrations (GfG Instrumentation, 2019). These values fell within the accuracy range defined for the GFG ozone sensor, so no adjustments were made to the data (GfG Instrumentation, 2019). The data from this calibration check is shown in Table 3.

Table 3. *Calibration data from re-checking the GFG one month after data collection was performed.*

Ozone generator concentration(ppb)	**GFG concentration (ppb)**	**Percent change (% error)**
0	0	0
100	120	+20%
200	210	+5%
400	400	0
800	780	-2.5%

Aim 1 – Differences among zones

Descriptive statistics

Table 1 shows the summary statistics for each of the study zones for the 1-minute averaged data and Table 2 shows the summary statistics for 15-minute averaged data. The highest concentrations of ozone were observed in zone 1. This was expected because the sensor was in closest proximity to the ozone source in this zone. A boxplot of ozone concentrations among the three zones demonstrates the variability in the ozone concentrations within and among each zone (Figure 2).

Zone 3 provided referent data on background ozone levels at the study site. Ultimately, the GFG did not detect any ozone in zone 3 and resulted in a consistent 0 ppb background level, therefore the data in this zone is not used in any statistical analysis performed and the data from the other zones was not adjusted as the background ambient ozone was assumed to be negligible.

Table 4. *Summary statistics of ozone concentrations among each zone using 1-min data.*

Zone	N	Mean (ppb)	Median (ppb)	Std. Dev (ppb)	Range (min, max)	IQR
1	2432	27.0	10	46.0	(0, 810)	40
2	2456	6.43	0	11.4	(0, 60)	10
3	2460	0	0	0	(0,0)	0

Table 5. *Summary statistics of ozone concentrations among each zone using 15- min TWA data*

Zone	N	Mean (ppb)	Median (ppb)	Std. Dev	Range (Min, Max)	IQR
1	167	26.6	20.7	21.8	(0, 122)	33.7
2	168	6.62	0	11.0	(0, 40)	10.7
3	169	0	0	0	(0,0)	0

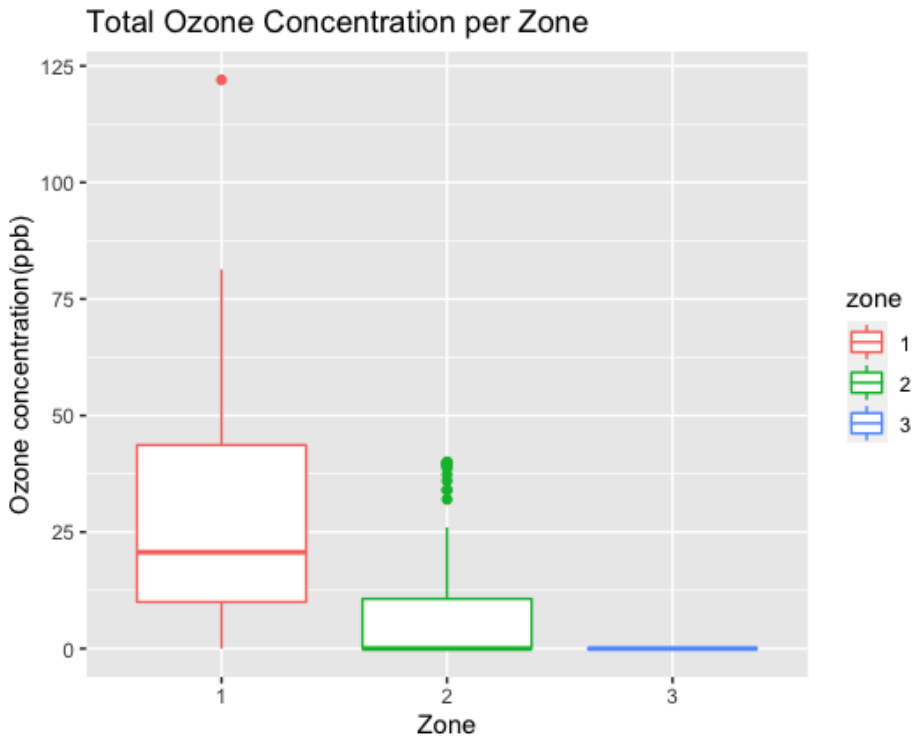

Figure 2. *Boxplot of ozone concentration in each zone using 15-minute averaged ozone data*

Inferential Statistics

The Mann-Whitney U test was used to test the null hypothesis of this aim that there is no statistical difference in ozone concentration between Zones 1 and 2. Results of this test showed that ozone concentration in Zone 1 is significantly higher compared to concentrations in Zone 2 (p-value < 2.2×10^{-16}).

Aim 2 – Assessing the effect of environmental conditions

Descriptive statistics

Part A of Aim 2 was to assess the potential relationship between temperature and ozone and relative humidity and ozone. A table showing mean, median, standard deviation, range, and sample sizes of temperature, relative humidity, and wind speed are shown in Table 6. Averaging wind direction can be complicated because wind direction is a vector. Wind direction is visually summarized in the wind roses shown in Figures 3 through 5. Wind roses show the frequency of each wind direction in the dataset along with the wind speeds associated with these wind directions. These are useful for summarizing wind behavior and understanding prevailing wind directions in each zone.

Table 6. *Summary statistics of temperature, relative humidity, and wind speed using 1-minute data*

Zone*	Environmental parameter	N	Mean	Median	Std. Dev	Range (min, max)	IQR
1	Temperature (F)	2432	58.5	57.2	5.5	(47.8, 71.1)	6.15
	Relative humidity (%)		70.9	78	17.9	(35, 97)	24
	Wind speed (mph)		2.4	1.8	2.4	(0, 12.8)	3.4
2	Temperature (F)	2460	55.4	53.6	6.6	(39, 68.9)	10.8
	Relative humidity (%)		72.9	70	14.2	(46, 96)	26
	Wind speed (mph)		1.8	0.9	2.0	(0, 9.2)	2.5
3	Temperature (F)	2460	54.4	55	3.5	(42.6, 60.8)	5
	Relative humidity (%)		77.6	79	15.4	(40, 99)	16
	Wind speed (mph)		3.3	3.1	2.2	(0, 17.2)	2.7

* Note that the weather station remained at the same location throughout the study period. Thus, the data summarized in this table represent local weather conditions at the site, during the time periods that the ozone sensor was in each of zone 1, zone 2, and zone 3.

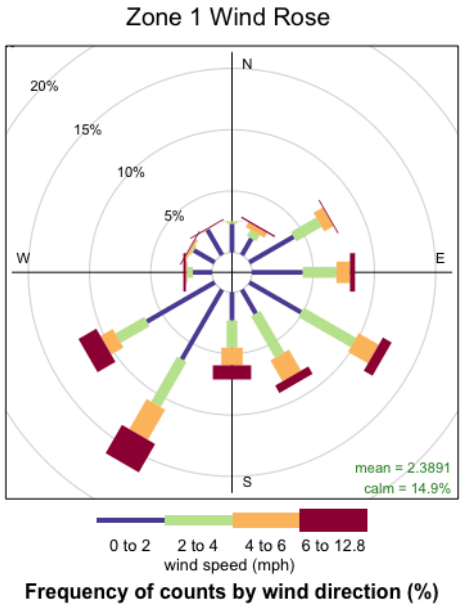

Figure 3. Wind rose for all 1-minute data when ozone sensor was in zone 1

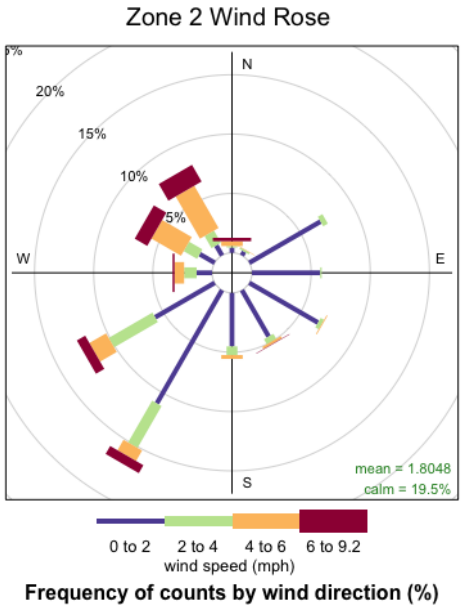

Figure 4. *Wind rose for all 1-minute data when ozone sensor was in zone 2*

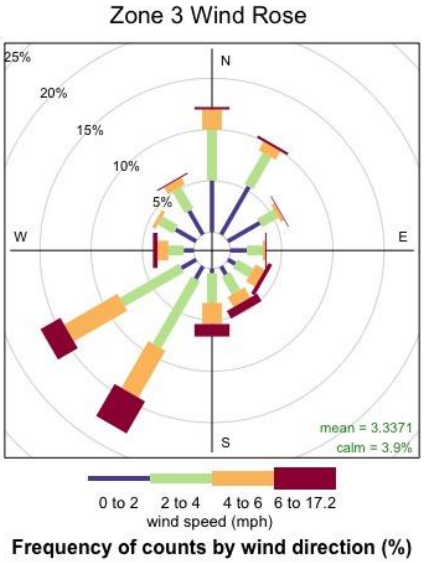

Figure 5. *Wind rose for all 1-minute data when ozone sensor was in zone 3*

Ozone and temperature, ozone and relative humidity

Spearman Rank-Order Correlation was used to determine the strength and direction of association between ozone and temperature and ozone and relative humidity. In zone 1, ozone and relative humidity (Figure 6) had a weak negative correlation (rho = -0.12, p-value = 0.13) and ozone and temperature (Figure 7) had essentially no relationship (rho = -0.01, p-value = 0.90). Both rho values in zone 1 were not statistically significant. For zone 2, the correlation coefficient (rho) of ozone and relative humidity (Figure 8) showed a negative relationship (rho = -0.73, p-value < 2.2×10^{-16}) and ozone and temperature (Figure 9) had a positive correlation (rho

= 0.59, p-value = 1.00 x 10^{-16}). These relationships were visually assessed to determine linearity of the relationship as well.

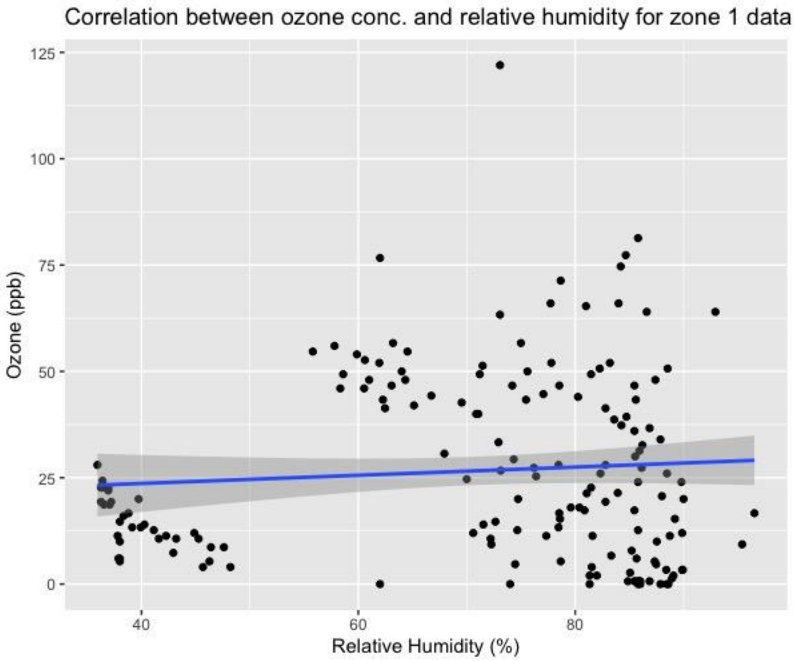

Figure 6*. Scatterplot of relative humidity versus ozone concentration in zone 1*

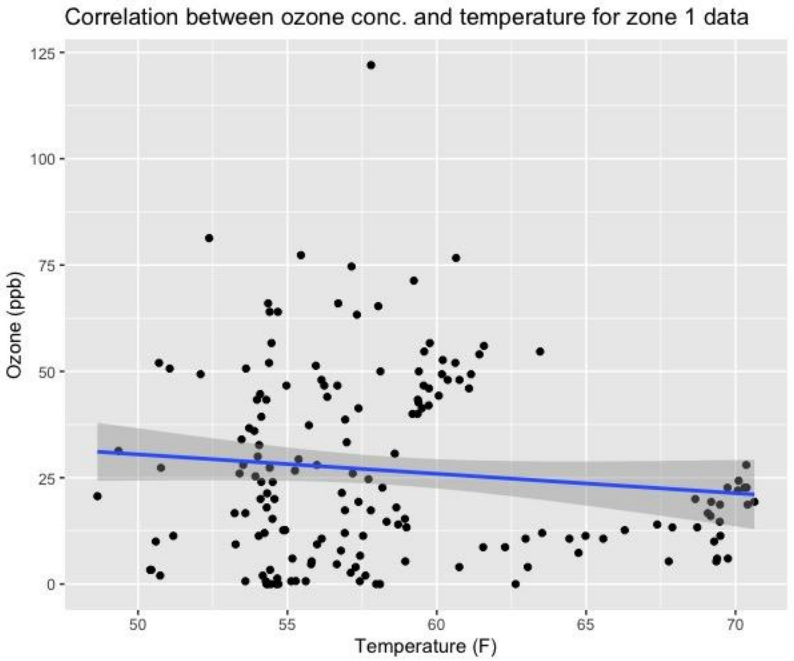

Figure 7*. Scatterplot of temperature versus ozone concentration in zone 1*

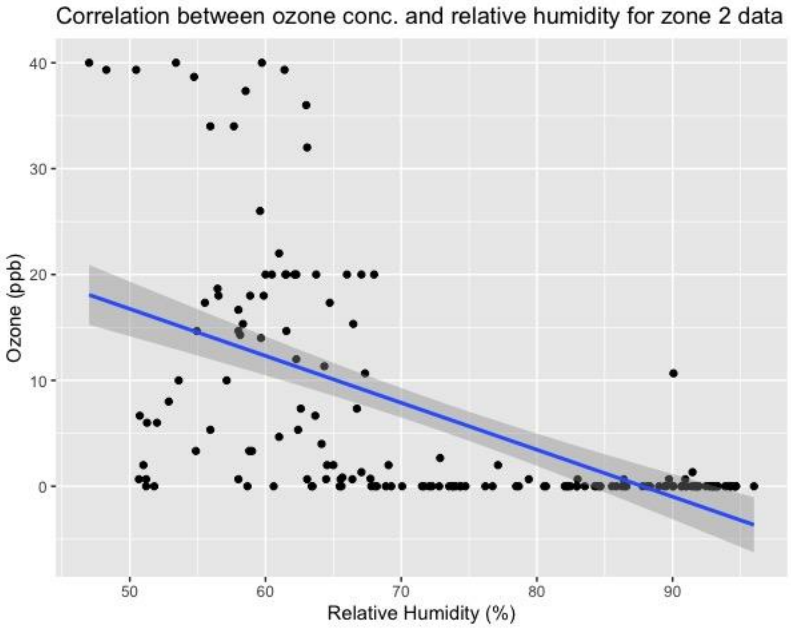

Figure 8. *Scatterplot of relative humidity versus ozone concentration in zone 2*

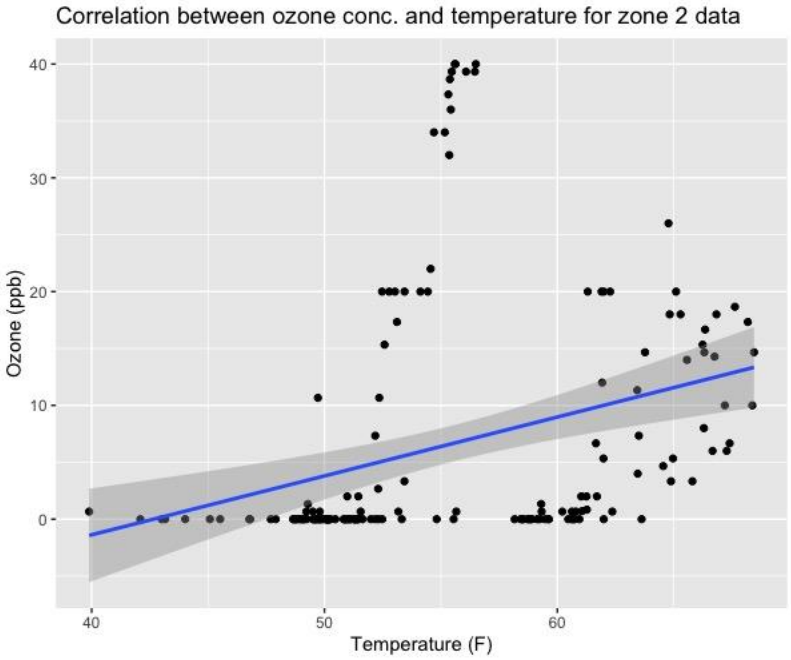

Figure 9. *Scatterplot of temperature versus ozone concentration in zone 2*

Ozone and wind speed, wind direction

In part B of Aim 2, we sought to understand the relationship between ozone concentration and wind speed and direction. It is important to note that the ozone sensor was located north of the ozone contact tower in zones 1 and 2. Therefore, upwind is considered to be wind coming from the north and downwind is considered to be wind coming from the south. The CBPF plots used to show potential sources of influence on ozone concentration are shown in Figures 10 and 11.

In our CBPF plots, we are focusing on wind behavior circumstances where ozone concentration will be in the 90th percentile for that zone. This is to understand the wind behavior parameters that may influence ozone to reach its highest concentrations or peak exposure scenarios and whether there are other O_3 sources contributing to the concentrations in Zones 1 and 2.

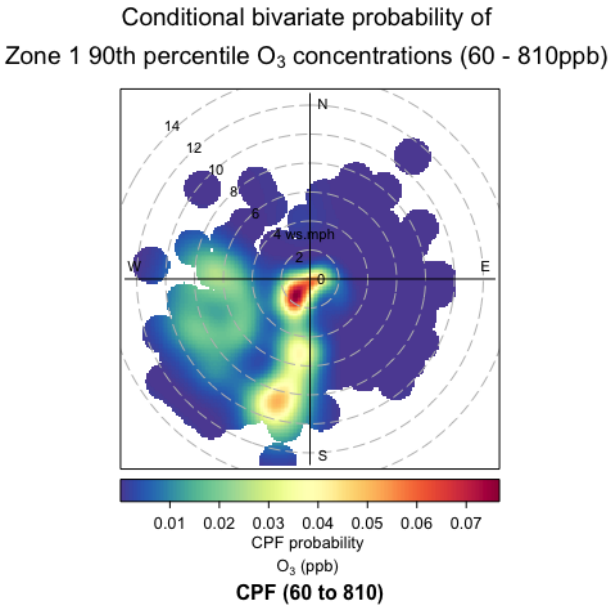

Figure 10. *Conditional bivariate probability plot showing likelihood of ozone concentration reaching 90th percentile under certain wind conditions in zone 1. Blue is a low probability, Red is a high probability.*

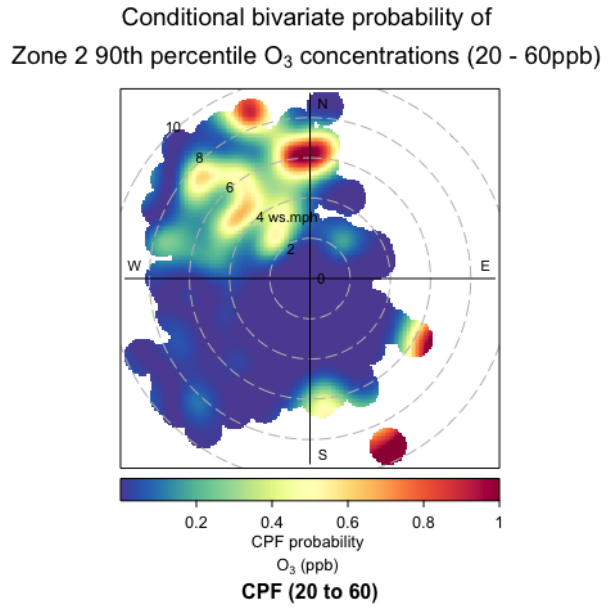

Figure 11. *Conditional bivariate probability plot showing likelihood of ozone concentration reaching 90th percentile under certain wind conditions in zone 2. Blue is a low probability, Red is a high probability.*

We also utilized a Quasi-Poisson model to fit the data and predict ozone concentrations using wind speed, wind direction, temperature, and relative humidity as the predictors and ozone concentration as the outcome. Quasi-Poisson models assume the data is non-normal and the variance is greater than the mean. We visualized the data in density plots (Figures 12 and 13) and determined that this type of model would work best for our data as it is right-skewed, and the variance is greater than the mean.

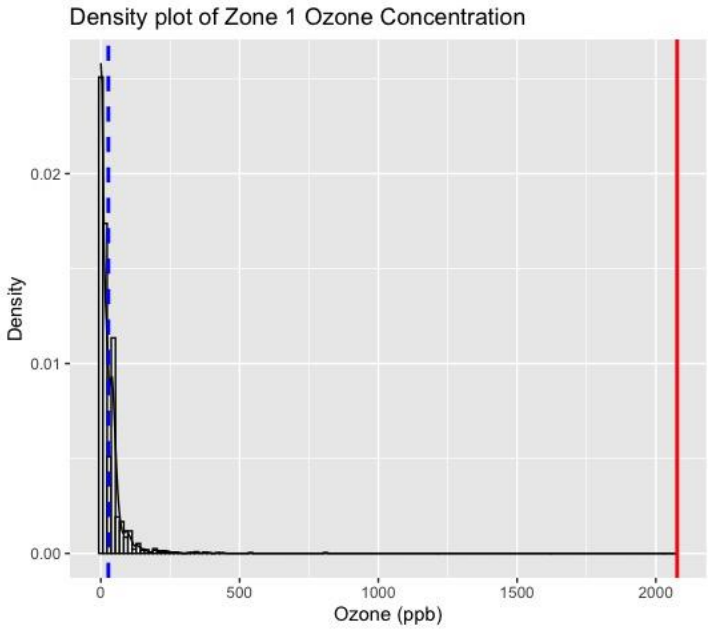

Figure 12. *Density plot of ozone concentration in zone 1. The dashed blue line represents the mean of the data, and the red solid line represents the variance.*

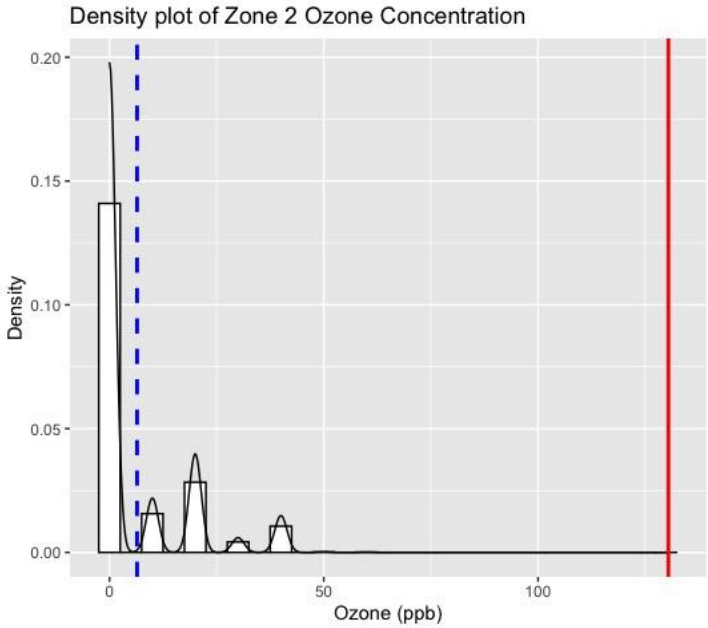

Figure 13. *Density plot of ozone concentration in zone 2. The dashed blue line represents the mean of the data, and the red solid line represents the variance.*

After determining that the Quasi-Poisson would be the best fit, we fit our data and performed the model analysis using the 1-minute averaged data. The regression outputs are summarized in Tables 7 and 8. The predictive models are shown in Figures 14 and 15. We assessed the fit of the regressions using r-squared and residual deviance. The zone 2 prediction model had an r-squared of 0.567 and the zone 1 model had an r-squared of 0.157. Residual plots for these models are in the Appendix.

Table 7*. Quasi-Poisson regression output for zone 1.*

Variable	Coefficient	Std. Error	P-value
Intercept	2.53	1.33	0.06
Temperature	0.03	0.02	0.05
Relative humidity	0.01	0.01	0.23
Wind speed	-0.10***	0.02	0.00
Downwind	-0.31**	0.11	0.01
Neither upwind/downwind	0.04	0.12	0.75

*,**,*** indicates significance at 90%, 95%, and 99% level, respectively

Table 8*. Quasi-Poisson regression output for zone 2.*

Variable	Coefficient	Std. Error	P-value
Intercept	0.91	1.40	0.52
Temperature	0.08***	0.02	2.42E-06
Relative humidity	-0.06***	0.01	9.08E-11
Wind speed	0.30***	0.03	< 2E-16
Downwind	-1.10***	0.23	1.57E-06
Neither upwind/downwind	-1.25***	0.19	2.15E-10

*,**,*** indicates significance at 90%, 95%, and 99% level, respectively

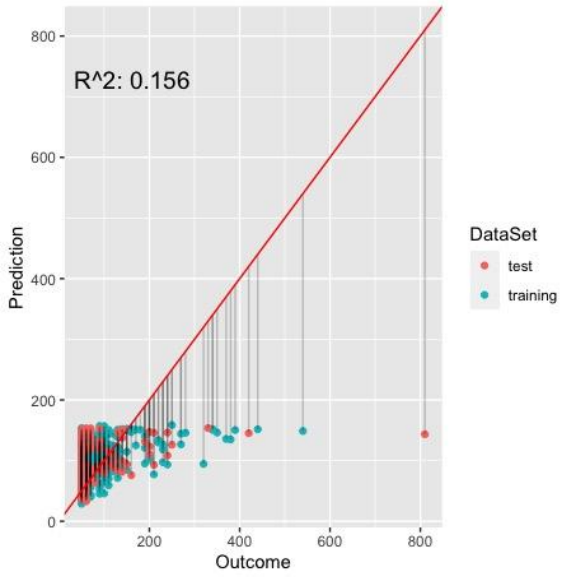

Figure 15. *Plot of ozone concentration outcome versus prediction in zone 1.*

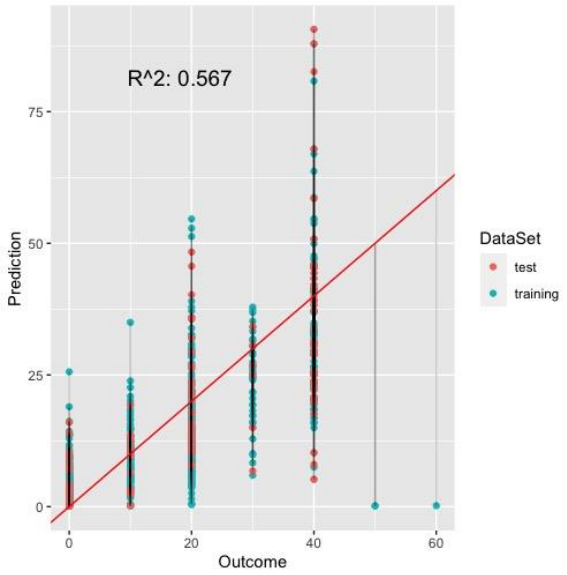

Figure 14*. Plot of ozone concentration outcome versus prediction in zone 2.*

Aim 3 – Characterizing peak exposures

Descriptive Statistics

The purpose of the third aim of this study was to characterize peak ozone concentrations and the environmental conditions with which they are associated. Peak exposures in this study are defined as high magnitude zone concentrations at or above 300 ppb. This is based off of the WA L&I Ceiling limit (300 ppb) and the study by Hennenberger et al. (2005) where peak exposures of 300 ppb and above were associated with asthma symptoms in pulp mill workers. Zone 1 had multiple instances of exceeding these limits in the 1-minute averaged data (Table 11).

Table 7. *1-minute averaged Ozone concentrations exceeding recommended and permissible exposure limits with the environmental conditions at the time of the peak exposures.*

Zone	Date	Time	Ozone (ppb)	Temp. (F)	Relative humidity (%)	Wind speed (mph)	Wind direction
1	10/19/21	9:50 AM	320	54.1	75	0	SE
	10/19/21	10:55 AM	380	54	76	0	W
	10/19/21	3:40 PM	420	57.7	72	0.2	W
	10/19/21	3:49 PM	540	57.4	74	0	E
	10/19/21	3:50 PM	810	57.4	74	0	NE
	10/22/21	9:34 AM	330	54.3	93	0	E
	10/22/21	9:49 AM	390	55.6	89	0	NE
	10/23/21	10:51 AM	370	52.3	85	0	E
	10/23/21	12:42 PM	350	54.7	87	0.2	E
	10/23/21	12:50 PM	340	56.5	80	0	E
	10/23/21	1:37 PM	440	55.6	85	0	E
	10/23/21	2:38 PM	340	57.2	83	0	N

Discussion

Overall, the results of our analyses provided us greater insight into the behavior of ozone at this study site and considerations to keep in mind when discussing recommendations for reducing the impact of occupational ozone exposure at SR^3. Environmental conditions had differing influences on the ozone concentrations in the two study zones we analyzed and were not able to explain all the variability in ozone concentrations. These conditions likely do not play a role in modulating the amount of ozone being produced by the water treatment system, but they could have a role in creating conditions where ozone can accumulate in the surrounding area and reach levels of concern. Also, there were some additional ozone sources that contributed to the concentrations at the study site of which we were previously not aware. These findings prompt a consideration of other parameters and nearby anthropogenic activities that may modulate the behavior of ozone and why the effect of the environmental parameters of interest were not able to explain more of the variability of the ozone seen at the study site.

Findings from Aim 1

Our hypothesis from aim 1 was that ozone levels would differ between each zone. The results of the Mann-Whitney test allowed us to reject the null hypothesis and, as hypothesized, ozone concentrations were significantly different among each zone. Zone 1 represented our "worst-case exposure scenario" and this zone had the highest average ozone concentrations and the greatest maximum ozone concentration. These high concentrations were expected in this zone because the ozone detector was closest to the ozone source in this study zone. At the study site, we did observe workers and employees occasionally having to get near the ozone source to troubleshoot the water treatment system or to get behind the system or underneath the pool deck to reach certain components, so there is a possibility that a worker could be exposed to ozone in these situations. The overall highest one-minute average concentration of ozone in Zone 1 was 810 ppb and the highest 15-minute averaged concentration was 122 ppb. Both of these concentrations exceeded the Ceiling limits recommended by NIOSH (100 ppb) and the 1-minute peak ozone concentration exceeded the ceiling limit set by Washington L&I (300 ppb) as well. These peak exposures are further discussed below.

In Zone 2, average and maximum ozone concentrations were a fraction of the levels measured in zone 1, and in zone 3 there was no ozone detected. Zone 2, which is the pool deck where employees undertake a variety of tasks, represents a more typical ozone exposure scenario. During the time period when our sensor was in zone 2 ozone concentrations in this zone never exceeded any permissible or recommended exposure limits. The maximum 1-minute ozone concentration was 60 ppb, and the maximum 15-minute averaged concentration was 40 ppb. While these concentrations do not exceed any permissible or recommended exposure limits, it is still possible that exposure could cause some irritation to the respiratory tract and eyes and possibly further health effects in more sensitive individuals such as those with asthma or other respiratory diseases. It is also possible that ozone concentrations can exceed the amounts we measured, as we only monitored for a brief period of time. A worker has also reported that they can smell the ozone occasionally when walking in the vicinity of the aquaria, and while odor threshold can be subjective based on an individual's sensitivity to odors, the odor threshold for ozone has been found to be as low as 15 to 20 ppb (Cain et al., 2007). While this is not an excessive level of ozone, for sensitive individuals it could be enough to exacerbate asthma or

cause mild to moderate symptoms of acute exposure (Lee et al., 1989). The worker's observation also suggests that there is a possibility of exposure in areas that we did not monitor in our study.

Findings from Aim 2

Part A

Results from Aim 2 support our hypothesis that there is a relationship between ozone and temperature and relative humidity – at least for zone 2. The strength of the relationships in zone 2 were moderate. These correlations were not observed in zone 1. The environmental factors themselves are highly correlated, such as the strong influence of temperature on relative humidity. Thus, the relationship between temperature and ground-level ozone is significantly influenced by relative humidity and this correlation can be biased (Chen et al., 2020).

While these factors are highly correlated, we can still glean some valuable information from comparing the Spearman Correlation results for each zone. Relationships between ozone and temperature or humidity were much stronger in Zone 2 when compared to Zone 1. This is likely because in Zone 1, the ozone detector was close enough to the ozone source that temperature and humidity did not play much of a role in the amount of ozone detected by the sensor. We did not gather data on the operation of the water treatment system (e.g. when it was off-gassing, or the amount of pool water moving into the system), which would likely have a stronger relationship with ozone concentrations in this zone. The manuals for the ozone systems state that ozone is generated and injected into the contact basin at constant levels, but perhaps if the burden of waste or microorganisms in the pool water is low there might be more excess ozone that did not react and therefore will lead to more ozone being off gassed (µplasmaO_3 Operation Manual, 2022).

Zone 2 was further away from the ozone sources, so these environmental conditions may have played a bigger role in affecting the amount of ozone that reached the detector on the pool deck (e.g due to dilution of the ozone plume emitted from the water treatment system or scavenging of the ozone emitted from the water treatment system by atmospheric processes). Alternatively, ambient ozone levels measured in Zone 2 may have been affected by temperature and relative humidity and contributed to the concentration of ozone measured on the pool deck (zone 2). Temperature and humidity can influence the half-life of ozone by decreasing it. This has been observed in closed chamber conditions, where the half-life time of ozone decreased under high temperatures and humidity conditions of 80% (McClurkin et al., 2013).

Part B

A Quasi-Poisson regression model was developed to provide predictions of ozone concentrations given all of the environmental parameters: temperature, humidity, wind speed, and wind direction. Using this predictive model, we were able to better understand the affect that wind behavior has on ozone concentrations in Zone 2 primarily - our model in Zone 1 did not fit the data well enough to explain the variability of the ozone concentrations or provide reliable predictions (r-squared = 0.16). By exponentiating the coefficients, we obtained the multiplicative factor by which the mean ozone concentration changes in response to a one unit change in a predictor variable (Equation 1).

Our hypotheses for this portion of Aim 2 were that, in Zone 1, ozone would also increase when wind direction changes from upwind to downwind and that ozone would be higher at lower wind speeds. We hypothesized that ozone would be higher at lower wind speeds in this zone because the detector was close to the source and at low or calm wind conditions, ozone would have the opportunity to accumulate in this area.

While the predictive model for the zone 1 data did not fit the data, we can still gain insight into the conditions surrounding the variability of the ozone to an extent by discussing why the model was not a good fit and exploring the coefficients that were statistically significant—wind speed and wind direction (downwind of the source). This model predicts that ozone decreases by 27% when wind direction changes from the sensor being upwind of the source to the sensor being downwind of the source. The model also predicts that ozone decreases by 10% when wind speed increases by 1 mph. It is unexpected that ozone would decrease when the sensor is downwind of the source. Perhaps the directional change from the sensor being upwind of the source to being downwind of the source also reflects a simultaneous increase in wind speed which pushes the ozone away from the GFG. For many pollutants, increasing wind speed generally results in lower concentrations due to the dilution effect (Camalier et al., 2007). This also supports the model's prediction that ozone will decrease when wind speed increases.

This model only used data where the ozone concentration was above 50 ppb, which we assumed would be concentrations attributable to the water treatment system. The residual plot for this zone demonstrated a considerable amount of variability that the model did not explain, which resulted in the poor fit of the model (Appendix Figure 5). Perhaps in this zone, the environmental conditions do not play a significant role in the ozone concentrations and do not have enough variability to account for the variability in the ozone. The act of the water treatment system working to disinfect the water and off-gas ozone is likely the biggest influence on the ozone concentrations seen here. In the future, it may be of interest to add a model variable that represents an aspect of the water treatment system. Taking the water treatment system into account with this model could explain the variability that the environmental parameters cannot. This zone is where the ozone concentrations exceeded recommended and permissible exposure limits on multiple occasions, so understanding the conditions surrounding these peak exposures is important in preventing occupational illness or injury as a result of exposures of this magnitude.

Looking at the CBPF for this zone (Figure 10), it appears that the dominant ozone source likely contributing to the higher concentrations (60 – 810 ppb) is the ozone contact tower. This is reflected by the red area that is dependent on calm wind and very low wind speeds which aligns with our models' prediction that ozone decreases when wind speed increases. The CBPF also shows that there is a lesser probability of these high concentrations with winds from the southwest. The plot does not imply that there are contributing sources from the north which is upwind of the ozone contact tower. This is interesting because the predictive Quasi-Poisson model output determined that ozone would decrease when the GFG was downwind relative to the ozone contact tower (southerly winds). In the wind rose for zone 1, the frequency of winds from the north were much lower compared to the frequency of winds from the southwest, south, and southeast. Perhaps the model data did not have enough wind direction data from the north to provide an accurate prediction regarding wind direction's influence on ozone.

In Zone 2, we hypothesized that ozone concentration will increase when the ozone detector is downwind of the ozone sources and when wind speed increases. Using our model to predict ozone behavior in Zone 2 resulted in some unexpected findings. The model predicts that ozone decreases when wind speed increases and when wind direction changes from the sensor being upwind of the source to being downwind of the source. Looking into this further using a CBPF (Figure 11), it appears that there are some unknown ozone sources that are north and northwest of Zone 2, where the CBPF shows that there is a high probability of ozone concentrations reaching 90^{th} percentile concentrations when wind speed is 6 mph from the north and 9 mph from the northwest as well as 9 mph from the southwest and 6 mph from the southeast. The CBPF plots show that these ozone sources are dependent on different wind speeds which shows that they are likely to be from separate sources. The source from the southeast is likely from the larger ozone contact tower, which was southeast relative to the GFG. Sources that are close by tend to be associated with lower wind speeds than sources that are further away (Uria-Tellaetxe & Carslaw, 2014). In a model without the wind direction parameter, the R-squared was 0.468. This demonstrates that the inclusion of the wind direction parameter does help the model attempt to explain the variability in the ozone. But, even with the wind direction variable there is some variability that is not described by the environmental parameters. The role of wind speed in ozone concentrations that were predicted by the model suggested that ozone would decrease when wind speed increased. Under calm wind conditions, ozone would have the opportunity to accumulate in the area surrounding the water treatment system and subsequently, the pool deck. When wind speed picks up, it may push the ozone further away from the sensor or dilute the ozone which could explain this model's prediction. The residual plot for zone 2 did demonstrate some variability that was not explained by the models which may support this explanation that the unknown ozone source played a larger role in the ozone concentrations at the site than expected (Appendix, Figure 6).

A possible explanation for the ozone hotspots dependent on higher wind speeds could be localized ambient ozone in that area, or perhaps another point source. The study site is located within the parking lot of a marina on the Puget Sound, where many boats are docked, and it is also near a busy street. Nearby, the study site is flanked by the water on the west/northwest and the road, located to the east and south of the study site. For the area to the south, car activity may provide the necessary precursors to ozone production (Placet, et al., 2000). From the west and north, boat activity may provide the necessary emissions to produce precursors to ambient ozone. Salts in ocean mists may accelerate the formation of ambient ozone by reacting with NO_x from ship emissions to form a highly reactive ozone precursor, nitryl chloride, which readily participates in the photochemical reaction to form ambient ozone (Potera, 2008). The majority of the boats docked at the Des Moines marina are recreational, which have been found to contribute to local NO_x concentrations (van der Zee et al., 2012). While the Puget Sound is not an ocean, it does contain salinity at about 28.5 parts per thousand (ppt) which is close to the salinity of the Northern Pacific Ocean, at 32 ppt (Banas, 2015). The Des Moines marina also has a boat fueling station, located approximately Northwest of the study site, that offers unleaded gasoline, diesel, and propane fuels (Des Moines Marina webpage, 2022) combustion of which does emit NO_x (Alvarez et al., 2008).

Findings from Aim 3

Understanding the context surrounding peak ozone concentrations during this study will provide insight into what types of conditions may play a role in these concentrations and how to ensure that the workers at this site avoid acute, high-magnitude ozone exposures that can result in negative health effects. In the 1-minute data, ozone reached peak exposure levels (>300ppb) on 12 occasions (Median = 375; Range = 810 ppb – 320 ppb). These peaks were limited to zone 1, which is understandable with the circumstances of this worst-case exposure scenario. The prevailing wind direction for these peak exposures came from the East but the wind speed was calm during these exposure scenarios (Median = 0 mph; Range = 0-0.2 mph), therefore wind direction likely did not play a significant role in modulating the ozone concentrations. Temperature was relatively stable (Range = 54 – 57.7F) and relative humidity was quite high (Range = 72-93) during these peaks. At low temperatures, the overall energy of the ozone molecule is lowered which results in resonance stabilization (Batakliev et al., 2014). This stabilization gives it some resistance against decomposition, which may play a role in preventing the off-gassed ozone from rapidly dissociating into oxygen and allowing it to accumulate in the area when there are calm winds, contributing to these peak exposures (Batakliev et al., 2014). Ozone is thermally stable in temperatures up to 482F (Dhandapani & Oyama, 1997). At temperatures below this, rapid decomposition of large amounts of ozone requires a catalyst, which is what is used in ozone destruction components of water ozonation systems (Dhandapani & Oyama, 1997). Humidity has been found to have no discernible effect on the decomposition of ozone in the presence of a catalyst (Mehandjiev & Naidenov, 1992; Batakliev et al., 2014). At the hospital, the water treatment system does not utilize an ozone destruction component. If humidity does not modulate ozone dissociation with the assistance of a catalyst, then it likely would not contribute to ozone destruction at this location. Five of the peak exposure instances occurred in the morning between 9:34 AM and 10:55 AM and the rest occurred throughout transiently throughout the afternoon. The clustering of high magnitude exposures in the mornings is interesting and unexpected. More data would be necessary to determine whether this a pattern and whether it could provide insight on a causative factor behind the peak exposures.

The brevity of these 1-minute peak exposures reduces the probability of a worker being in that area in such close proximity to the ozone tower at the exact time to receive that dose of ozone. But it is still a concern because if a worker did happen to be exposed to an acute ozone concentration of that magnitude, that worker would very likely experience the acute effects described previously. While many acute effects are reversible, repeated peak exposures can result in chronic conditions in previously healthy individuals such as the development of asthma, as seen in pulp mill workers after repeated peak exposures of 300 ppb (Hennenberger 2005).

Limitations of the study

This study had several limitations that should be addressed in future work. This study measured the ozone concentration in the workplace environment, which may not be an accurate representation of individual worker's ozone exposures. Future studies should consider undertaking personal exposure monitoring on the workers.

This study relied on measurements from one marine mammal hospital, which may not be representative of occupational ozone exposure at other marine mammal hospitals. Future studies should recruit additional marine mammal hospitals, in order to evaluate the generalizability of the findings from the current study.

While we did take data to represent the background ozone conditions on the site in zone 3, perhaps the regional ozone was lower that week of data collection or the ozone reacted with the plastic surfaces within zone 3 (Appendix, Image 3), artificially lowering the ambient ozone concentration at this location. Therefore, our background data may not have reflected true background ozone concentrations.

Time and budget constraints made it such that only 5, 8-hour days of data could be collected for each zone. Also, the data collection was not randomized, each zone's data was collected in 5 consecutive days because it was nearing the end of the season and SR^3 were preparing to turn off the water treatment system when animal levels dwindled so we prioritized completion of the Zones close to the water treatment system and this might have introduced some bias. There was only one direct reading device, therefore we could not simultaneously monitor every zone. Finally, the training and test data used in the model were autocorrelated and while we did attempt to account for this in the predictive model, this may still result in reduced precision of these results.

Recommendations for the workplace

The results of this study found that there is potential for harmful exposures above the Ceiling limit specified by WA L&I and above the ceiling exposure limit recommended by NIOSH. These exposures occurred in zone 1, our worst-case exposure scenario, where the GFG was close to the top of the smaller ozone tower. To prevent harmful peak exposures, an ozone destruct device could be installed. This is a more expensive strategy but is a more effective control strategy than the other options because it would help keep ozone concentrations below harmful levels by dissociating the ozone into oxygen (Ozonetech, 2022).

Another strategy would be to set a perimeter extending about two or more feet from around the smaller ozone tower where only authorized persons may enter. I recommend setting this perimeter within the pool deck area that the ozone tower is right next to as well and trying not to store items here, to prevent individuals from getting too close to the ozone source. If the area within that perimeter needs to be accessed, for example to perform maintenance, it would be beneficial to measure the ozone concentration using a monitoring device to ensure it is below levels of concern prior to starting work. Also prior to working in this area, it would be beneficial to perform a job hazard analysis. This is a technique that focuses on identifying hazards associated with a job task before they occur and ideally controlling any uncontrolled hazards that are identified to eliminate them or reduce them to an acceptable level (OSHA, 2002). This will bring awareness to the hazard posed by ozone and will provide an opportunity to create a plan to mitigate it or to at least have a response plan if there is an overexposure during the job task.

Hazard communication would also be beneficial in preventing harmful exposures. This can be done in the volunteer training as well as some signage near the ozone sources to bring awareness to the potential hazard posed by ozone and help people understand how to avoid harmful

exposures and the health risks associated with ozone, especially for those with asthma or other respiratory diseases. If there is a circumstance where an individual must be close to the small ozone tower, such as during maintenance, they should be made aware of the risks through signage and the system should be turned off, if possible, to eliminate the chance of exposure. In addition to the hazard communication methods mentioned previously, an additional item to consider is establishing a respiratory health awareness program (NIOSH CDC, 2022). The goal of this program would be to educate and bring awareness to workers and volunteers about the respiratory hazards associated with this job, particularly with the use of ozone. This is particularly valuable to those with asthma or other respiratory diseases that work or volunteer here, so that they are aware of this hazard and understand that they are at a higher risk of having a more severe reaction to an exposure.

Currently, the strategy controlling ozone concentrations at this site is natural ventilation from the system being outside. If this control is attenuated or removed by enclosing the facility, ozone concentrations will build up to levels that will likely further exceed exposure limits. Awareness should also be brought to any future additions to the hospital that may result in people spending more time close to the ozone contact-basins, such as installing new equipment or storing items nearby that people would have to interact with. There should be care in ensuring that no task or obstacle is making people spend an extended time in the area closest to the ozone contact-basins.

While ozone concentrations did reach levels of concern, these were very brief and sporadic, and they did not occur in the area representing a typical exposure scenario where there was more foot traffic. If the water treatment system remains outside, un-enclosed in the same location, the use of administrative controls to restrict access to the ozone contact towers and hazard communication should be adequate in ensuring workers are not over-exposed.

Conclusions

The use of ozone in water treatment and disinfection processes is a reliable and effective way to disinfect water in marine mammal husbandry. In this study, we performed a risk assessment and quantified the concentration of ozone in three zones of a marine mammal hospital to relate to worker exposures. This study aimed to characterize the ozone concentrations at this site and understand how environmental conditions might modulate ozone. A major goal of this study was to understand the behavior of the ozone coming from the water treatment system, and to use insight from the data to provide recommendations to the marine mammal hospital on how to safely work around the water treatment system. Currently, there are not many studies that have characterized the risk of occupational ozone exposures, and there are even less focusing on an animal husbandry setting. Our results showed that in close proximity to the source, ozone did exceed ceiling exposure limits and further away the concentration was much lower, but if sustained for 8-hours, it would have exceeded a recommended exposure limit regarding a heavy workload. Our results also showed that there seems to be a source of ozone not associated with the water treatment system which may be associated with ambient ozone concentration levels in the region. We found that at locations further away from the water treatment ozone source, temperature and relative humidity had a significantly stronger relationship with ozone and closer to the source, wind speed and direction played a bigger role in determining exposure concentrations. The small sample size of the study was a limitation in understanding correlation between ozone concentrations and environmental parameters. Our study demonstrated the

importance of adequate ventilation in having an ozone-based water treatment system, if this system was indoors or enclosed under the same conditions, ozone would have undeniably reached more consistent, concerning levels.

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Appendix

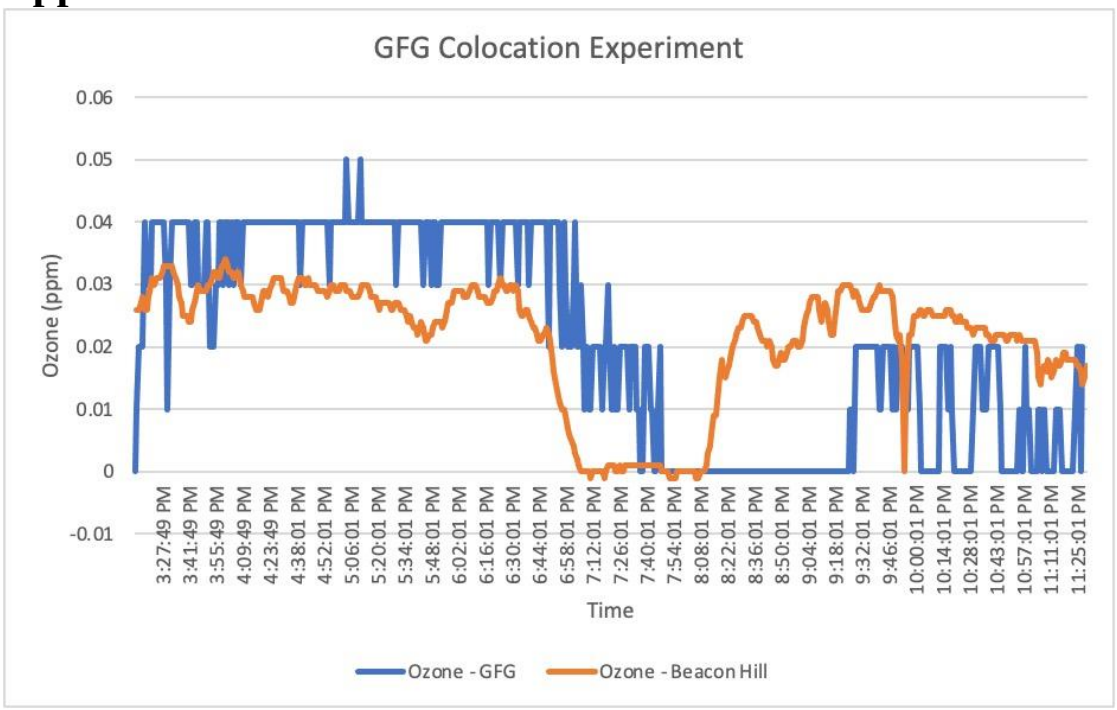

Figure 1. Colocation experiment results. Blue is GFG data and orange is WA Department of Ecology data.

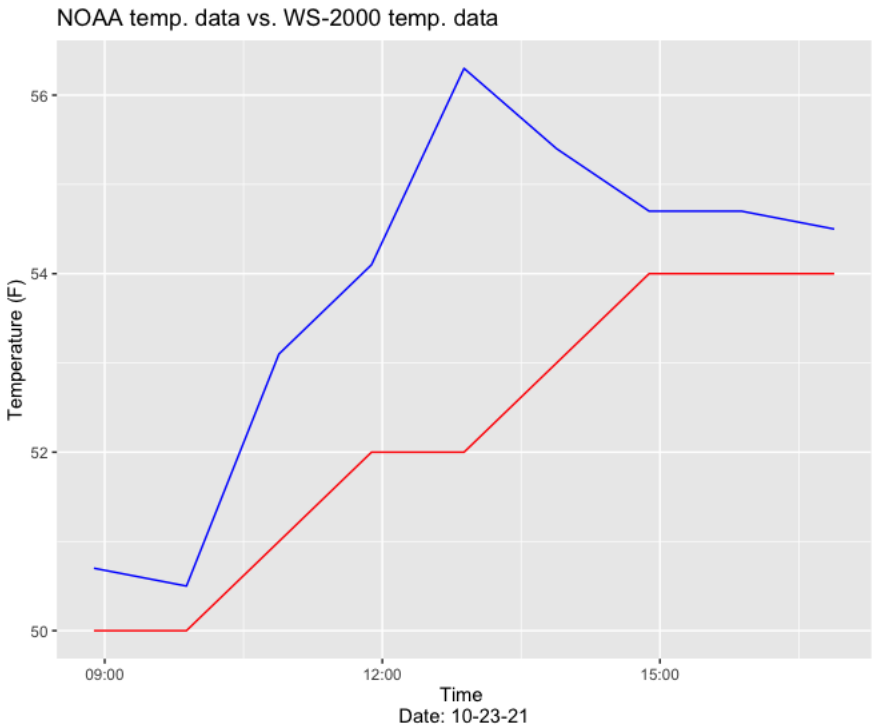

Figure 2. NOAA temperature data versus WS-2000 temperature data. Blue is WS-2000 and red is NOAA.

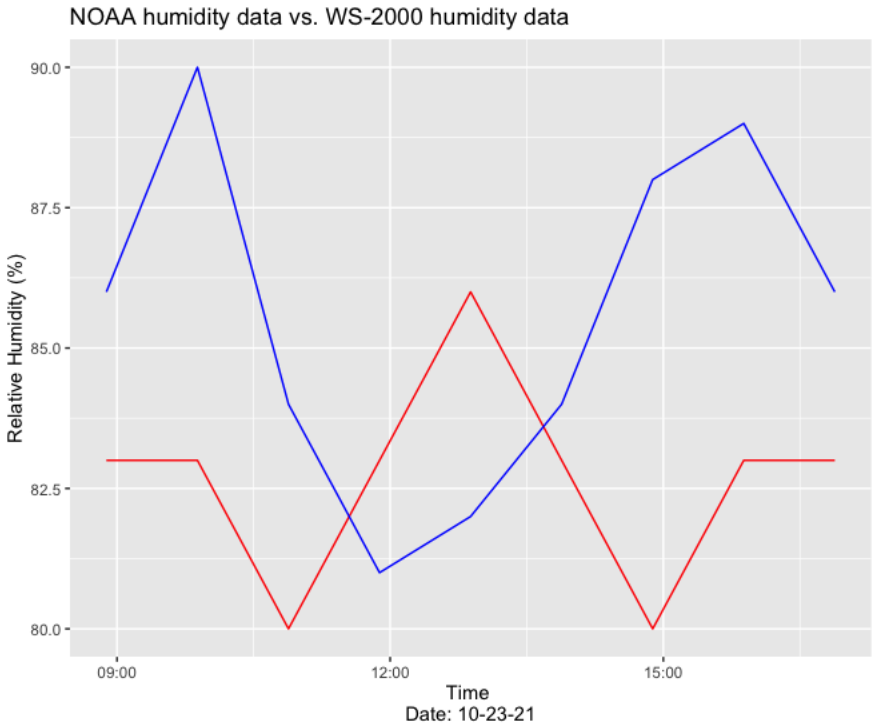

Figure 3. NOAA relative humidity data versus WS-2000 relative humidity data. Blue is WS-2000 and red is NOAA.

Table 1. Table of wind data from NOAA and wind data from WS-2000

Date, time	Wind dir., NOAA	Wind dir., WS-2000	Wind speed (mph), NOAA	Wind speed (mph), WS-2000
10/23/21 8:53	ESE	S	12	2.2
10/23/21 9:53	SE	E	8	1.1
10/23/21 10:53	SE	SE	8	0.9
10/23/21 11:53	S	SE	8	0.9
10/23/21 12:53	SE	S	7	0.2
10/23/21 13:53	SE	N	5	0
10/23/21 15:53	SW	NW	6	0.7
10/23/21 16:53	SW	SW	6	0.2

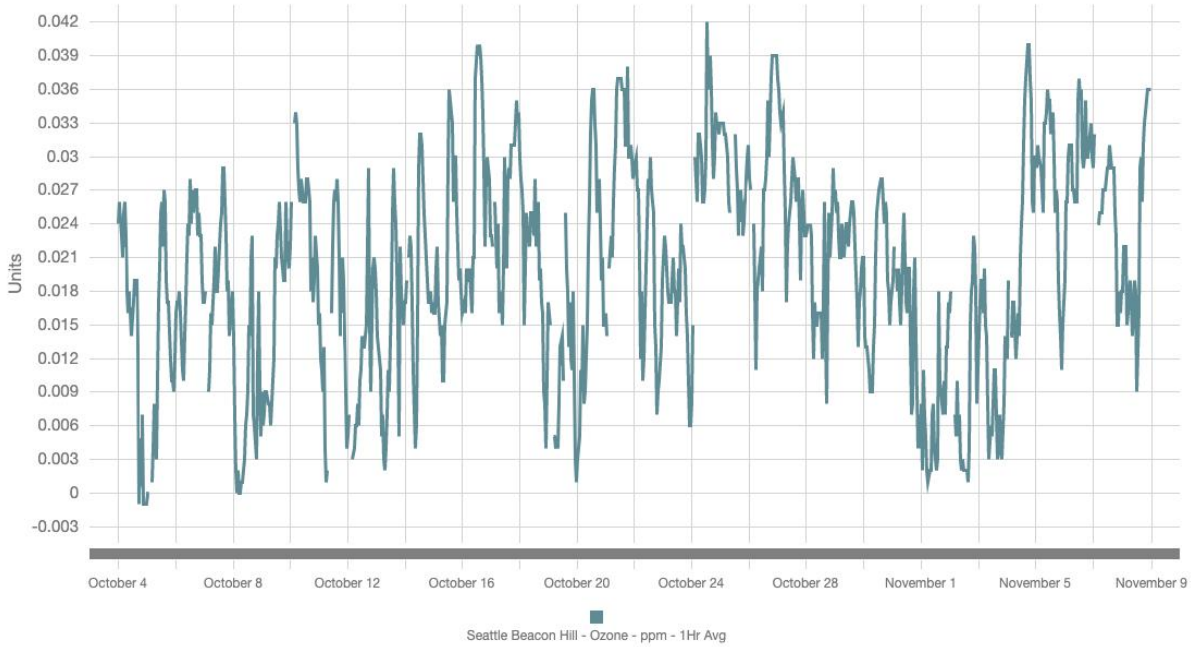

Figure 4. King-county regional Ozone concentration data (ppm) over the period of October and November 2021.

Image 1. Inside of the white tent (zone 3). This tent contains numerous smaller plastic pools for isolating patients which may have reacted with ozone, artificially lowering background levels.

Figure 5. Residual plot of Zone 1 Quasi-Poisson regression

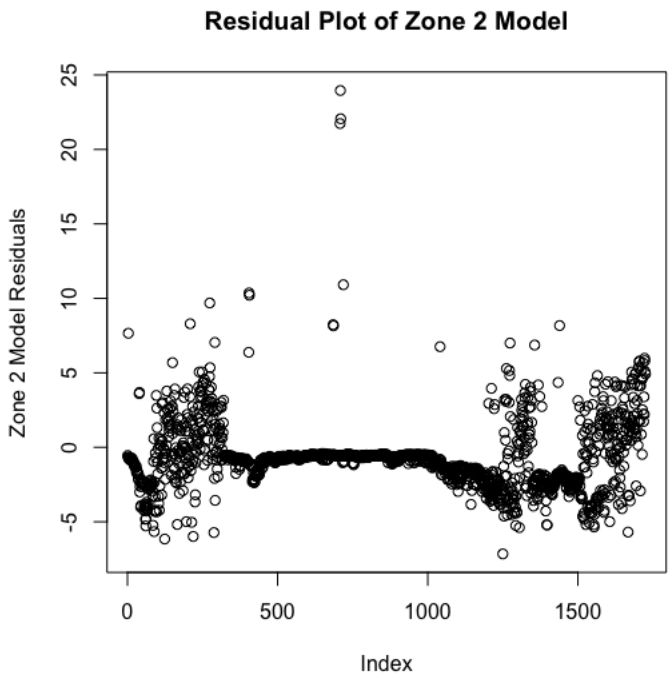

Figure 6. Residual plot of Zone 2 Quasi-Poisson regression

Text 1. Regression output for zone 2 model including wind direction

```
Call:
glm(formula = o3.ppb ~ temp.F + ws.mph + hum, family = "quasipoisson",
    data = modeldata_zone1)


Deviance Residuals:
    Min       1Q   Median       3Q      Max
-6.2565  -1.9010  -0.8421  -0.5215  23.0096

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  6.535242   0.880809   7.420 1.84e-13 ***
temp.F       0.004566   0.010707   0.426     0.67
ws.mph       0.341180   0.022171  15.389  < 2e-16 ***
hum         -0.093143   0.007557 -12.326  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

(Dispersion parameter for quasipoisson family taken to be 29.17655)

    Null deviance: 30180 on 1724 degrees of freedom
Residual deviance: 11205 on 1721 degrees of freedom
  (3 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 7

Text 2. R code for creating training and testing datasets for the predictive model

```
zone1_alldays_TBL[, "5mindate" := floor_date(date, "5 mins")]
set.seed(123)
unique_dates_zone1 <- unique(zone1_alldays_TBL$`5mindate`)
N.train.zone1 <- ceiling(0.7 * length(unique_dates_zone1))
N.test.zone1 <- length(unique_dates_zone1) - N.train.zone1
trainset.zone1 <- sample(seq_len(length(unique_dates_zone1)), N.train.zone1)
testset.zone1 <- setdiff(seq_len(length(unique_dates_zone1)), trainset.zone1)
modeldata_zone1 =
(zone1_alldays_TBL[`5mindate`%in%unique_dates_zone1[trainset.zone1],])
```