

# Microbiological Water Quality of Ko Olina Lagoons

By  
Marek Kirs  
Jaline Sergue

May 2020

Special Report SR-2020-04

PREPARED FOR  
Clean Water Branch  
Hawai'i State Department of Health

WATER RESOURCES RESEARCH CENTER  
University of Hawai'i at Mānoa  
Honolulu, Hawai'i 96822

Cover caption: Aerial view of Ko Olina lagoons, O‘ahu, Hawai‘i by Marek Kirs

# **Microbiological Water Quality of Ko Olina Lagoons**

By  
Marek Kirs  
Jaline Sergue

May 2020

Special Report SR-2020-04

PREPARED FOR  
Clean Water Branch  
Hawai'i State Department of Health

Principal Investigator: Marek Kirs

WATER RESOURCES RESEARCH CENTER  
University of Hawai'i at Mānoa  
Honolulu, Hawai'i 96822

AUTHORS:

Marek Kirs  
Associate Researcher  
Water Resources Researcher Center  
University of Hawai'i at Mānoa  
2540 Dole St., Holmes Hall 283  
Honolulu, Hawai'i 96822  
Tel.: 808/956-8272  
Email: [kirs@hawaii.edu](mailto:kirs@hawaii.edu)

Jaline Sergue  
Research Assistant  
Water Resources Researcher Center  
University of Hawai'i at Mānoa  
2540 Dole St., Holmes Hall 283  
Honolulu, Hawai'i 96822  
Tel.: 808/956-7847

Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the Water Resources Research Center.

## ABSTRACT

The Ko Olina Resort lagoons are extremely popular for recreational activities, however, they have not been extensively monitored for water quality. The objective of this study is to address this lack of water quality information by examining and evaluating the lagoons' microbiological water quality over a one-year period. In 2019 (January 14 to December 11), 128 water samples were collected from the four lagoons (Kohola, Honu, Nai'a, and Ulua). The results indicated generally good water quality as (1) enterococci were not detected in a large proportion of the samples (88%), and (2) only one sample exceeded the Hawai'i Beach Action Value for enterococcus concentrations (130 MPN per 100 ml). In addition, no human-associated *Bacteroides* marker was detected in this sample, nor were *Clostridium perfringens* concentrations elevated, which suggested the source was not human fecal matter. The areas of brown water found in late August 2019 in the Kohola and Nai'a lagoons were due to the extensive growth of a dinoflagellate, tentatively identified as *Gymnodinium*. Fecal indicator bacteria (enterococci and *C. perfringens*) were not associated with the blooms. The saxitoxin levels were low (0.039  $\mu\text{g}$  per L) in all four lagoons and were not associated with the observed blooms, nor did it present any plausible direct risk to human health. The localized blooms were probably linked to the elevated nitrogen levels associated with the two lagoons. The blooms may possibly have resulted from the extensive use of irrigation and fertilizers during the dry summer months, and local hydrology and water exchanges may have also contributed to the observed differences. The linkage between irrigation sources, fertilizer use, and local hydrology should be further studied to more definitively determine the cause of the dinoflagellate blooms if mitigating periodic nuisance blooms in the Ko Olina lagoons is of interest.



## CONTENTS

---

Abstract .....	v
1.0. Introduction .....	1
2.0. Methodology .....	1
2.1. Sample Collection .....	1
2.2. Sample Analyses .....	1
3.0. Results and Discussion .....	2
3.1. Bacteriological Analyses .....	2
3.2. Phytoplankton Analyses .....	4
4.0. Conclusions .....	5
Acknowledgments .....	5
References .....	5
Figures .....	7
Tables .....	15

## Figures

---

1. Water samples collected from the four lagoons at Ko Olina resort .....	9
2. Geometric mean and standard error of the (A) enterococcus and (B) <i>C. perfringens</i> concentrations detected in the Ko Olina lagoons over a one-year period (01/14/19–12/11/19) .....	10
3. Puddles pictured next to palm tree contained >24,196 MPN of enterococci per 100 ml and 35 CFU of <i>C. perfringens</i> per 100 ml .....	11
4. Brown water colorization due to the extensive phytoplankton growth (bloom) (top) in the Nai'a Lagoon. Dinoflagellate (bottom left and right), tentatively identified as <i>Gymnodinium</i> was the dominant taxon in the samples collected from the lagoons impacted by the blooms .....	12
5. Mean concentrations of (A) ammonia, nitrate+nitrite, total nitrogen, and phosphorus and (B) chlorophyll A in the Ko Olina lagoons over a one-year period (01/14/19–12/11/19) .....	13

## Tables

---

1. Concentration of enterococci and <i>C. perfringens</i> in the Ko Olina lagoons, 14 January 2019 to 11 December 2019 .....	17
--	----

2. Concentration of dinoflagellate, tentatively identified as <i>Gymnodinium</i> , in Ko Olina lagoons during phytoplankton bloom episodes in 2019 .....	17
3. Concentration of nutrients and chlorophyll A in Ko Olina lagoons during phytoplankton bloom peak, 19 August 2019 .....	17
4. Concentration of saxitoxin in Ko Olina lagoons during phytoplankton bloom episodes in 2019 .....	18

## 1.0. INTRODUCTION

The lagoons at the Ko Olina Resort (Kohola, Honu, Nai‘a, and Ulua) are extremely popular and equally utilized by tourists as well as local residents as they provide safe environments for swimming, snorkeling, and paddle boarding. These calm tropical blue lagoons as well as the adjacent beautifully maintained resort’s grounds are treasured and revered by many, including those who choose not to or are not able to directly enjoy the lagoons.

The lagoons have no direct point source discharges of pollutants such as stormwater pipes or streams flowing into them. However, microbes potentially originating from non-point sources such as leaching from the surrounding soils, shedding by the swimmers, or leaching from aging sewer lines servicing adjacent bathrooms and showers may be of concern.

Water quality information available for the lagoons is limited [1]. Even with the limited data, recently elevated enterococci concentrations, exceeding the State’s Beach Action Value (BAV) of 130 MPN of enterococci per 100 ml was detected in one of the lagoons [1]. As a result, caution signs were posted along the lagoon. In addition, during the dry summer months, brown water patches develop in some of the lagoons and has been reported to HDOH. This is a potential concern as the visitors’ numbers are projected to increase as the resort grows, thereby increasing the pressure on the local environment.

The objective of this one-year project was to fill in these knowledge gaps and address the HDOH and community concerns by studying the microbiological water quality in all four Ko Olina lagoons.

## 2.0. METHODOLOGY

### 2.1. Sample Collection

Over the period of one year (14 January 2019 to 11 December 2019), 128 water samples were collected from the four lagoons (Kohola, Honu, Nai‘a, and Ulua) at the Ko Olina Resort (Fig. 1). For the bacteriological tests, one liter of water was collected using sterilized polypropylene (PP) bottles. For the chemical analyses, pre-cleaned two-liter brown high-density polyethylene (HDPE) bottles were used. This sampling effort yielded a total of 32 samples for each of the four lagoons. The samples were transferred on ice to the laboratories for analyses.

The physical and chemical parameters were measured at the site, including (1) temperature, (2) conductivity, and (3) pH using the handheld Pro1030 Instrument (YSI Inc., Yellow Springs, OH); and (4) oxygen concentrations (mg/L and %) using the Pro20i Dissolved Oxygen Instrument (YSI Inc.). In addition, salinity was calculated based on the conductivity and water temperature measured and used to adjust oxygen measurements. The instruments were calibrated before each use. Turbidity was measured using 2100Q Portable Turbidimeter (Hach Co., Loveland, CO) at each site, and the instrument was calibrated every two months. Number of people swimming, as well as presence of animals, such as seals and dogs, were also recorded. Rainfall data (3h, 6h, 12h, and 24h) was downloaded after each sampling event from <http://weather.hawaii.edu/current/hawobs.cgi?banner=uhmet> (Department of Meteorology, SOEST, University of Hawai‘i at Mānoa) for the weather stations Lualualei (LUAH1) and Palehua (PLHH1).

### 2.2. Sample Analyses

The samples were analyzed for the bacteriological parameters (concentrations of enterococci and *Clostridium perfringens*) at the Water Resources Research Center (WRRC) laboratory (University of

Hawai'i at Mānoa) within three hours of the sample collection. Chemical analyses (total nitrogen, ammonia, nitrate and nitrite, total phosphorous, and chlorophyll A) was conducted by the State Laboratories Division (SLD) (2725 Waimano Home Road, Pearl City, Hawai'i 96782).

The concentration of enterococci as the most probable number (MPN) were determined using Enterolert® kit in Quanti-Tray 2000® format according to the manufacturer's protocol (IDEXX Laboratories, Westbrook, ME). *C. perfringens* concentrations as colony forming units (CFU) were determined using membrane filtration based technique and mCP medium as described in Bison and Cabelli [2], except 30 mg of indoxyl- $\beta$ -D-glycoside was added per 100 ml medium. Our earlier experiments using 16S RNA gene sequencing of environmental isolates have shown 94% correct identification rate for this method. The lower limit of quantification for the enterococci was 10 MPN per 100 ml, and for *C. perfringens* 1 CFU per 100 ml. For the statistical analyses, samples containing <10 MPN enterococci were treated as 2.3 MPN per 100 ml, a concentration derived based on the historical membrane filtration-based sampling data and conventionally used by the HDOH. *C. perfringens* samples containing <1 CFU per 100 ml were treated as 0.5 CFU per 100 ml.

Samples containing bacteria at levels exceeding the BAV for enterococci or 50 CFU of *C. perfringens* per 100 ml, were analyzed for the human-associated *Bacteroides* marker. This was done using the bacterial materials collected on a separate set of membrane filters. This set was collected by filtering 500 ml of sample through the filter, and storing the folded filters at -80°C. DNA was extracted from the filters originating from the samples with the elevated concentrations of indicator bacteria using DNeasy PowerSoil kit (Qiagen, Hilden, Germany) and analyzed on CFX96 Touch Real-Time PCR detection system (Bio-Rad, Hercules, CA) using SSOAdvanced Universal Probes chemistry (Bio-Rad) and primers (500 nM each) and probe (80 nM) as specified in Green et al. [3]. The Sketa assay [4] was used to test for DNA loss and PCR inhibition. The lower limit of quantification for the qPCR test was 5 gene copies per 100 ml.

Phytoplankton analyses were conducted on the selected samples when brown discoloring of the water was observed. In the laboratory, 100 ml of the water samples was fixed with the Lugol's Solution (2–3% final concentrations). Samples were analyzed using Sedgewick-Rafter Counting cells (1 ml volumes) as well as after 12 h sedimentation in Utermöhl-Chambers (50 ml volumes) (Hydro-Bios, Kiel-Altenholz, Germany) on Axiovert A1 inverted microscope (Zeiss, Oberkochen, Germany). Live observation and photography were also conducted before the material was fixed. Two sets of the samples were tested for the saxitoxin level using Saxitoxin ELISA test kits (Eurofins, Luxembourg). Absorbance was read on EMax® Plus Microplate Reader (Molecular Devices, San Jose, CA) at 450 nm within 15 min after the addition of the stopping solution provided with the kit. The lower limit of quantification for this test was 0.02  $\mu$ g of saxitoxin per L. A positive control (0.075  $\mu$ g per L) was tested along the standard.

Standard quality assurance and operating procedures developed for the WRRC laboratory were followed and are available upon request ([kirs@hawaii.edu](mailto:kirs@hawaii.edu)). Digital file with the raw data in a spreadsheet format will be submitted to the Clean Water Branch along with this report.

## 3.0. RESULTS AND DISCUSSIONS

### 3.1. Bacteriological Analyses

Enterococci were detected in 18% of the samples, and *C. perfringens* was detected in 67% of the samples (Table 1). The geometric mean of enterococci concentrations was 3.2 MPN per 100 ml, and the geometric mean of *C. perfringens* concentrations was 1.5 CFU per 100 ml. Rolling geometric mean of

enterococci for five consecutive samples remained below the lower limit of quantification (10 MPN per 100 ml) throughout the study period, except for the Ulua Lagoon where the geometric mean increased to 11.53 MPN per 100 ml after a sample with high levels of enterococci was collected on 12 November 2019. The concentrations of enterococci and *C. perfringens* were not significantly different between the lagoons ( $P = 0.968$  and  $P = 0.146$ , respectively) (Fig. 2).

Only one of the samples (0.78% from total samples collected) exceeded the BAV of 130 CFU enterococci per 100 ml. Beach notifications such as caution signs are currently posted in Hawai'i when concentrations of enterococci exceed this value. The sample collected on 12 November 2019 from the Ulua Lagoon, contained 408 MPN of enterococci per 100 ml. Although the sample was collected during the rainy season, no significant rainfall (>1 mm) was observed over the 24 h period prior to sampling. Nutrient levels (nitrogen and phosphorous) were typical for the lagoon. Also, the number of swimmers was comparable to the number of swimmers observed in other lagoons. Therefore, no apparent source of elevated enterococcus concentrations could be determined.

Similarly, only one of the samples (0.78% from total samples collected) exceeded *C. perfringens* based on an advisory value of 50 CFU per 100 ml. Currently, this value is no longer used by the HDOH when making beach management decisions, although several studies have demonstrated that *C. perfringens* concentrations may correlate better with the health risk [5]. This sample was collected on 4 December 2019 from the Kohola Lagoon. Although the sample was collected during the rainy season, no significant rainfall (>1 mm) was observed over the 24 h period prior to sampling. Nutrient levels (nitrogen and phosphorous) were typical for the lagoon. Also, the number of swimmers was comparable to the number of swimmers observed in other lagoons. Therefore, no apparent source of elevated *C. perfringens* concentrations could be determined.

Both samples (elevated concentration of enterococci and elevated concentration of *C. perfringens*) were analyzed for the presence of the human-associated fecal *Bacteroides* marker. The marker was not detected in those two samples, hence indicating that the source of the indicator bacteria was unlikely to be human fecal matter.

There was no significant relationship between the additional parameters measured (rainfall, temperature, dissolved oxygen, pH, conductivity, salinity, turbidity, ammonia, nitrate + nitrite, total nitrogen, total phosphorous, number of swimmers) and the indicator bacteria (enterococci and *C. perfringens*) concentrations (Pearson correlation;  $P > 0.05$ ; for all comparisons, both bacterial indicators). This analysis was repeated by removing all the samples where enterococci concentrations were below the lower limit of quantification, yet no significant relationship was detected between the additional parameters and both indicator bacteria (Pearson correlation;  $P > 0.05$ ; for all comparisons, both bacterial indicators). It should be noted that although samples were collected without any considerations of weather conditions there was no significant rainfall before any of the sampling events as the 24 h rainfall preceding the sampling event remained below 1.8 mm throughout the study.

Collectively, bacteriological analyses indicated good water quality in all of the Ko Olina lagoons throughout the study period. The elevated concentrations of both indicator bacteria in single samples appears to be from non-human sources; however, the study was not focused on tracking the microbial contaminant sources. In this regard, the environment (soils, animals including seals) can be a source of indicator bacteria. Earlier studies have shown that enterococci can grow in Hawaiian soils and impact water quality measurements [6,7]. It could be speculated that some of the indicator bacteria detected were transmitted from the adjacent environments to the lagoons by humans (feet transport). For example, although a single sample was collected, enterococci concentrations in the puddles next to the beach

shower (Fig. 3) exceeded 24,196 MPN per 100 ml, and concentrations of *C. perfringens* were 35 CFU per 100 ml. A person wandering through those puddles could transfer indicator bacteria into the lagoon.

### 3.2. Phytoplankton Analyses

The typically tropical blue water turned brown in two of the lagoons (Kohola and Nai'a) during a late summer month (Fig. 4, top). It was first observed on 19 August 2019 during routine sampling, which triggered extra sample collections on 20 and 29 August—no nutrient or chlorophyll analyses were conducted for the extra samples. Additional samples were collected on 5 September 2019 when the brown water had more or less dissipated.

Analyses of the samples under the microscope revealed high concentrations of dinoflagellates. A single dominant taxon was tentatively identified as *Gymnodinium* (Fig. 4, bottom). Further confirmation and species identification would require electron microscopy and/or molecular analyses.

Throughout this period, the dinoflagellate blooms were only associated with the Kohola and Nai'a lagoons. The highest concentration of *Gymnodinium* cf. was detected in the Nai'a Lagoon, and the concentrations decreased in both lagoons during the next sampling events (Table 2). Intermittent (daily-variable) color change over this and an earlier period were reported by two of the Marriott's Ko Olina Beach Club's staff members interviewed at the Nai'a Lagoon.

As stated earlier, nutrient and chlorophyll data were available only for two out of four sets of samples collected over this period, hence statistical analyses could not be conducted. Nevertheless, it should be noted that higher total nitrogen levels were observed in the Nai'a as well as the Kohola lagoons when compared to the other lagoons on 19 August 2019 (Table 3). As a function of phytoplankton biomass, chlorophyll A concentrations were also elevated in both lagoons (Table 3), which correlated with the cell concentration estimates (Table 2). Furthermore, when the nutrient data for all four lagoons over the entire study period were analyzed, the nitrate + nitrite and total nitrogen levels were significantly different between all lagoons (Kruskal-Wallis ANOVA, Tukey;  $P < 0.001$ ,  $n = 34$ ) (Figs. 5A and 5B), except no difference was detected between the Honu and Nai'a lagoons for both parameters ( $P = 0.280$  (nitrate + nitrite) and  $P = 0.709$  (total nitrogen)). Collectively, these data appear to indicate that elevated nitrogen is found during blooms at both lagoons. Potentially, fertilizers (used on the lawns surrounding the lagoons) coupled with the extensive irrigation during the hot and dry summer season when the blooms occurred (late August), were contributing to the bloom formation. The difference in local hydrology resulting in differential transport of fertilizers through the groundwater system, as well as difference in water exchange with the open ocean, may also be contributing factors.

There was no evidence that the blooms were driven by direct input of sewage-borne contaminants as enterococci concentrations remained very low, between <10 to 40 MPN per 100 ml in the Kohola Lagoon and were <10 MPN per 100 ml in the Nai'a Lagoon, and concentrations of *C. perfringens* also remained very low, between <1 to 3 MPN per 100 ml in the Kohola Lagoon and were <1 to 1 MPN per 100 ml in the Nai'a Lagoon.

Many dinoflagellates can produce phycotoxins, which can be harmful to marine life, including humans. Humans are typically exposed by eating seafood containing phycotoxins, although accidental swallowing or inhaling the water when swimming may have some potential risk. During the blooms, we measured concentrations of saxitoxin, the most common phycotoxin produced by the dinoflagellates in two sets of samples (Table 4). The saxitoxin concentrations were low ( $0.039 \mu\text{g}$  per L) and uniform throughout all four lagoons (Table 4), ergo an unlikely result of the blooms observed in the two lagoons.

Only a limited number of states have set limits for saxitoxin concentrations for recreational waters. The state of Ohio is posting Recreational Public Health Advisories or Elevated Recreational Public Health Advisories when saxitoxin levels exceed  $0.8 \mu\text{g per L}$  and  $3 \mu\text{g per L}$ , respectively [8]. The Oregon Health Authority (OHA) recommends  $8 \mu\text{g per L}$  [9], and Washington State's Department of Health recommends  $75 \mu\text{g per L}$  threshold [10] for recreational waters. The World Health Organization is currently considering  $30 \mu\text{g per L}$  as the guidance limit for recreational waters [11]. The observed saxitoxin levels of  $0.039 \mu\text{g per L}$  in the Ko Olina lagoons were several orders of magnitude lower than any of the aforementioned threshold levels. The  $\text{LD}_{50}$  for saxitoxin is  $5.7 \mu\text{g per kg}$  when ingested directly [12]. Tolerable daily intake of saxitoxin has been suggested to be about  $0.05 \mu\text{g per kg}$  for healthy humans [9]. An average 80 kg person would need to drink in excess of 100 L of the lagoon water to reach that level. Therefore, the toxin level detected had no plausible risk to human health. Nevertheless, as phycotoxins can accumulate in seafood, and as it is unknown what environmental factors trigger toxin production, it would be advisable to continue the testing of phytoplankton bloom impacted waters.

#### 4.0. CONCLUSIONS

- Good water quality, based on fecal indicator bacteria and nutrient analyses, was observed in all four Ko Olina lagoons over the study period (14 January 2019 to 11 December 2019).
- Elevated nitrogen levels may support formation of phytoplankton blooms and related color change (i.e., brown).
- Dinoflagellate, tentatively identified as *Gymnodinium*, was the single dominant taxon producing the blooms.
- The saxitoxin levels were low and uniform in all four lagoons, and were not associated with the observed blooms, nor presented any plausible direct risk to human health.
- It is advisable to monitor water quality in the lagoons for any potential change.

#### ACKNOWLEDGMENTS

We would like to sincerely thank Dr. Danny Licudine and the analyses team at the State Laboratories Division (SLD) for providing the nutrient and chlorophyll A data used in this report. The project was funded by the Clean Water Branch, HDOH.

#### REFERENCES

1. State of Hawaii, Department of Health. n.d. Clean Water Branch: Water Quality Data–Microbiology. <http://cwb.doh.hawaii.gov/CleanWaterBranch/WaterQualityData/default.aspx> (accessed October 2018).
2. Bisson, J.W., and V.J. Cabelli. 1979. Membrane filter enumeration method for *Clostridium perfringens*. *Appl. Environ. Microbiol.* 37(1): 55–66.

3. Green, H.C., R.A. Haugland, M. Varma, H.T. Millen, M.A. Borchardt, K.G. Field, W.A. Walters, R. Knight, M. Sivaganesan, C.A. Kelty, and O.C. Shanks. 2005. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. *Appl. Environ. Microbiol.* 80(10): 3086–3094.
4. Haugland, R.A., S.C. Siefring, L.J. Wymer, K.P. Brenner, and A.P. Dufour. 2005. Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. *Water Res.* 39(4): 559–568.
5. Viau, E.J., D. Lee, and A.B. Boehm. 2011. Swimmer risk of gastrointestinal illness from exposure to tropical coastal waters impacted by terrestrial dry-weather runoff. *Environ. Sci. Technol.* 45(17): 7158–7165.
6. Hardina, C.M., and R.S. Fujioka. 1991. Soil: the environmental source of *Escherichia coli* and enterococci in Hawaii's streams. *Appl. Environ. Microbiol.* 6(2): 185–195.
7. Fujioka, R.S., and M.N. Byappanahalli. 2003. *Proceedings and Report: Tropical Water Quality Indicator Workshop, Honolulu, Hawaii, March 1–2, 2001*. Final report to USEPA and Hawaii State Department of Health. Special Report SR-2004-01, Water Resources Research Center, University of Hawaii at Manoa, Honolulu.
8. Ohio Department of Health. 2018. Advisories for HABs-contaminated recreational waters. <https://odh.ohio.gov/wps/portal/gov/odh/know-our-programs/harmful-algal-blooms/resources/habs-ohio-advisories> (accessed March 2020).
9. Oregon Health Authority (OHA). 2019. *Oregon Harmful Algae Bloom Surveillance (HABS) Program. Recreational Use Public Health Advisory Guidelines Cyanobacterial Blooms in Freshwater Bodies*. Public Health Division, Center for Health Protection, Environmental Public Health Section. 27 p.
10. Hardy, J. 2011. *Washington State Provisional Recreational Guidance for Cylindrospermopsin and Saxitoxin*. Washington, D.C.: Washington State Department of Health. 36 p.
11. World Health Organization (WHO). 2019. Cyanobacterial toxins: Saxitoxins. In *Background Document for Development of WHO Guidelines for Drinking-Water Quality and Guidelines for Safe Recreational Water Environments* (Draft). [https://www.who.int/water\\_sanitation\\_health/water-quality/guidelines/chemicals/saxitoxin-gdwq-bd-for-review-20191125.pdf](https://www.who.int/water_sanitation_health/water-quality/guidelines/chemicals/saxitoxin-gdwq-bd-for-review-20191125.pdf)
12. Nguyen, H.V.N., M.E. Smith, and H.D. Swoboda. 2020. Shellfish Toxicity. [Updated 2020 Mar 31]. In *StatPearls* [Internet]. Treasure Island, Florida: StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK470225/>

---

## Figures

---





Note: ★ indicates where water samples were collected.

Figure 1. Water samples collected from the four lagoons at the Ko Olina resort.

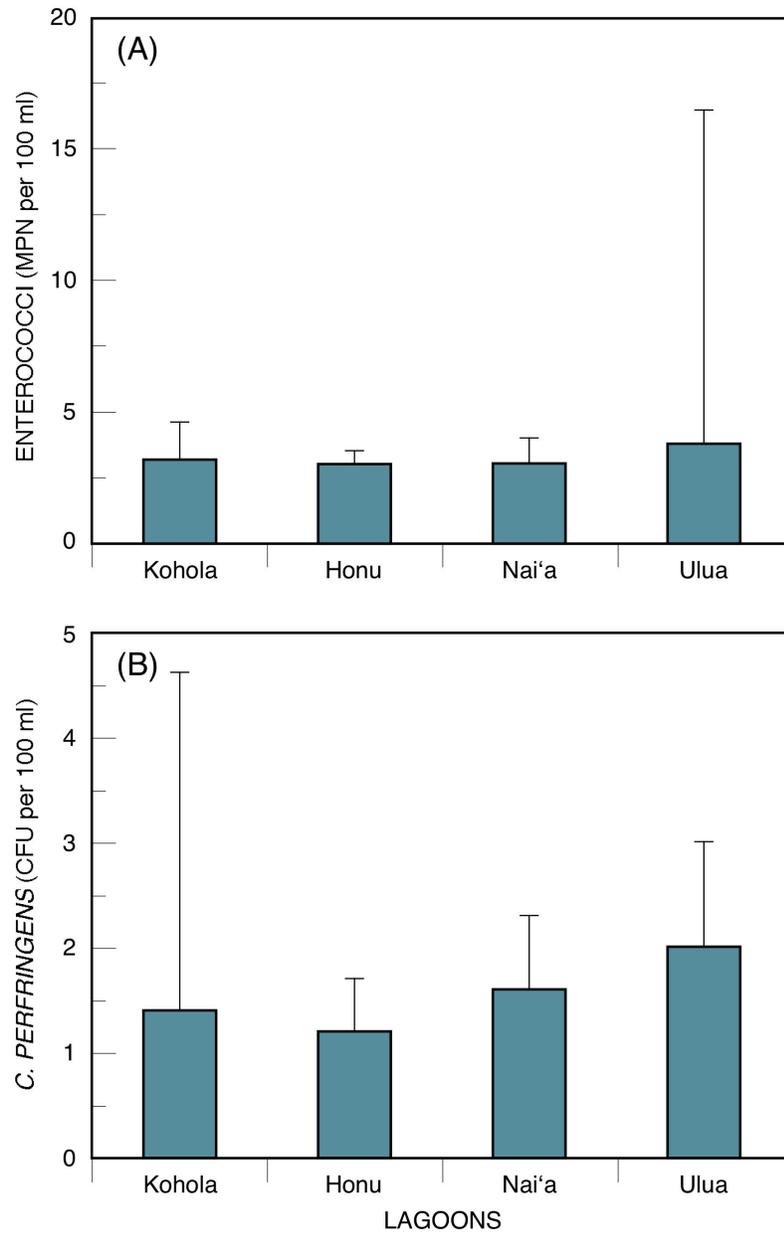


Figure 2. Geometric mean and standard error of (A) enterococcus and (B) *C. perfringens* concentrations detected in the Ko Olina lagoons over a one-year period (01/14/2019 – 12/11/2019).



Figure 3. Puddles pictured next to palm tree contained >24,196 MPN of enterococci per 100 ml and 35 CFU of *C. perfringens* per 100 ml. These puddles could potentially be a source of indicator bacteria found in the lagoons.

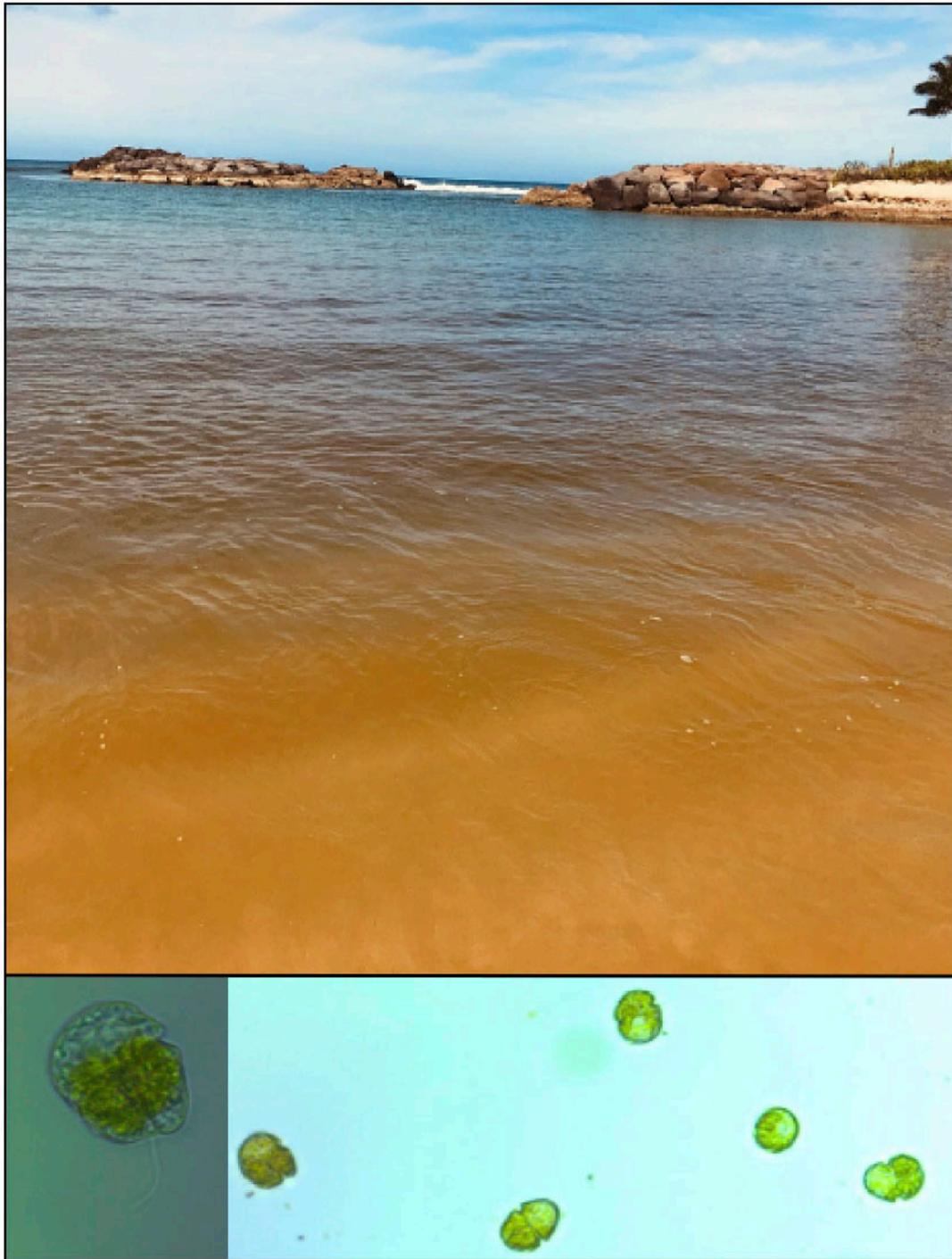


Figure 4. Brown water colorization due to the extensive phytoplankton growth (bloom) (top) in the Nai'a Lagoon. Dinoflagellate (bottom left and right), tentatively identified as *Gymnodinium* was the dominant taxon in the samples collected from the lagoons impacted by the blooms.

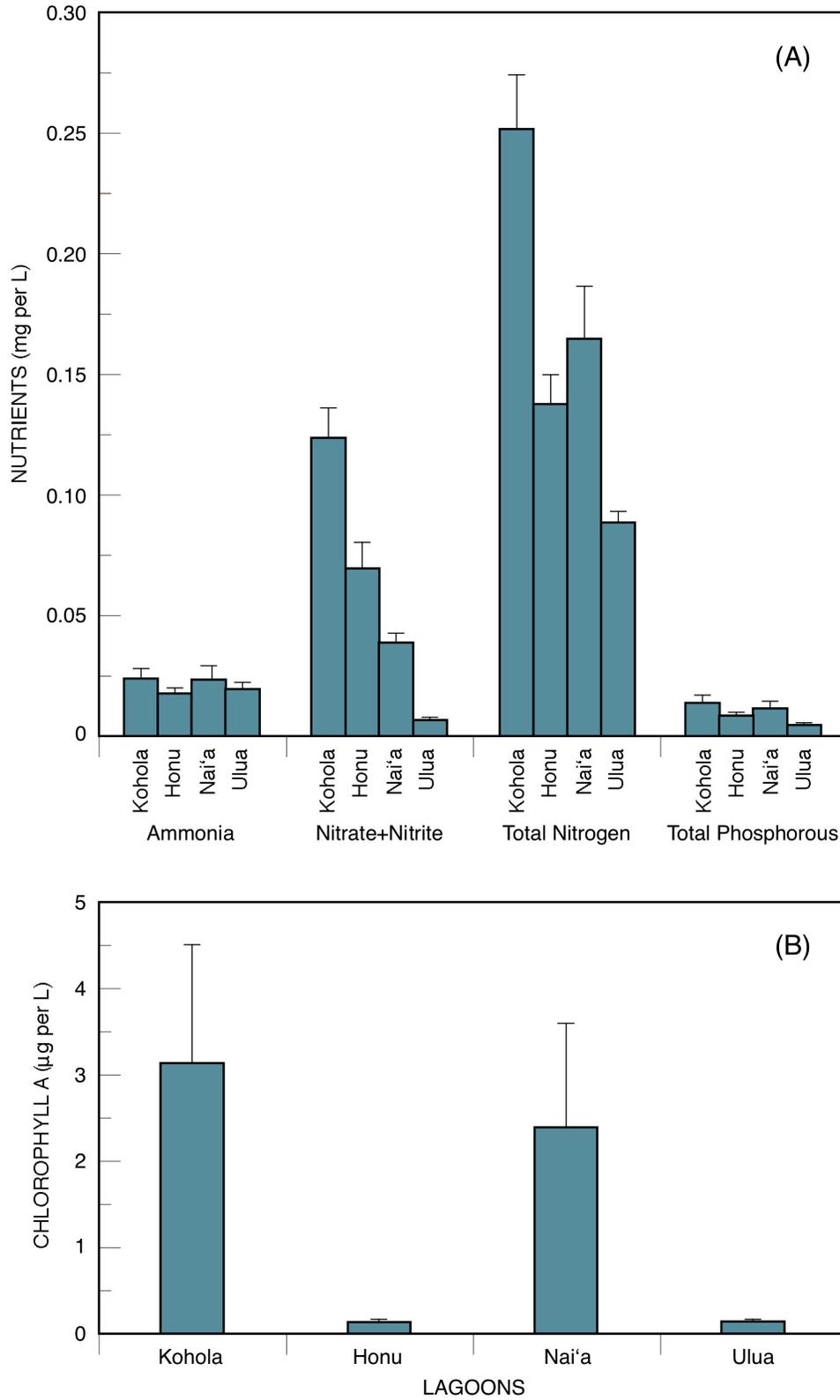


Figure 5. Mean concentrations of (A) ammonia, nitrate+nitrite, total nitrogen, and phosphorous and (B) chlorophyll A in the Ko Olina lagoons over a one-year period (01/14/2019 – 12/11/2019). Error bars correspond to standard errors.



---

## Tables

---



Table 1. Concentration of enterococci and *C. perfringens* in the Ko Olina lagoons, 14 January 2019 to 11 December 2019.

Lagoons	Enterococci (MPN/100 ml)				<i>C. perfringens</i> (CFU/100 ml)			
	No. of Samples	No. of Positive Samples	Geometric Mean	Range (min to max)	No. of Samples	No. of Positive Samples	Geometric Mean	Range (min to max)
Kohola	32	6	3.2	<10–41	32	21	1.4	<1–104
Honu	32	6	3.0	<10–10	32	17	1.2	<1–10
Nai'a	32	5	3.0	<10–31	32	23	1.6	<1–14
Ulua	32	6	3.8	<10–408	32	25	2.0	<1–33

Table 2. Concentration of dinoflagellate, tentatively identified as *Gymnodinium*, in Ko Olina lagoons during phytoplankton bloom episodes in 2019.

Lagoons	Dinoflagellate (cells/ml)			
	August 19	August 20	August 29	September 5
Kohola*	18,417	14,383	326	0.5
Honu	<1	<1	<1	<1
Nai'a*	24,263	6,645	<1	<10–31
Ulua	<1	<1	<1	<1

\*Indicates brown phytoplankton blooms were visible.

Table 3. Concentration of nutrients and chlorophyll A in Ko Olina lagoons during phytoplankton bloom peak, 19 August 2019.

Lagoons	Ammonia (mg N/L)	Nitrate + Nitrite (mg N/L)	Total Nitrogen (mg NP/L)	Total Phosphorous (mg P/L)	Chlorophyll A ( $\mu$ g/L)
Kohola*	0.022	0.002	0.768	0.087	75.998
Honu	0.019	0.051	0.127	0.009	0.210
Nai'a*	0.015	0.005	0.553	0.051	53.834
Ulua	0.018	0.007	0.091	0.009	0.322

\*Indicates brown phytoplankton blooms were visible.

Table 4. Concentration of saxitoxin in Ko Olina lagoons during phytoplankton bloom episodes in 2019.

Lagoons	Saxitoxin ( $\mu\text{g/L}$ )			
	August 19	August 20	August 29	September 5
Kohola	0.039	0.039	NT	NT
Honu	0.039	0.039	NT	NT
Nai'a	0.039	0.039	NT	NT
Ulua	0.038	0.039	NT	NT

Note: NT = not tested. *Gymnodinium* cf. concentrations decreased in the samples collected on August 29th and September 5th, hence saxitoxin concentrations were not tested in those samples.