CHARACTERIZING NITROGEN AND $\delta^{15}N$ VALUES IN THE BLADE TISSUES OF A WINDWARD POPULATION OF *AVRAINVILLEA LACERATA*, AN INVASIVE GREEN ALGA

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DEDICATION

For my sisters, Lan and Diana, who are always on my side.

And for my parents, Hoa and Dang, I love you both and owe you everything.

To my partner, Evan, thank you for the unconditional support and love.

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ABSTRACT

The green alga *Avrainvillea lacerata* was first discovered in Hawaiʻi by Brostoff in 1989. The plant is invasive, and its history has not been well documented. It has continued to proliferate along the shores of Oʻahu, and is projected to invade a quarter of all coastlines in the Main Hawaiian Islands (MHI) due to climate change. To understand the fundamental relationship between nitrogen (N) availability and algal growth, in opposing wet and dry seasons, *A. lacerata* blade tissues were sampled to examine $\delta^{15}N$ (‰) and %N in photosynthetic tissue and compared with dissolved inorganic nitrogen (DIN) in the water column at Kualoa Regional Beach Park (KBP) on the northeastern side of Kāneohe Bay. Tissue values in this study were compared with data collected from a leeward population of *A. lacerata* in Maunalua Bay. *A. lacerata* as well as other invasive algae can be used to assess the availability of sources in coastal environments.

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INTRODUCTION

Disturbances can greatly alter community composition within coastal ecosystems. Various sources of nutrient loading in coastal water bodies, including terrestrial runoff, fertilized pasture, agricultural land, polluted rivers and groundwater, and improper sewage treatment and discharge, are significant disturbances to manage. Eutrophic conditions can create a phase shift as populations of fleshy macroalgae become dominant because of excess nutrients such as inorganic nitrogen (N) and phosphorus (P) entering reef ecosystems (Stimson et al. 2001). Blooms can be of both indigenous or nonindigenous algae, as seen with *Hypnea musciformis* on Maui (Smith et al. 2002), *Dictyosphaeria cavernosa* in Kāneʻohe Bay (Stimson et al. 2001), and the mats of *Avrainvillea lacerata* in Maunalua Bay (Peyton 2009; Minton at el. 2012). The succession of invasive algae in coastal environments may result from ecological, physiological, and reproductive characteristics.

For decades, *Avrainvillea lacerata* (Bryopsidales, Chlorophyta) has been the focus of statewide concern as its distribution expanded along the shores of Oʻahu. Gradually, this perennial alga has proliferated around Oʻahu extending from Mokuleʻia in the north to the eastern side of Kahuku to Waimānalo, and from ʻEwa to Maunalua Bay (Veazey et al. 2019). More recently, some parts of Kauaʻi (Smith et al. 2002; Veazey et al. 2019) have seen the arrival of this species. This invasive species was thought to have arrived before 1981. It was initially classified as *A. amadelpha* (Brostoff 1989; Godwin et al. 2000) on two separate reefs on Oʻahu, Kahe Point, and Koko Head. In 2018, a molecular taxonomic revision established that records of *Avrainvillea amadelpha* species in Hawaiʻi are, in fact, *Avrainvillea lacerata* (Wade 2019).

1.1 INVASIVE ALGAE

The common name of *A. lacerata* is "leather mudweed" which derives from the plant's thick, dark olive blades and below-sand holdfast – a morphology that can trap particles, allowing an increase in sediment accumulation. The current distribution of *A. lacerata* in Hawaiʻi extends across broad environmental habitats ranging from exposed, shallow intertidal to deep mesophotic zones (0 - 90 m). In areas once dominated by the native seagrass, *Halophila hawaiiana* (Peyton 2009; Unabia 2011) have been replaced by *A. lacerata* (Langston and Spalding 2017). The presence of *A. lacerata* on reefs is alarming because its weedy characteristics alter benthic structures (Minton et al. 2012), decrease biodiversity (Peyton 2009; Veazey et al. 2019), and it is not palatable to herbivores such as native urchins and fishes (Van Heukelem 2016, S. Chulakote pers. comm). This alga is presumed to disperse successfully via sexual reproduction, fragmentation, and regenerative holdfast, similar to other Bryopsidalean species (Smith et al. 2002).

A continuous tubular network forms the thallus of unicellular *A. lacerata* to create differentiated structures that resemble holdfast, stipes, and blades. Mat forms are constructed with multiple stipes, although each stipe usually bears a single terminal blade (Verlaque et al. 2017). Energy is not expended on building cellular cross walls. Instead, these single-celled organisms invest in growth (Vroom and Smith 2001). Growth can be expedited in this one-compartment organization by strategically distributing nutrients and organelles (e.g., chloroplast and multiple nuclei) through cell expansion via turgor pressure. However, it remains unclear if land-based nitrogen sources impact *A. lacerata* occurrence.

1.2 NITROGEN SOURCES

To date, the information about *A. lacerata* in Hawaiʻi is limited to the ecosystem surveys, including mesophotic depths (Foster et al. 2019; Langston & Spalding 2017; Magalhães & Bailey-Brock 2017), as a part of Mālama Maunalua cleanups (Minton & Conklin 2012; Peyton 2009), modeled changes in distribution (Veazey et al. 2019), taxonomic assessments (Wade 2019; Brostoff 1989), and a UROP funded project (Albright 2017). The fundamental processes of the plant remain understudied. To grow, a plant must capture nutrients, especially in relatively oligotrophic environments (Rosenberg & Ramus 1984). Nitrogen is a critical component in algal proteins and enzymes and can be a limiting factor for plant growth and biomass (Fong et al. 1993; Hein et al. 1995). Therefore, to grow, this macroalga must obtain nutrients from its environment. The availability of nutrients in coastal marine habitats is impacted by terrestrial over-land water flow of agricultural runoff, municipal wastewater, and as well as underground wastewater discharges (Derse et al. 2007; Dailer et al. 2010; Cox et al. 2013; Amato et al. 2016, 2019). To understand whether anthropogenic nutrients are potential sources for invasive species the first step is to document possible identities of nutrient sources.

Stable isotopes are ideal for studying element cycling in ecosystems (Peterson & Fry 1987). Nitrogen occurs as two stable isotopes: ^{14}N and ^{15}N. The ratio between the two-stable isotopes in a sample is compared to the standard ratio in atmospheric nitrogen (N_2) and represented as $\delta^{15}N$ and has the units of parts per thousands (Peterson & Fry 1987), as shown in equation 1.

(1)

$$\delta^{15}N\ (‰) = \left(\frac{Rsample}{Rstandard} - 1\right) \times 10^3, \text{ R is the ratio } {}^{15}N/{}^{14}N$$

Atmospheric nitrogen is used as the standard for all nitrogen samples, giving atmospheric samples a $\delta^{15}N$ value of 0‰ (Peterson & Fry 1987). The Haber process incorporates fixed atmospheric N^2 isotopes to assemble synthetic fertilizer, giving the final product a low isotopic signature equivalent to its atmospheric source (Kendall & McDonnell 1998). Meanwhile, biological sources such as wastewater contain active microbes that preferentially metabolize ^{14}N over ^{15}N because of the slight difference in mass where ^{14}N is assimilated more quickly (Peterson & Fry 1987). For this reason, biologically processed material, such as wastewater, tends to be enriched in the heavier isotope ^{15}N and have larger values of $\delta^{15}N$. Macroalgae exposed to fertilizer from nearby agricultural practices on Hanalei, Kauaʻi exhibited $\delta^{15}N$ values as low as - 4‰ (Derse et al. 2007), compared to macroalgae enriched in sewage ranging from 7‰ to 38‰ (Dailer et al. 2010).

Stable isotopic values and ranges can illuminate the biological transfer of natural, fertilizer, and biological waste in ecosystems. Distinctive $\delta^{15}N$ value from algal bioassays can determine the origin of the anthropogenic or natural nutrient source (Costanzo et al. 2001). There is increasing evidence that macroalgae assimilate nitrogen with little to no fractionation, especially in oligotrophic, tropical settings (Cohen & Fong 2005; Derse et al. 2007; Dailer et al. 2010). The ambient nutrient concentration in all oligotrophic water bodies is low. Therefore, it is less likely for macroalgae to fractionate towards a specific nitrogen isotope. As a result, the algal $\delta^{15}N$ signatures collected in Hawaiʻi have been successfully used to discern between natural and anthropogenic nitrogen sources (Amato et al. 2019; Dailer et al. 2010; Derse et al. 2007).

1.3 SIGNIFICANCE

Over $2 million dollars have been spent to remove 1.3 million kg. of *A. lacerata* over a 90,000 m^2 area in Maunalua Bay (Kittinger et al. 2016). This extensive removal effort by

Mālama Maunalua, The Nature Conservancy, and Pono Pacific Land Management LLC ultimately demonstrated an unexpected resiliency by *A. lacerata* to manual removal; regrowth from its extensive holdfast system is a likely explanation. This alga inhabits intertidal, subtidal, and mesophotic ecosystems in the Main Hawaiian Islands (MHI), altering benthic ecosystems and often out-competing native species (Godwin et al. 2000; Smith et al. 2002). In the next decades, this alga is predicted to encroach on one-quarter of all coastlines in the MHI based on the present-day distribution and environmentally susceptible coastlines (Veazey et al. 2019).

Avarinvillea lacerata is dependent on its ability to harness and capture nutrients, especially in relatively oligotrophic environments like Hawaiʻi. Compared to the algal population in Maunalua Bay, the basal holdfast in this population of *A. lacerata* is relatively smaller. Analogous to alga with a traditional streamline morphology, we assume that N will be distributed evenly throughout the thallus of this windward population of *Avrainvillea*. Therefore, we expect to see a streamlined relationship between N acquisition (% N) and seasonal plant response. Additionally, $\delta^{15}N$ isotopic analysis will serve to identify the origin(s) of N on the reefs of Kualoa Regional Beach Park. When combined, we hope to better understand the fundamental features of nitrogen use and storage for *A. lacerata* to help future conservation efforts protect Hawaiian reef biodiversity.

2.0 MATERIAL AND METHODS

2.1 DESCRIPTION OF KUALOA REGIONAL BEACH PARK

Kualoa Regional Beach Park (KBP; latitude 21.5161111, longitude -157.8416667) is surrounded by shallow-water reefs and is on the windward side of Oʻahu in Kāneʻohe Bay. The monthly mean rainfall at the Kualoa Station between 1948 to 1974 was 127 mm from October to

April wet season (Giambelluca et al. 2013). In the dry season, the monthly mean rainfall in May to September was 77 mm at the Kualoa Station (Giambelluca et al. 2013). To determine whether there was a seasonal effect on nitrogen sources, two fine-scale surveys on *A. lacerata* blade tissue N were conducted on February 15, 2020, and July 18, 2020, during the wet and dry seasons of Kualoa. We predict that elevated N values (%N and $\delta^{15}N$) will be observed in organisms located near the public septic restrooms in both seasons.

Following a similar approach to Albright (2017), *A. lacerata* collections surveyed %N and $\delta^{15}N$ at 12 sites, parallel to the shore (Figure 1c). The first transect was 25 m from the shoreline; the outer transect was 150 m from the shoreline, in water 0.5 – 2 m deep. An approximate distance of 50 m separated each site (n=12). Four sites (1, 6, 7, and 12) were in front of the public recreational bathrooms for the park. On February 15, 2020, the wet season collection was conducted between 11:00 to 13:00, with tides estimated to be 0.2 - 0.5 inches (NOAA Tide Prediction, Waikane, Kāneʻohe Bay). On July 18, 2020, the dry season collection was conducted between 7:00 to 9:00, with tides estimated to be -0.2 - 0.3 inches (NOAA Tide Prediction, Waikane, Kāneʻohe Bay).

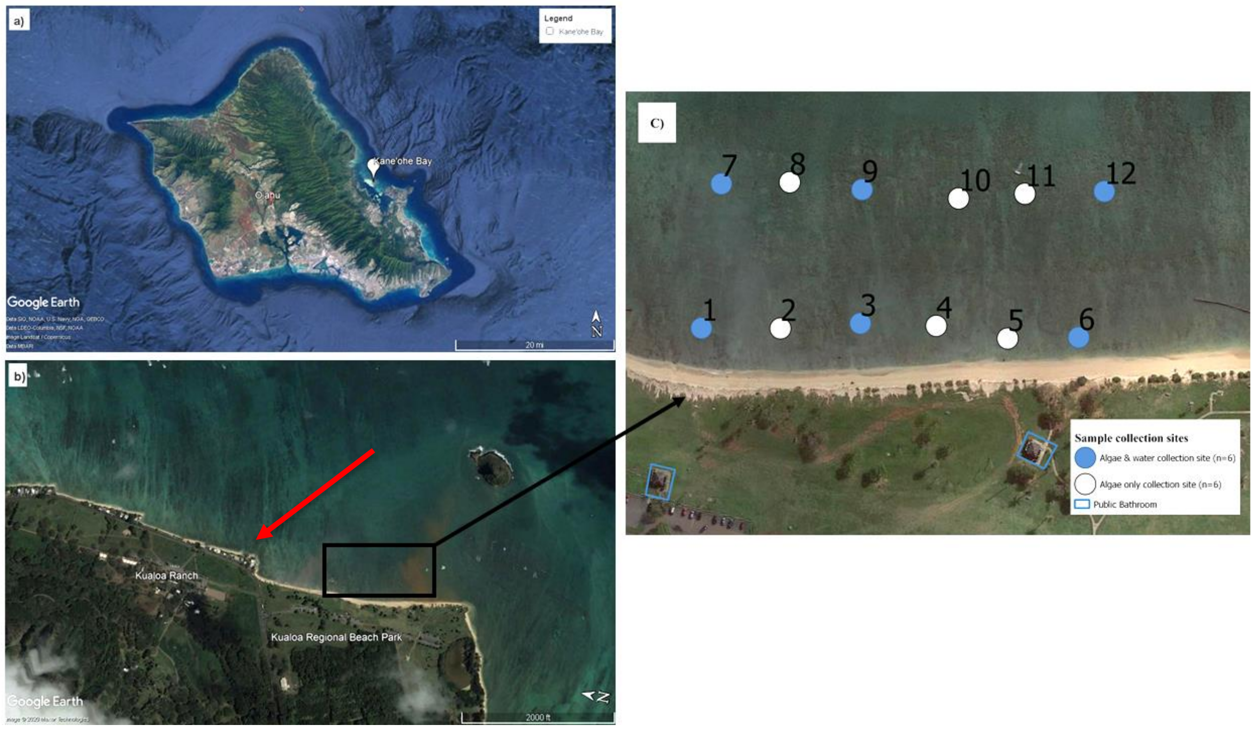

Figure 1: a) Study location is on the Windward side of O‘ahu. b) Kualoa Regional Beach Park is located adjacent to Kualoa Ranch, a privately owned cattle ranch/tourist destination, as well as private homes with cesspools on the northwest side of the park depicted by the red arrow (c) The black rectangle identifies the specific sampling sites at Kualoa Regional Beach Park.

2.2 NITROGEN CONTENT AND δ^{15}N VALUE DETERMINATION

Plant tissue samples were prepared in triplicates (n=3) per collection site for tissue % N and δ^{15}N analysis. Each thallus (total n=36) was rinsed with deionized water, cleared of epiphytic algae, invertebrates, and sediment. Plant tissues were dried to a constant weight at 60°C for 14-days, and only the blades were powdered into fine, homogeneous particles ranging between 0.2- 0.6 µg using a mortar and pestle.

Ground tissue samples were sent to the Biogeochemical Stable Isotope Laboratory, the University of Hawai‘i at Mānoa, for tissue total nitrogen, total carbon, and δ^{15}N measurements using an Exeter Analytical CE 440 Elemental Analyzer. The Exeter Analytical CE 440

Elemental Analyzer provides an automated analysis of carbon, hydrogen, nitrogen, and sulfur following the methodologies of Gordon (1969) and Sharp (1971).

Figure 2: (a-b): *Avrainvillea lacerata* specimens collected in Kualoa display a range of plant sizes and the plants lack prominent holdfast (black arrows) compared to the population of this species in Maunalua Bay (c-d): photos provided by Scott Van De Verg. Photosynthetic blade tissues (red arrows) were processed for δ^{15}N and %N.

Published δ^{15}N values from algae collected in the Ka‘ena Point region in Amato et al. (2019) serves as a baseline for this study with natural nitrogen sources dentified with δ^{15}N values between 0.5‰ - 2.8‰, while values $\geq$ 6.0‰ serve to indicate wastewater nitrogen sources for O‘ahu (C. M. Smith, pers. comm, unpublished data). Data from the algal tissue were analyzed using a Kruskal-Wallis One Way ANOVA test to determine if δ^{15}N and %N values statistically differ between sites, designating algal δ^{15}N and %N the dependent variable and site as the categorical factor. After a significant result ($p < 0.05$) was obtained, a Dunn's pairwise test was used to determine differences among sites.

2.3 WATER COLUMN NUTRIENT CONCENTRATIONS

To observe potential nutrient influx from variable rainfall sampling occurred respectively in February and July. At both 25 and 150 m sections, ambient water from sites 1, 3, 6, 7, 9, and

12 were collected in duplicates (n=2, total n=12) using acid-washed 500-ml bottles. The initial three 500-mL fluids withdrawn were disposed of to remove potential impurities. Collections were made from the water column surrounding the site and transported on ice to UHM, where 50 mL of filtered seawater were withdrawn using sterile syringes with GF/F 0.25 mm 0.45 µm filters. Filtered water samples were frozen in preparation for analysis at SOEST Lab for Analytical Biogeochemistry (S-LAB) at UHM and analyzed for dissolved inorganic nutrients (DIN: NO^{3-} + NO^{2-}, and NH^{4+}, PO_4^{3-}, and H_4SiO_4) using a Seal Analytical AA3 Nutrient Autoanalyzer.

The Seal Analytical AA3 Nutrient Autoanalyzer measures nutrients in oligotrophic seawaters. Following the methods by Kerouel and Aminot (1997), ammonium concentrations were measured fluorometrically using a reaction with o-phthalaldehyde (OPA) at 75°C to form a fluorescent species in the presence of a borate buffer and sodium sulfite (S-LAB). Nitrate and nitrate analysis was based on Armstrong et al. (1967) and Grasshoff et al. (1983) method using a diazo reaction, where the copper-cadmium reductor column reduced the nitrate to nitrite. In acidic conditions, the nitrite in the sample reacts with the sulfanilamide to form a diazo compound, coupled with N-(1-Naphthyl)ethylenediamine dihydrochloride to form a purple azo dye. The concentration of nitrite was then determined colorimetrically at 550 nm. Silicate concentration was determined colorimetrically at 820 nm based on reducing silicomolybdate in an acidic solution to molybdenum blue by ascorbic acid (Grashoff et al. 1983).

2.4 ANALYSES

Statistical analyses were performed on total N content and $\delta^{15}N$ values on *A. lacerata* tissue from each season. Data did not meet the requirements for normality and homogeneity of variance. A non-parametric One-way Kruskal-Wallis analysis of variance (ANOVA) was run to compare algal values and water column samples between 25 and 150 m in the dry and wet seasons. This same approach was used when comparing total N content and $\delta^{15}N$ values on *A. lacerata* tissue from Kualoa Regional Beach Park and Albright's (2017) Paikō Reef study.

3.0 RESULTS

3.1 WET SEASON SAMPLING

The overall mean algal $\delta^{15}N$ values from the 25 m sites (1-6) were 4.0 ± 0.4‰, which is significantly higher than $\delta^{15}N$ values 3.0 ± 0.8‰ from 150 m sites (7-12) (p-value = 0.0007) (Figure 3). Sites 2 and 6 had the highest mean value of $\delta^{15}N$, at 4.1 ± 0.20‰ and 4.2 ± 0.56‰ respectively (Figure 3). According to Dunn's test of pairwise comparisons of ranks, the $\delta^{15}N$ values at corresponding sites 1 and 7 (p-value = 0.50) and sites 5 and ll (p-value = 0.82) were not significantly different. The mean %N along the 25 m sites was 1.21 ± 0.30%, significantly lower (p-value = 0.007) than the 1.41 ± 0.22% along the 150 m sites. In the wet season, the overall mean %N in *A. lacerata* blade tissues were 1.31 ± 0.28%.

Dissolved inorganic nutrients nitrate + nitrite (N+N) and ammonia in collected water samples between 25 and 150 m did not differ significantly. Average N+N concentrations for 25 and 150 m were 1.27 ± 0.40 µmol/L (n=6) and 1.40 ± 0.43 µmol/L (n=6), respectively. Average ammonia concentrations for 25 and 150 m were 0.18 ± 0.10 µmol/L (n=6) and 0.28 ± 0.28 µmol/L (n=6), respectively.

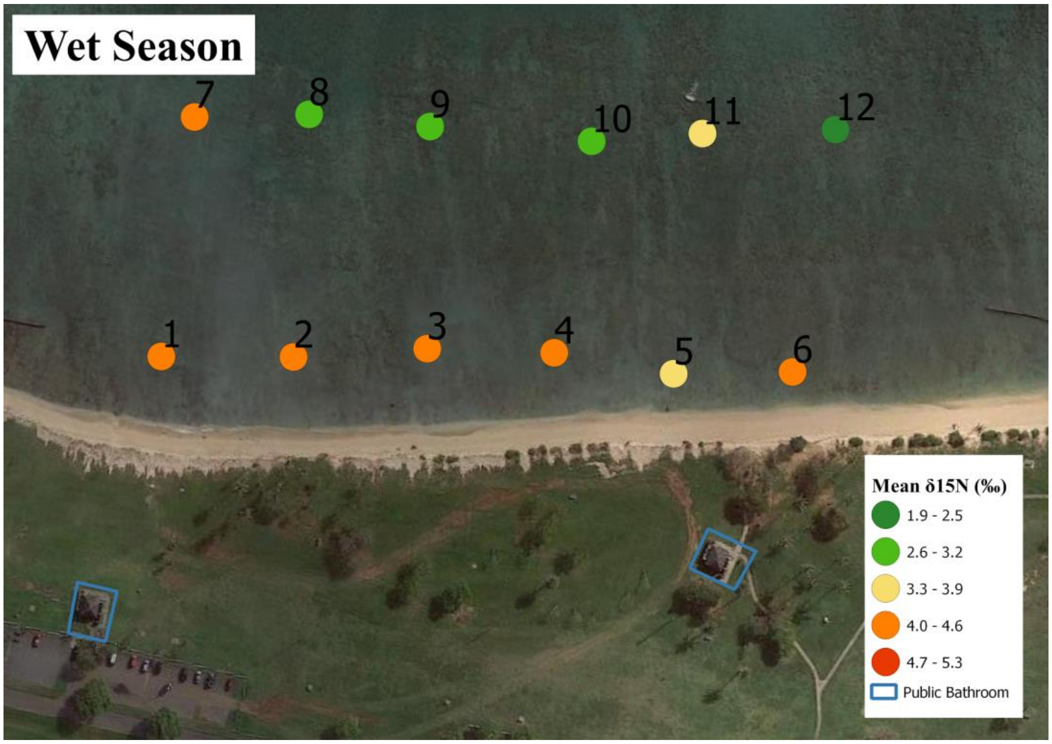

Figure 3: Mean $\delta^{15}N$ (‰) values in *A. lacerata* individuals collected in the wet season on February 2020 from the reefs of Kualoa Regional Beach Park.

3.2 DRY SEASON SAMPLING

The mean algal $\delta^{15}N$ values from the 25 m sites ranged from 3.2 ± 0.2‰ to 5.2 ± 0.5‰, which were significantly higher than sites 7-12 (p-value =1.43E-05) (Figure 4). The highest value of $\delta^{15}N$ (mean $\delta^{15}N$=5.2) was located at site 2 (Figure 4). The mean %N between 25 and 150 m sites differed (p-value = 1.12E-05) with respective values of 0.91 ± 0.19% and 1.28 ± 0.15%. In the dry season, the overall mean %N in *A. lacerata* blade tissues were 1.11 ± 0.25%.

Dissolved inorganic nutrients nitrate + nitrite (NO_2^- + NO_3^-) and ammonia in collected water samples between 25 m and 150 m did not differ significantly. Mean (NO_2^- + NO_3^-) concentrations for 25 m and 150 m are respectively, 1.79 ± 0.27 µmol/L (n=6) and 1.71 ± 0.14 µmol/L (n=6). Mean ammonia concentrations for 25 m and 150 m are respectively, 0.25 ± 0.18 µmol/L (n=6) and 0.24 ± 0.06 µmol/L (n=6).

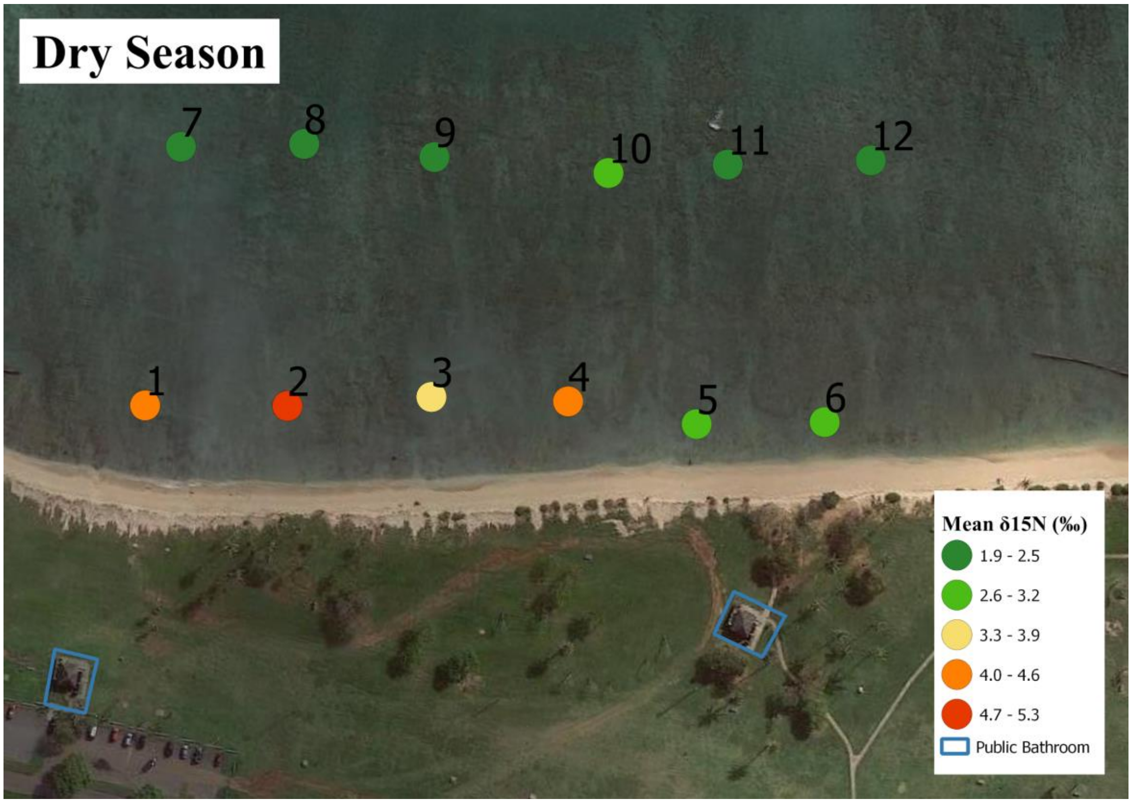

Figure 4: Mean $\delta^{15}N$ (‰) values in *A. lacerata* individuals collected in the dry season on July 2020 from the reefs of Kualoa Regional Beach Park.

3.3 NUTRIENT CONCENTRATIONS BETWEEN WET AND DRY SEASON

The surface waters of Kualoa Regional Beach Park had DIN (NO^{3-} + NO^{2-}, and NH^{4+}) PO_4^{3-}, and H_4SiO_4 concentrations were relatively low in the dry and wet seasons (Table 1). Yet, the average concentrations of NO_2^- + NO_3^- and H_4SiO_4 in the dry season were much higher than in the wet season (p-value < 0.05, One-way Kruskal-Wallis ANOVA). The mean concentration of PO_4^{3-} in the wet season was somewhat higher than the dry season concentrations. No distinct differences in NH_4+ and Total P concentrations were detected between the two seasons (p-value > 0.05, One-way Kruskal-Wallis ANOVA).

Table 1: Nutrient concentrations (mean ± SE) in coastal waters at the Kualoa Regional Beach Park study site in the wet and dry seasons.

Kualoa Regional Beach Park Surface Waters					
µmol/L	**n**	**Wet Season**	**n**	**Dry Season**	**p-value**
$NO_2^- + NO_3^-$	12	1.33 ± 0.40	12	1.75 ± 0.21	0.003*
NH_4^+	12	0.20 ± 0.21	12	0.24 ± 0.13	0.20
H_4SiO_4	12	2.58 ± 0.94	12	8.68 ± 2.42	0.0000323*
PO_4^{3-}	12	0.07 ± 0.01	12	0.04 ± 0.01	0.0000435*
Total P	12	0.18 ± 0.03	12	0.16 ± 0.02	0.05

4.0 DISCUSSION

4.1 SEASONAL ALGAL $\delta^{15}N$ VALUES

Published values can serve as a baseline for Oʻahu, where marine algae collected from the remote and undeveloped Kaʻena Point had $\delta^{15}N$ values between 0.5‰ - 2.8‰ (Amato et al. 2019). The mean algal $\delta^{15}N$ values in this study were slightly higher than the remote baseline values seen in reef plants at Kaʻena Point. In addition, the nitrogen values collected were not within the range for wastewater and therefore does not support our prediction that specimens collected near the septic restrooms will exhibit elevated N values. Rather, the moderately lower values may reflect the much lower use of Kualoa Regional Beach Park during the COVID-19 pandemic; restroom access were closed off and post-restrictions tourism activities were at a decline. In such a situation, KPB $\delta^{15}N$ values from *A. lacerata* more likely reflect a combination of fine-scale nitrogen sources located nearby as well as continued drainage from the bathrooms' septic tanks, associated with rain and groundwater movement.

The N content and $\delta^{15}N$ values in algae were often higher close to the shoreline along the 25 m sites (1-6) in both seasons. Possible interaction between terrestrial N sources and the adjacent marine waters during the wet and dry season may increase or decrease along with changes in rainfall and water motion. During the wet season, plants collected along the 25 m sites exhibit isotopic values that most likely indicate mixed N sources. While in the dry season, reductions in rainfall may concentrate potential N sources delivered by groundwaters, as well as reduced surface discharges. Potential mixed sources at KBP may derive from the nearby septic restrooms, Kualoa Ranch located across the beach, and the cluster of private homes known to be on cesspools within 10 m of the shore. However, blade tissues collected from both seasons remain in low ranges for %N suggesting that during the collection period, a low volume of N is entering the reef.

The wet and dry seasons are likely to differ in underground and overground water flow because of hydrological features. *A. lacerata* specimens collected during the wet season at site 7 and 11 had higher mean $\delta^{15}N$ values than the other 150 m sites. Sand channels can be seen connecting sites 1 and 7 as well as sites 5 and 11. Coincidentally, these sites recorded $\delta^{15}N$ values relatively higher than sites without sand channel links (8, 9, 10, 12) in the wet season. However, this feature was not observed in specimens collected in the dry season where all the 150 m sites $\delta^{15}N$ values are within the natural N range. The absence of elevated $\delta^{15}N$ values at sites 7 and 11 in the dry season may suggest less hydrological interactions via sand channels. We propose that seasonal changes attributed to rainfall, coastal currents, and water motion could be a dynamic factor impacting nutrient distribution along the reef.

4.2 SEASON VARIATION IN WATER CHEMISTRY

Mean concentrations of $NO_2^- + NO_3^-$ and H_4SiO_4 in the dry season are respectively, 1.33 ± 0.40 µmol/L (n=6) and 8.68 ± 2.42 µmol/L (n=6), which are significantly higher than the wet season concentrations, again suggesting possible concentrating impacts during low rainfall. Low mean concentrations of PO_4^{3-} , NO_2^- + NO_3^-, and H_4SiO_4 in the wet season could be attributed to increased rainfall, diluting concentrations of pollutants entering coastal waters.

4.3 KUALOA BEACH PARK AND PAIKO REEF COMPARISON

Values for mean $\delta^{15}N$ (‰) and total %N from *A. lacerata* collected either at Kualoa Beach Regional Park, Kāneʻohe Bay from the wet and dry season, and Albright's (2017) Paikō Reef, Maunalua Bay data in the dry season is summarized in Table 2. Data from the fine-scale mapping survey were not normally distributed and had an unequal sample size. For the dry season sampling at KBP, the target alga, *A. lacerata*, at site 1 was limited, with only one specimen retrieved for analysis. Regardless of season variability, $\delta^{15}N$ and total %N in the blade tissues of *A. lacerata* between Kualoa and Paikō Reef were significantly different (Table 2). $\delta^{15}N$ values analyzed in *A. lacerata* individuals collected from Paikō Reef in the dry season had an average value of 0.5 ± 0.7‰ that is within the low range of fertilizer influence. The collective distribution of $\delta^{15}N$ values relative to %N values from Kualoa and Paikō Reef best represents a polynomial relationship (Polynomial Regression analysis: n =80, r_s=0.2507, p-value=0.00019 (Figure 5).

Table 2: Mean nitrogen parameters values of *Avrainvillea lacerata* from this study. Paikō Reef, Maunalua nitrogen values are from Albright (2017).

Site					
	n	**δ^{15}N (‰)**		**Total N (%)**	
Kualoa *Wet Season*	36	3.5 ± 0.8	**A**	1.31 ± 0.28	
Kualoa *Dry Season*	34	3.1 ± 1.0	**A**	1.11 ± 0.25	
Paikō *Dry Season*	10	0.5 ± 0.7		1.67 ± 0.43	

One-way Kruskal-Wallis ANOVA detected significant differences among site collections for δ^{15}N (‰) and total N (%). Locations that share boldface letters are not significantly different (p-value > 0.05) according to Dunn's test of pairwise comparisons of ranks. Therefore, all parameters except for δ^{15}N values between Kualoa wet and dry seasons were statistically different.

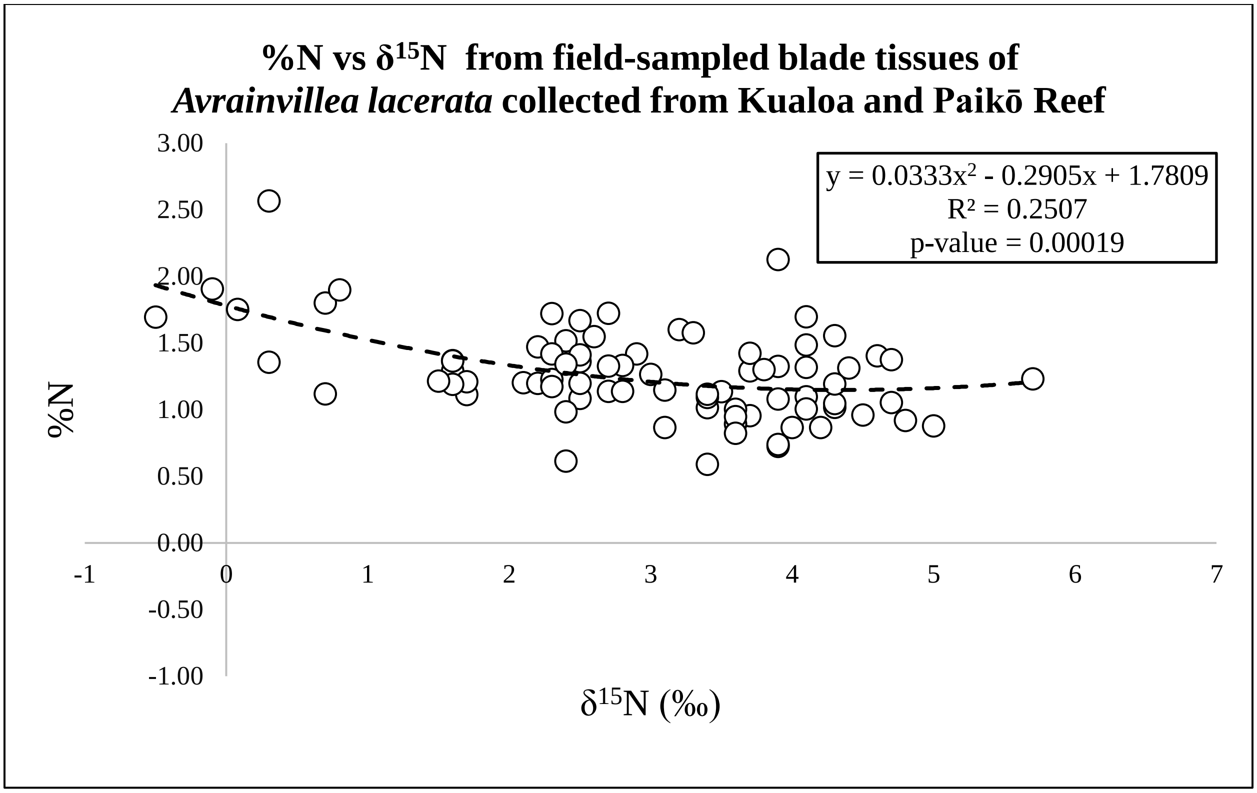

Figure 5. Tissue parameters %N vs. δ^{15}N (‰) from field-sampled tissues of *A. lacerata* at two reef regions, Kualoa and Paikō Reef.

The geographic fine-scale distribution of δ^{15}N values in algal tissues detected during the two seasons and two grouped sites (25 and 150 m) reveals that N sources in these areas act on local scales. KBP has two septic restrooms on land near the shore, Kualoa Ranch private cattle ranch located across the highway, and private homes that are known to be on cesspools north-west of the beach. Therefore, a diverse range of N sources could be influencing the δ^{15}N values variation in *A. lacerata* collected from Kualoa Regional Beach Park, where the mean value for all 12 sites varied between 1.9‰ to 5.2‰.

In contrast, residential homes along Paikō Reef are serviced by sewage lines, and the algal blades' δ^{15}N values in Albright (2017) study ranged from -0.05‰ to 1.8‰. The low tissue δ^{15}N values observed in the *A. lacerata* population at Paikō Reef during a dry season may indicate soil N, NO_3^-, NH^{4+} in fertilizer and rain sources (Kendall & McDonnell 1998). In that study, it is thought that possible anthropogenic N source linking nearby residential fertilizer usage with algal $\delta^{15}N$ and %N values observed in the tissues of the blades and holdfast. However, in this study, the $\delta^{15}N$ values of *A. lacerata* at Kualoa Regional Beach Park were often related to the fine-scale nitrogen sources located where samples were collected and values may be low due to the COVID-19 restrictions in place during the sampling period.

Stable isotopic values ($\delta^{15}N$) and total (% N) within the blade tissues of *A. lacerata* from Kualoa and Paikō Reef were coupled to observe any unifying relationship in the species (Figure 5). The polynomial relationship between $\delta^{15}N$ and %N may suggest that the alga can perform at low N concentrations and is adept at acquiring nutrients from various N sources. The morphological difference between the windward and leeward populations are distinct. *A. lacerata* individuals collected from KBP had relatively small holdfasts compared to its blade region; therefore, this study primarily focused on the photosynthetic region of the plant in relation to its N storage. On the other hand, individuals collected from Paikō Reef had a proportionally larger holdfast region than its upright blades. Albright (2017) showed that %N and $\delta^{15}N$ between basal holdfast and bladed regions significantly differed. Building on her results, *A. lacerata* may have the ability to collect and store nitrogen from otherwise accessed nutrients such as groundwater. To continue these linked investigations, future research should further investigate the partitioning of nutrients between the basal holdfast and blade regions for *A. lacerata*.

5.0 CONCLUSION

5.1 INVASIVE SPECIES MANAGEMENT

This invasive alga is dynamic and resilient. As an invasive Bryopsidalean species, *A. lacerata* can proliferate using several mechanisms (e.g., sexual reproduction, fragmentation and regenerative holdfast, and lack of palatability) to overcome removal, posing a significant threat to native communities in Hawai‘i (Smith et al. 2002). Furthermore, in response to climate change scenarios, the distribution of *A. lacerata* is projected to increase, leaving a quarter of all coastlines in the MHI susceptible for an invasion (Veazey et al. 2019). Regions of concern and removal efforts of this alga should be prioritized in order to protect reef biodiversity in Hawai‘i.

5.2 NUTRIENT MANAGEMENT

Environmental management in Hawai‘i currently uses algal isotopic values to assess risks, take preventative measures, and formulate remediation plans. In the State of Hawai‘i, groundwater reservoirs serve the majority of the community as an essential drinking water resource, and to protect that natural resource, all cesspools will be upgraded, converted, or closed by 2050. In the 2017 legislative session, Act 125 was passed to address the severe health and environmental impacts of cesspool pollution (State of Hawai‘i Department of Health Environmental Management Division, 2017). Stable isotope analysis and other multitracer approaches served as validated assessment tools to change and uphold the Clean Water Act in Hawai‘i Wildlife Fund v. County of Maui. These valuable assessments can be used to identify regions of land where on-site disposal system (OSDS) upgrades will result in improved ecosystem health. The study by Amato et al. (2019) did not include KBP area; therefore, future studies using algal isotopic analysis should include areas with several private class IV (cesspools) such as the ones considered to be high risks located north of the KPB (Figure 6) (Hawaii Statewide GIS Program, data from 2008).

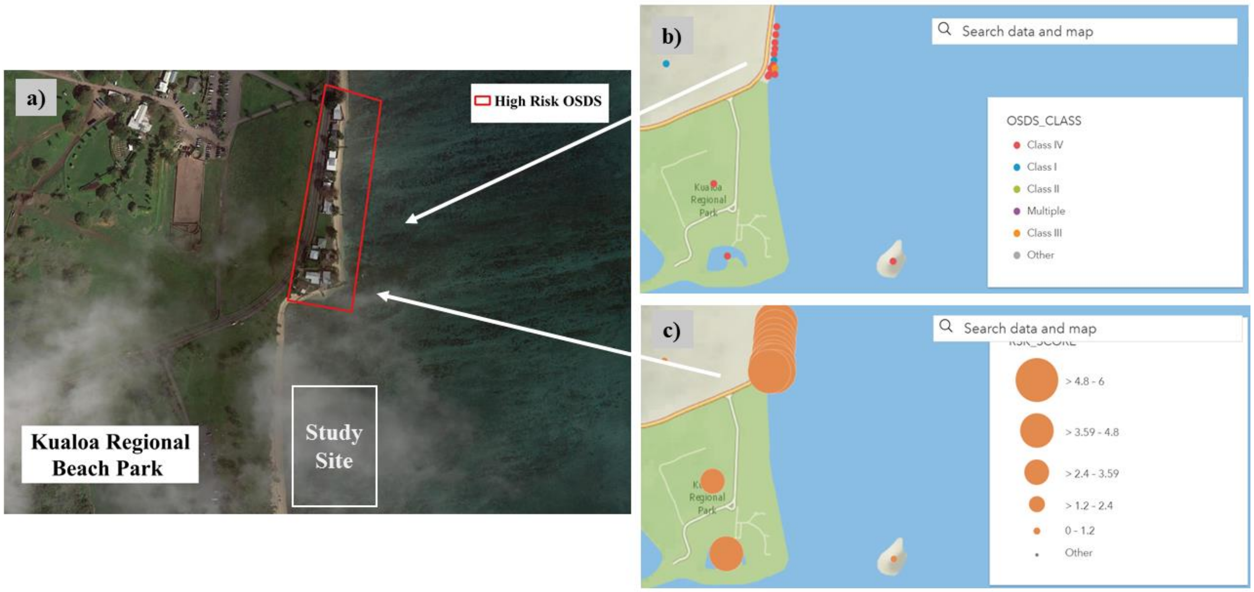

Figure 6 a) Private on-site disposal systems (OSDS) north from KBP. b) OSDS types are classified as Class IV (wastewater discharged with no treatment; cesspool), Class III (anaerobic treatment), and Class I (soil treatment). (c) These residential OSDS are considered to be high risk according to 2008 data from the Hawaiʻi Statewide GIS Program.

Surface currents following the prevailing northeasterly (NE) wind direction can move water along the peninsula where private residentials serviced by OSDS are located. These prevailing currents could transport and deliver seawater contaminated with waste into adjacent recreational waters at Kualoa Regional Beach Park. Such flow could pose significant health risks to the public and nearby coastal ecosystems.

APPENDIX

Table 1. Tissue nitrogen and $\delta^{15}N$ (‰) concentrations from *A. lacerata* specimens collected on February 15, 2020 (wet season) at Kualoa Regional Beach Park.

Wet Season Sample ID	**Weight (mg)**	**µg N**	**%N**	**$\delta^{15}N$ (‰ vs. AIR)**	**µg C**	**$\delta^{13}C$ (‰ vs. VPDB)**
1.1	3.2195	42.3	1.31	4.4	592.7	-13.5
1.2	3.1057	35.3	1.14	3.5	543.4	-11.7
1.3	2.8438	42.3	1.49	4.1	544.7	-12.4
2.1	2.9876	63.6	2.13	3.9	700.6	-17.4
2.2	2.9978	46.7	1.56	4.3	615.3	-12.7
2.3	2.8854	31.7	1.10	4.1	514.5	-11.4
3.1	3.0250	29.1	0.96	4.5	513.3	-8.4
3.2	3.0423	32.9	1.08	3.9	550.6	-11.3
3.3	2.8053	26.8	0.96	3.7	486.5	-9.9
4.1	3.1827	32.0	1.01	3.6	548.6	-10.7
4.2	3.0241	42.5	1.41	4.6	588.6	-12.5
4.3	3.1991	42.4	1.33	3.9	608.4	-11.7
5.1	3.0612	31.1	1.02	3.4	526.4	-9.2
5.2	3.0838	33.7	1.09	3.4	528.2	-9.9
5.3	3.1200	40.3	1.29	3.7	571.6	-12.3
6.1	2.9498	26.4	0.89	3.6	451.6	-9.4
6.2	3.1761	33.5	1.05	4.7	546.3	-10.0
6.3	3.2023	32.6	1.02	4.3	530.3	-9.4
7.1	3.0264	51.4	1.70	4.1	642.1	-15.4
7.2	3.0906	32.4	1.05	4.3	527.4	-10.1
7.3	2.8411	39.1	1.38	4.7	557.0	-12.2
8.1	3.0151	46.7	1.55	2.6	619.1	-14.8
8.2	2.9140	41.4	1.42	2.9	578.3	-14.0
8.3	2.9720	51.2	1.72	2.3	675.7	-16.6
9.1	2.8991	31.5	1.09	2.5	505.6	-10.6
9.2	2.8604	42.1	1.47	2.2	559.0	-14.9
9.3	3.1275	50.1	1.60	3.2	641.0	-13.0
10.1	2.8773	32.8	1.14	2.7	507.3	-11.8
10.2	2.8953	33.0	1.14	2.8	490.2	-12.1
10.3	3.0793	53.1	1.72	2.7	726.7	-13.9
11.1	3.1875	41.5	1.30	3.8	600.4	-11.5
11.2	3.0787	48.6	1.58	3.3	613.4	-14.5
11.3	3.0906	44.0	1.42	3.7	630.1	-14.0
12.1	3.0265	36.4	1.20	2.1	550.2	-12.3
12.2	2.9636	38.3	1.29	1.6	558.7	-13.1
12.3	3.1355	47.6	1.52	2.4	625.9	-14.5

Table 2. Tissue nitrogen and $\delta^{15}N$ (‰) concentrations from *A. lacerata* specimens collected on July 18, 2020 (dry season) at Kualoa Regional Beach Park.

Dry Season Sample ID	**Weight (mg)**	**µg N**	**%N**	**$\delta^{15}N$ (‰ vs. AIR)**	**µg C**	**$\delta^{13}C$ (‰ vs. VPDB)**
1.1	3.0882	36.9	1.19	4.3	545.9	-9.8
2.1	3.0777	28.3	0.92	4.8	492.7	-12.7
2.2	3.0602	26.9	0.88	5.0	475.9	-9.2
2.3	3.0047	37.0	1.23	5.7	536.7	-9.9
3.1	3.0456	26.4	0.87	4.2	465.2	-7.7
3.2	2.9981	17.7	0.59	3.4	433.1	-3.5
3.3	3.0312	22.0	0.73	3.9	466.6	-4.3
4.1	3.0569	26.5	0.87	4.0	495.8	-6.7
4.2	3.0783	22.8	0.74	3.9	465.6	-4.9
4.3	3.1366	31.6	1.01	4.1	541.3	-8.2
5.1	3.0655	29.1	0.95	3.6	476.9	-8.6
5.2	3.0464	25.1	0.82	3.6	468.0	-6.1
5.3	3.0881	19.0	0.62	2.4	435.0	-3.5
6.1	3.1143	34.8	1.12	3.4	533.1	-9.9
6.2	3.1791	36.5	1.15	3.1	555.9	-9.1
6.3	3.0240	26.2	0.87	3.1	480.9	-6.4
7.1	3.0965	51.7	1.67	2.5	613.8	-13.7
7.2	3.0871	37.0	1.20	2.5	555.9	-10.1
7.3	2.9945	42.5	1.42	2.3	552.8	-13.4
8.1	3.0999	42.2	1.36	2.5	643.5	-14.0
8.2	3.0222	40.3	1.33	2.8	561.8	-11.6
8.3	3.0198	36.2	1.20	2.2	562.2	-11.1
9.1	3.0867	34.4	1.11	1.7	543.5	-8.1
9.2	3.0596	37.6	1.23	2.3	555.7	-9.2
9.3	3.0580	35.9	1.17	2.3	555.3	-9.0
10.1	3.0926	40.8	1.32	4.1	589.5	-10.5
10.2	3.0998	43.8	1.41	2.5	577.3	-13.6
10.3	3.0977	30.5	0.98	2.4	518.2	-8.7
11.1	3.0260	40.2	1.33	2.7	574.9	-12.6
11.2	3.0081	38.1	1.27	3.0	549.9	-11.3
11.3	3.1548	38.2	1.21	1.7	570.9	-10.1
12.1	3.0311	41.5	1.37	1.6	525.8	-10.9
12.2	3.0180	40.5	1.34	2.4	565.0	-11.5
12.3	3.0379	36.2	1.19	1.6	559.9	-10.5

Table 3. Dissolved inorganic nutrients (DIN: NO^{3-} + NO^{2-}, and NH^{4+}, PO_4^{3-}, H_4SiO_4) water samples collected on February 15, 2020 (wet season) at Kualoa Regional Beach Park.

Wet Season SLAB	**Total P**	**Phosphate**	**Silicate**	**N+N**	**Ammonia**
I.D.	**µmol/L**	**µmol/L**	**µmol/L**	**µmol/L**	**µmol/L**
1.1	0.19	0.08	3.55	0.59	0.03
1.2	0.16	0.06	2.95	0.55	0.16
3.1	0.23	0.08	4.18	1.80	<0.02
3.2	0.16	0.09	3.46	1.50	0.35
6.1	0.20	0.07	2.88	1.49	<0.02
6.2	5.45	0.15	0.06	2.44	1.28
7.1	0.15	0.06	3.31	1.16	0.15
7.2	0.19	0.08	1.77	1.69	0.59
9.1	0.19	0.08	2.00	1.43	0.04
9.2	0.16	0.07	1.52	1.33	0.10
12.1	0.21	0.06	1.74	1.71	0.22
12.2	0.19	0.07	1.20	1.47	0.64

Table 4. Dissolved inorganic nutrients (DIN: NO^{3-} + NO^{2-}, and NH^{4+}, PO_4^{3-}, H_4SiO_4) in water samples collected on July 18, 2020, (wet season) at Kualoa Regional Beach Park.

Dry Season SLAB	**Total P**	**Phosphate**	**Silicate**	**N+N**	**Ammonia**
I.D.	**µmol/L**	**µmol/L**	**µmol/L**	**µmol/L**	**µmol/L**
1.1	0.14	0.02	4.65	1.53	0.13
1.2	0.17	0.05	4.89	1.83	0.37
3.1	0.16	0.04	7.88	1.48	0.13
3.2	0.16	0.03	9.10	2.11	0.12
6.1	0.15	0.03	10.49	1.71	0.56
6.2	0.17	0.04	12.22	2.11	0.17
7.1	0.14	0.05	8.25	1.67	0.18
7.2	0.14	0.04	6.36	1.46	0.21
9.1	0.16	0.04	8.83	1.81	0.32
9.2	0.23	0.06	9.48	1.77	0.27
12.1	0.15	0.03	11.07	1.73	0.18
12.2	0.17	0.05	10.94	1.82	0.25

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