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Nitrogen stable isotopes (δ^{15}N) and tissue nitrogen in shallow-water and mesophotic macroalgae differ between the Main Hawaiian Islands and the Northwestern Hawaiian Islands

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Abstract

The Hawaiian Archipelago stretches 2500 km from the Main to the Northwestern Hawaiian Islands, represents a complex gradient of oceanographic and anthropogenic drivers, and has a high abundance and diversity of native and invasive macroalgae. These photosynthetic organisms occur in intertidal to mesophotic (30–150+ m) depths and absorb nitrogen with limited fractionation associated with their physiology and source. Our goal was to examine nitrogen dynamics from shallow to mesophotic reefs using compositional patterns of two well-characterized macroalgal tissue parameters: stable isotope ratio of nitrogen and tissue nitrogen content. We collected 813 macroalgal samples from 13 islands/atolls between 0 and 117 m depths. Within the Main Hawaiian Islands, macroalgal tissue stable N isotope ratios were higher in mesophotic depths; N content was higher in shallow depths. However, within the Northwestern Hawaiian Islands, no differences in stable N isotope ratios and N content were found between shallow and mesophotic depths. Regionally, stable N isotope ratios varied along a gradient of anthropogenic and oceanographic processes (in Main and Northwestern Hawaiian Islands, respectively), while N content reflected elevated nitrogen in the Main compared with the Northwestern Hawaiian Islands. Additionally, the invasive macroalga *Avrainvillea lacerata* had significantly higher N content than co-occurring native bryopsidalean macroalgae at similar depths, and may be reshaping nutrient dynamics from shallow to mesophotic depths in the Main Hawaiian Islands. Nitrogen dynamics at mesophotic depths may be influenced by nearshore anthropogenically derived nitrogen via submarine groundwater discharge and/or inputs from deeper water within the Main Hawaiian Islands.

The Hawaiian Archipelago spans 2500 km from the island of Hawai'i at the southernmost end of the Main Hawaiian Islands to Hōlanikū (Kure Atoll) at the northernmost end of the Northwestern Hawaiian Islands (Fig. 1). The Northwestern Hawaiian Islands are contained within the Papahānaumokuākea Marine National Monument—one of the largest and most remote marine protected areas in the world. The range in anthropogenic impact from the nearly pristine Northwestern Hawaiian Islands to the more heavily populated (> 1.4 million people) (https://www.populationu.com/us/hawaii-population) Main Hawaiian Islands creates a gradient for examining nitrogen dynamics in macroalgal-dominated systems (Vroom and Braun 2010; Jouffray et al. 2015; Spalding et al. 2019b) across multiple scales of depth, island/atoll, and region. Effective coral reef conservation requires establishing baselines across gradients of human impact, particularly regarding the impact of pollution and overfishing (Sandin et al. 2008).

The Main Hawaiian Islands coastal reefs have a high abundance of gently sloping hard and soft-bottom habitats at mesophotic depths, while the Northwestern Hawaiian Islands reefs have steeper terrain (Rooney et al. 2010; Pyle et al. 2016; Spalding et al. 2019b). Macroalgal, coral, and fish composition within mesophotic coral ecosystems varies with depth and between the Main Hawaiian Islands and Northwestern Hawaiian Islands (Spalding et al. 2019b). At 30–50 m depths, large beds of the green alga, *Microdictyon setchellianum*, and brown macroalgae (*Dictyopteris* sp. or *Sargassum* sp.) are present in the Northwestern Hawaiian Islands (Parrish and Boland 2004). In

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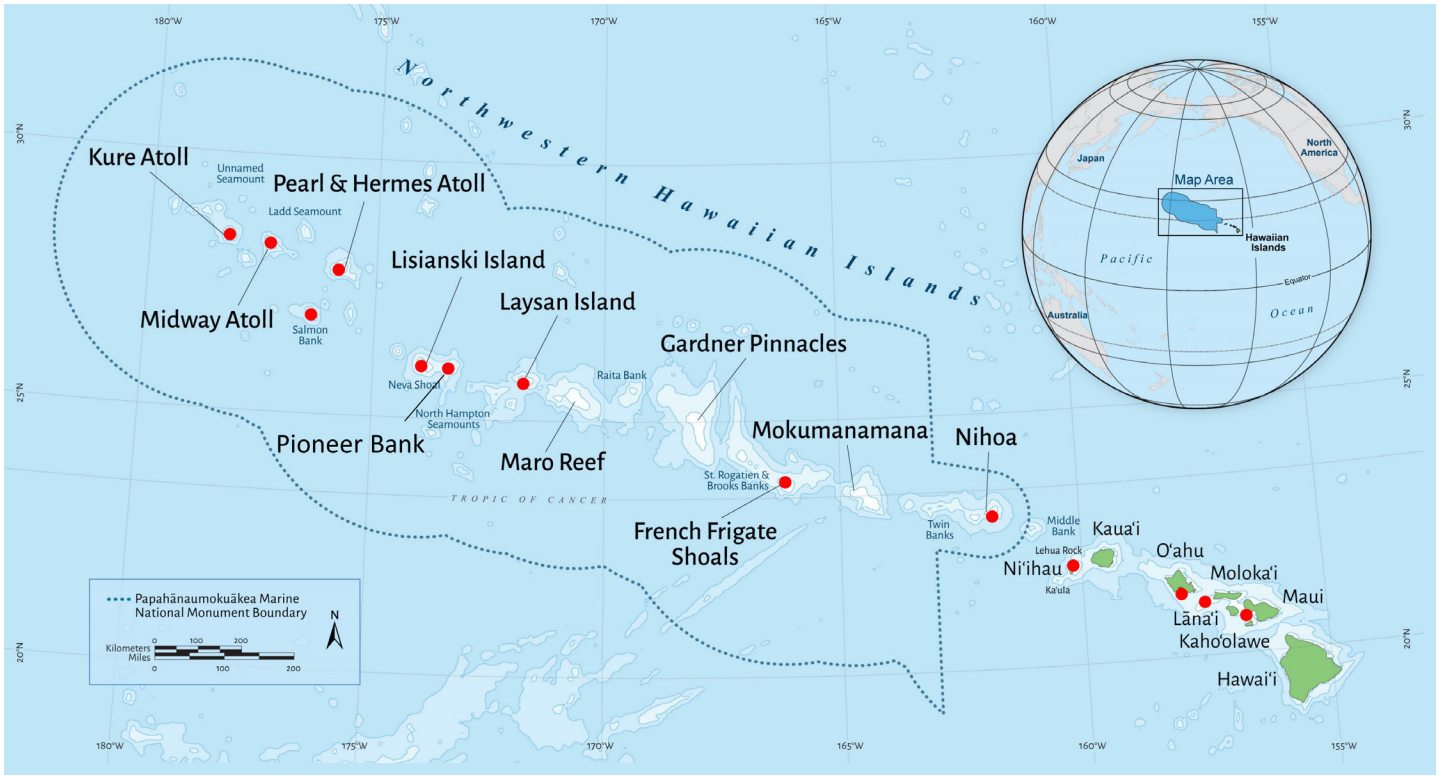

Fig. 1. The Hawaiian Archipelago. Green islands represent the Main Hawaiian Islands. Islands within the Northwestern Hawaiian Islands (dotted outline) compose the Papahānaumokuākea Marine National Monument. Red dots indicate sample locations. Map courtesy of Papahānaumokuākea Marine National Monument.

the Main Hawaiian Islands, psammophytic meadows of the calcified green alga, *Halimeda kanaloana* (Verbruggen et al. 2006; Spalding et al. 2019a) and low-relief branching coral are the most abundant species over unconsolidated sediments and scattered carbonate substrate with increasing depth until ~ 80 m, where *Leptoseris* coral is abundant to 130 m depths. Scleractinian coral (~ 22%) and macroalgae (~ 36%) are abundant from 50 to 70 m depths in the Northwestern Hawaiian Islands (Rooney et al. 2010) but found in highest abundance at 30–40 m (Scleractinian coral; ~ 17%) and 40–50 m (macroalgae; 43%) in the Northwestern Hawaiian Islands (Rooney et al. 2010).

The factors influencing the abundances and differences in mesophotic macroalgal cover across the archipelago are unclear, as these low-light and normally oligotrophic ecosystems would not be expected to support such diverse and abundant macroalgal cover (Spalding et al. 2016, 2019a,b). We hypothesize that an increase in nitrogen within mesophotic coral ecosystems contributes toward the high abundance of macroalgae in these low-light environments. Likely mechanisms in high oceanic islands include submarine groundwater discharge (SGD) transporting elevated nitrogen levels to mesophotic reefs, or nitrogen imported from deeper waters beyond mesophotic depths.

With the exception of the recent discovery of the cryptogenic, invasive-like alga *Chondria tumulosa* (Sherwood et al. 2020), the Northwestern Hawaiian Islands do not contain invasive macroalgae. In comparison, the Main Hawaiian Islands contain several species of invasive Chlorophyta and Rhodophyta (Smith et al. 2002; Foster et al. 2019). Invasive macroalgae may negatively impact communities because of their abilities to alter biotic and abiotic factors (Bellgrove et al. 2017). The siphonous green alga *Avrainvillea lacerata* has transformed Hawaiian reefs (both shallow and mesophotic) into muddy seascapes and has outcompeted the native flora (Smith et al. 2002; Peyton 2009; Wade et al. 2018; Foster et al. 2019; Wade 2019). This species has a unique mound-building feature that sequesters soft sediments around the holdfast (Littler et al. 2004) enabling this alga to grow in soft sediments or on hard substrate (Littler et al. 2004; Peyton 2009; Wade et al. 2018; Foster et al. 2019; Wade 2019).

Nitrogen stable isotopes and tissue nitrogen are commonly used to determine sources and flow of nutrients within a food web. δ^{15}N can be used to trace nitrogen through food webs (Peterson and Fry 1987; Gillies et al. 2012), and in some cases, indicate specific nitrogen sources (McClelland et al. 1997; Costanzo et al. 2005; Dailer et al. 2010, 2012a,b; Lapointe and Bedford 2011; Lapointe et al. 2021a). Non-anthropogenic signatures of δ^{15}N typically range from 0‰ to 4‰, while

nitrogen from natural fertilizer, synthetic fertilizer, and sewage sources generally ranges from 0‰ to 4‰, −4‰ to 4‰, and 7‰ to 38‰, respectively (Kendall and McDonnell 1998; Gartner et al. 2002; Dailer et al. 2010). However, non-anthropogenic values can fluctuate outside of the 0–4‰ range due to denitrification, nitrification, upwelling, or excrement from animal populations. Macroalgae are commonly used to probe sources of nitrogen in coastal regions because of their ability to incorporate nitrogen with little to no isotopic fractionation or discrimination of source, especially in low nutrient tropical environments (Gartner et al. 2002; Cohen and Fong 2005; *see* summary in Dailer et al. 2010). Percent nitrogen (%N) in algal tissues is used as a proxy for N-flux into the environment (Amato et al. 2016) and has been used to determine anthropogenic nitrogen enrichment within macroalgal tissue (Lapointe and Bedford 2011). Values exceeding 2.0% N typically indicate nitrogen enrichment from anthropogenic (Amato et al. 2016) or non-anthropogenic sources. δ^{15}N and %N may be used in combination to determine the source of nitrogen more accurately in macroalgal tissue (Amato et al. 2016, 2020).

Species or genus-specific differences in δ^{15}N fractionation may exist depending on an alga's physiological mechanisms for nitrogen processing (Vaughn et al. 2021) or biological uptake of nitrate (Mariotti et al. 1982), requiring caution when interpreting results across different species or genera. However, most macroalgae have heterogeneous, patchy distributions (Pyle et al. 2016; Spalding et al. 2019b) that make collecting replicates of the same genera or species at every site difficult, especially when examining trends across broad scales, such as the Hawaiian Archipelago. This lack of replication in sample size at the species level is particularly difficult in mesophotic coral ecosystems where access is challenging and limited to submersibles or technical diving. The repeated collection of the most abundant members of macroalgal communities across multiple sites and depths allow for the interpretation of broad scale patterns, and more detailed analyses of commonly encountered species.

We extensively sampled the most abundant macroalgae throughout the Hawaiian Archipelago and focused on analyzing macroalgal δ^{15}N and %N data at multiple scales: gross spatial trends at the genus level across the region at mesophotic vs. shallow depths, refined spatial patterns of the most abundant species regionally, and site-specific differences in species in an extensively sampled location. We were also interested in the nitrogen content and sources used by the invasive *A. lacerata* as compared to native macroalgae, and between shallow and mesophotic depths. Investigating the tissue nitrogen of common macroalgae in mesophotic coral ecosystems and shallow-water communities will deepen our understanding of these two habitats. This is the first comprehensive study of nitrogen quantities and sources in macroalgal tissue across the photic zone in a tropical archipelago.

Materials and methods

Study sites

Mesophotic (30–117 m) macroalgae samples were collected from 13 islands/atolls across the Hawaiian Archipelago, while shallow-water (0–30 m) macroalgae samples were collected from six islands/atolls (Fig. 1). In the Northwestern Hawaiian Islands, the specific location and depth of samples was dependent on the predetermined dive locations of previous and existing support (NOAA, National Fish and Wildlife Foundation) and the management priorities of the Papahānaumokuākea Marine National Monument. Sites in the Main Hawaiian Islands were selected based upon the availability of hard, gently sloping substrate from 40 to 200 m depths, with an emphasis on sites around the islands of O'ahu and the Maui Nui Island complex. Samples ($n = 813$) were collected during the summer (May to July) and fall (August and September) months from 2012 to 2019 during periods of calm weather.

Collection methods

Mesophotic collections in the Northwestern Hawaiian Islands were conducted by technical divers using closed circuit rebreathers. Samples in the Main Hawaiian Islands were collected by submersibles (*Pisces* IV and *Pisces* V submersibles, Hawai'i Undersea Research Laboratory) and technical divers using closed circuit rebreathers. Shallow-water collections were collected via open-circuit SCUBA. Typically, three replicates of each algal species at each site and depth were collected by hand or the submersible manipulator arm and placed in plastic bags or individual containers per depth, respectively. The most visually abundant macroalgal species (5–10% cover) were collected at each depth in an area $\sim$ 10–20 m^2 depending upon time at depth.

Sample processing/isotope analysis

Samples were sorted to the genus or species level based on morphology and identified using Hawaiian macroalgal taxonomic references (Abbott 1999; Abbott and Huisman 2004; Huisman et al. 2007). After sorting, species were rinsed with deionized water, and preserved for stable isotope analyses (see below) and in silica, DMSO, or both for future molecular analyses. Only clean (without epiphytes or encrusting invertebrates) macroalgal thalli representative of new growth were used for analyses. Holdfast regions were excluded from the analysis.

Several genera of macroalgae had calcified tissue, which impacted the weight of the samples. Acidification is generally known to degrade nitrogen within calcified macroalgae (Strait and Spalding 2021), suggesting that acidification of calcified macroalgae would produce variable nitrogen parameters. Thus, calcified samples produced slightly lower %N due to an increased sample weight from $CaCO_3$ (Strait and Spalding 2021). We attempted to reduce this effect in *H. kanaloana*, *Halimeda*

spp., and *Udotea geppiorum* samples by selecting apical regions of new growth on thalli with little to no calcification for tissue nitrogen analyses.

Samples used for isotope analyses were rinsed with deionized water, dried with paper towels, and then dried at 60°C to a constant weight in a drying oven (Dailer et al. 2010; Lapointe and Bedford 2011; Amato et al. 2016). Dried samples were ground with a mortar and pestle into a fine powder, weighed, and packaged into a tin capsule. Tissue δ^{15}N and nitrogen of the packaged samples were determined using a Costech ECS 4010 Elemental Combustion System/Zero Blank Autosampler/ThermoFinnigan MAT Conflo IV/ThermoFinnigan DeltaXP at the Biogeochemical Stable Isotope Laboratory, University of Hawai'i at Mānoa. Ratios of ^{15}N : ^{14}N are expressed relative to atmospheric nitrogen and calculated as in Sweeney et al. (1978) using:

$$\delta^{15}\mathrm{N} = \left[\left(R_{\mathrm{sample}}/R_{\mathrm{standard}}\right) - 1\right] \times 10^3$$

$$R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N}$$

Dry weight of the algal tissue and nitrogen in algal tissue was used to determine %N.

Data analysis

Analyses of δ^{15}N and %N by algal taxon, location, and depth were performed in R version 1.4.1106. Data were visually inspected for normality and Levene tests were used to assess variance. For data that fit the assumptions of parametric testing, one- and two-way ANOVAs (with and without interaction terms) and Tukey's post hoc tests were used to compare samples. Datasets that were not normally distributed were log-transformed. Randomization tests were used on the remaining data that did not meet parametric assumptions. The variable of interest (δ^{15}N or %N), in one- and two-way interaction ANOVAs, was randomized and the test underwent 10,000 permutations. New *p*-values were calculated using the new *f* statistics and number of permutations. Bonferonni correction was applied to the α values to correct for multiple comparisons. Tukey's post hoc tests were used to compare randomized samples.

We analyzed the data on multiple spatial scales. First, we assessed regional differences (Main Hawaiian Islands vs. Northwestern Hawaiian Islands) in δ^{15}N and %N values, followed by regional and depth differences using an interaction term (region × depth). Second, because samples were collected from different islands/atolls, data were analyzed by location to determine the importance of location. Lastly, to address site- and depth-specific variability during a single time period, we determined the differences in %N values from collection sites at Manawai (Pearl and Hermes Atoll). Manawai was sampled extensively (14 sites and 77 samples) in August 2019.

Most analyses were conducted at the genus level for genera with cryptic species that were difficult to differentiate morphologically or for genera with few species' replicates. We determined if genus was a significant factor using one-way ANOVAs for all samples and subsets of Main Hawaiian Islands, Northwestern Hawaiian Islands, shallow, and mesophotic samples. The most abundant genera collected that occurred across the photic zone were compared by region with an interaction term (genus × depth). Blades from *A. lacerata* and slightly calcified apical regions of *H. kanaloana*, and *Halimeda* spp. were selected from the Main Hawaiian Islands. Calcified *U. geppiorum* was also selected for comparison but was only found at mesophotic depths limiting an analysis of genus by depth. *Caulerpa* sp., *Dasya* sp., *Halimeda* spp., and *Microdictyon* sp. were selected from the Northwestern Hawaiian Islands. Additionally, we analyzed mesophotic *A. lacerata*, *H. kanaloana*, *Halimeda* spp., and *U. geppiorum* tissue δ^{15}N and %N to determine if differing holdfast structures influence tissue nitrogen content.

Results

A total of 813 macroalgal samples from 13 islands/atolls throughout the Hawaiian Archipelago were collected and analyzed for δ^{15}N and %N. We collected 26 different macroalgal genera which comprised 679 Chlorophyta, 85 Rhodophyta, and 49 Phaeophyceae samples. The taxa with the greatest sample sizes from the Main Hawaiian Islands were *H. kanaloana*, *A. lacerata*, *Halimeda* spp., and *Ulva/Umbraulva* spp. (Table 1). *Ulva/Umbraulva* spp. produced the highest average δ^{15}N values (4.5‰ ± 0.2‰; Table 1), while *A. lacerata* produced the highest average %N values (4.5% ± 0.1%; Table 1) and had several samples from mesophotic depths that exceeded 5.0%. The genera with the greatest sample sizes from the Northwestern Hawaiian Islands were *Halimeda* spp., *Microdictyon* spp., *Codium* sp., and *C. tumulosa*. *Chondria* sp. (5.0‰ ± 0.0‰; Table 2) and *Caulerpa* sp. (2.2% ± 0.1%; Table 2) produced the highest average δ^{15}N and %N respectively from the Northwestern Hawaiian Islands.

δ^{15}N differed significantly between the Main Hawaiian Islands and Northwestern Hawaiian Islands ($F = 76.0$, df = 1, $p < 0.0001$), with averages of 2.4 ± 0.06‰ and 3.2 ± 0.08‰, respectively. Samples collected from both regions had elevated δ^{15}N levels; however, samples from the Main Hawaiian Islands had a greater range (−2.4‰ to 8.5‰; Northwestern Hawaiian Islands: −0.6‰ to 6.8‰) of values and higher variation (Fig. 2a). %N also differed significantly between the regions ($F = 200.4$, df = 1, $\alpha = 0.025$, $p = 0.0001$). The range of %N was greater within the Main Hawaiian Islands (0.2–5.7%; Avg: 2.4% ± 0.05%), with 18 samples from south O'ahu and 1 sample from Maui exceeding 5.0%. Few samples from the Northwestern Hawaiian Islands (0.0–3.6%; Avg: 1.2% ± 0.04%) exceeded 3.0% and most remained under 2.0% (Fig. 2b).

Table 1. Average and range of δ^{15}N and %N of macroalgal taxon collected from the Main Hawaiian Islands. Islands/atolls are listed north (Niʻihau) to south (Maui). "-" indicates $n = 0$. Only the apical tips or new growth of calcified algae (*) were used for analyses.

Taxon	δ^{15}N ± SE (‰)	Min (‰)	Max (‰)	% N ± SE (%)	Min (%)	Max (%)	Niʻihau (*n*)	Oʻahu (*n*)	Molokaʻi (*n*)	Maui (*n*)	Total (*n*)
Shallow											
Avrainvillea lacerata	1.2±0.2	−1.7	3.2	3.2±0.1	2.4	4.7	-	41	-	-	41
*Halimeda kanaloana**	2.4±0.1	−2.4	5.2	2.6±0.0	0.9	5.2	-	-	-	222	222
Halimeda spp.*	3.2±0.2	1.5	4.4	1.9±0.2	0.8	2.8	1	8	-	3	12
Mesophotic											
Amansia glomerata	2.9±0.3	2.2	3.4	2.6±0.1	2.3	2.8	-	-	-	4	4
Avrainvillea lacerata	1.5±0.1	−0.1	3.6	4.5±0.1	0.7	5.7	-	61	-	-	61
Codium sp.	1.9±0.5	−0.4	3.7	1.3±0.1	0.7	2.0	-	1	-	11	12
Distromium sp.	3.3±0.2	3.0	3.9	1.6±0.2	1.3	2.4	2	-	-	3	5
*Halimeda kanaloana**	2.9±0.2	1.4	4.2	2.2±0.1	1.5	3.5	-	-	-	22	22
Halimeda spp.*	2.3±0.1	0.0	4.9	0.9±0.1	0.2	2.7	-	34	18	19	71
Spatoglossum macrodontum	3.1±0.2	2.6	3.5	1.6±0.2	1.2	2.1	-	4	-	1	5
*Udotea geppiorum**	3.0±0.1	1.3	4.0	0.7±0.1	0.3	1.7	-	-	-	34	34
Ulva/Umbraulva spp.	4.5±0.2	3.0	8.5	1.7±0.1	0.5	3.2	-	1	18	25	44

Separating shallow and mesophotic samples revealed a significant difference in both δ^{15}N and %N ($p < 0.0001$) for both the Main Hawaiian Islands and Northwestern Hawaiian Islands. Because of differences in δ^{15}N and %N between the two regions, an interaction term (region × depth) was added to the ANOVA analyses. The factor δ^{15}N did not produce a significant interaction ($F = 2.28$, df = 1, $p = 0.1310$; Fig. 3a). However, there was an additive effect of region ($F = 61.5$, df = 1, $p < 0.0001$) and enrichment with depth ($F = 50.8$, df = 1, $p < 0.0001$) on δ^{15}N. The Main Hawaiian Islands shallow-water samples from Oʻahu and Maui produced the most and lowest negative δ^{15}N values, although all regions and depths included negative values. Main Hawaiian Islands mesophotic samples produced values > 6.0‰ (Fig. 3a).

Both depth ($F = 25.0$, df = 1, $\alpha = 0.0125$, $p = 0.0001$) and region ($F = 208.4$, df = 1, $\alpha = 0.0125$. $p = 0.0001$) were significant, and a significant interaction ($F = 9.3$, df = 1, $\alpha = 0.0125$, $p = 0.0026$) was found for %N (Fig. 3b). The Main Hawaiian Islands samples had greater %N averages and ranges than the Northwestern Hawaiian Islands samples. Main Hawaiian Islands mesophotic samples (2.1% ± 0.1%) from Oʻahu, Maui, Molokaʻi, and Niʻihau produced the greatest range and differed significantly from the Main Hawaiian Islands shallow-water samples (2.6% ± 0.04%; Tukey's post hoc, $p < 0.0001$). Main Hawaiian Islands samples across the photic zone exceeded 4.0% N with most shallow-water samples exceeding 2.0% N in tissues (Fig. 3b). Samples from the Northwestern Hawaiian Islands had a smaller range and did not differ between shallow and mesophotic depths (Tukey's post hoc, $p = 0.9874$) in %N. Nine Northwestern Hawaiian Islands samples of various genera and from five different islands/atolls exceeded 3.0%, but most samples were < 2.0% N.

Multiple one-way ANOVA tests produced p values < 0.0001 for the parameter genera. The green algae *A. lacerata*, *H. kanaloana*, and *Halimeda* spp. were the most sampled genera across the photic zone from the Main Hawaiian Islands. *Udotea geppiorium* was selected for comparison with the other three bryopsidalean genera. A randomization test of two-way ANOVAs with interaction terms (genus × depth) revealed there was an interaction ($F = 7.7$, df = 2, $\alpha = 0.025$, $p = 0.0001$) for δ^{15}N, and significant differences between the most sampled Main Hawaiian Islands genera ($F = 50.8$, df = 2, $\alpha = 0.0125$, $p < 0.0001$) and depth ($F = 1.85$, df = 1, $\alpha = 0.025$ $p < 0.0001$). All genera did not differ in δ^{15}N between shallow and mesophotic depths (Fig. 4a). Analysis of %N data produced significant differences for both genera ($F = 478.0$,

Table 2. Average and range of δ^{15}N and %N of macroalgal taxon collected from the Northwestern Hawaiian Islands. Islands/atolls are listed from north (Hōlanikū) to south (Nihoa). "-" indicates $n = 0$. The names for the Northwestern Hawaiian Islands are Hōlanikū (Kure Atoll), Kuaihelani (Midway Atoll), Manawai (Pearl and Hermes Atoll), Kapou (Lisianski), Kamole (Laysan), and Lalo (French Frigate Shoals). Only the apical tips or new growth of calcified algae (*) were used for analyses.

Genus	δ^{15}N ± SE (‰)	Min (‰)	Max (‰)	% N ± SE (%)	Min (%)	Max (%)	Hōlanikū (*n*)	Kuaihelani (*n*)	Manawai (*n*)	Salmon Bank (*n*)	Kapou (*n*)	Pioneer Bank (*n*)	Kamole (*n*)	Lalo (*n*)	Nihoa (*n*)	Total (*n*)
Shallow																
Caulerpa sp.	3.6±0.1	3.4	3.9	1.8±0.0	1.7	1.8	-	-	-	-	3	-	-	-	-	3
Chondria tumulosa	2.8±0.1	2.2	3.4	1.6±0.1	0.9	2.7	-	-	24	-	-	-	-	-	-	24
Dasya atropurpurea	3.4±0.1	3.2	3.6	2.2±0.1	2.1	2.3	-	-	-	-	3	-	-	-	-	3
Halimeda spp.*	2.8±0.2	0.9	6.2	1.1±0.1	0.4	2.4	-	-	12	-	6	-	-	12	-	30
Laurencia sp.	3.4±0.6	0.9	5.0	1.5±0.1	1.1	2.0	-	-	6	-	-	-	-	2	-	8
Liagora sp.*	3.0±0.1	2.4	3.4	0.5±0.1	0.0	0.8	-	-	-	-	3	-	-	8	-	11
Microdictyon spp.	1.7±0.2	−0.2	3.0	1.2±0.1	0.9	2.0	-	-	9	-	3	-	-	3	-	15
Nemacystus sp.	3.2±0.6	2.1	4.3	0.8±0.0	0.7	0.8	-	-	3	-	-	-	-	-	-	3
Mesophotic																
Amansia sp.	2.0±0.7	0.7	2.8	2.1±0.2	1.7	2.4	-	2	-	-	-	-	-	1	-	3
Caulerpa sp.	4.1±0.3	2.4	5.2	2.2±0.1	1.8	2.9	-	-	4	-	-	1	1	2	-	8
Chondria sp.	5.0±0.0	5.0	5.0	2.0±0.0	2.0	2.0	1	-	-	-	-	-	-	-	-	1
Cladophora sp.	3.4±0.2	−0.3	4.6	1.6±0.1	0.8	3.1	-	-	-	-	7	4	-	10	-	21
Codium sp.	3.7±0.2	1.1	5.5	1.0±0.1	0.5	1.9	3	3	9	2	5	-	1	4	1	28
Dasya atropurpurea	4.3±0.3	2.7	6.3	1.7±0.2	0.8	3.6	5	-	3	2	1	1	-	-	-	12
Dichotomaria sp.*	3.6±0.2	3.1	4.6	0.7±0.1	0.5	1.0	-	-	1	-	3	-	1	-	1	6
Dictyota sp.	3.9±0.6	0.8	4.9	1.0±0.1	0.5	1.4	4	-	-	-	-	1	-	-	1	6
Distromium sp.	3.2±0.4	0.4	5.0	1.1±0.1	0.6	1.9	1	-	-	-	-	-	4	3	1	9
Galaxaura sp.*	4.2±0.3	3.6	4.8	0.4±0.0	0.4	0.5	-	-	3	-	-	-	-	-	-	3
Gracilaria sp.	4.8±0.5	3.8	5.8	1.4±0.2	1.1	1.8	1	-	-	1	-	2	-	-	-	4
Halimeda spp.*	2.6±0.3	0.3	5.3	1.2±0.1	0.2	2.3	1	-	3	-	5	1	3	6	1	20
Microdictyon spp.	2.2±0.3	−0.6	5.2	1.1±0.1	0.7	3.0	2	1	3	1	5	3	1	5	-	21
Padina spp.*	3.9±0.4	2.3	6.8	0.7±0.1	0.4	1.1	1	1	4	-	-	1	1	2	-	10
"*Peyssonnelia*" sp.*	3.3±0.3	2.8	3.6	0.9±0.2	0.6	1.4	1	-	2	-	-	-	-	-	-	3
Sargassum obtusifolium	3.2±0.3	2.4	4.5	1.1±0.2	0.7	1.8	3	-	2	1	-	-	-	-	-	6
Scinaia sp.	4.8±0.3	4.2	5.2	0.8±0.2	0.5	1.3	-	-	1	-	-	-	-	1	1	3
Sporochnus sp.	3.5±0.5	1.8	4.7	1.4±0.1	0.9	1.7	2	-	1	-	-	2	-	-	-	5
Ulva/Umbraulva spp.	4.6±0.3	2.4	5.8	1.1±0.1	0.3	2.0	4	-	4	-	1	2	-	3	-	14

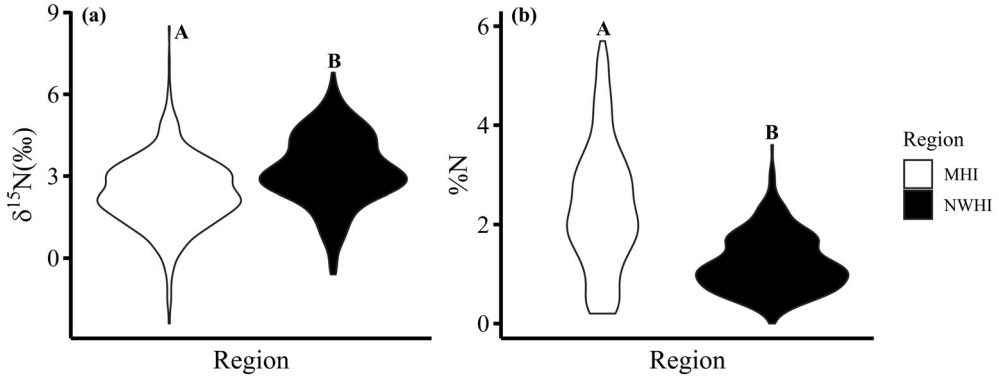

Fig. 2. Violin plots of (**a**) δ^{15}N and (**b**) %N from shallow and mesophotic macroalgae samples collected from the Main Hawaiian Islands (MHI) ($n = 533$) and Northwestern Hawaiian Islands (NWHI) ($n = 280$). Letters indicate significant differences from (**a**) a one-way ANOVA ($p < 0.0001$) and (**b**) a randomization test of a one-way ANOVA ($\alpha = 0.025$, $p = 0.0001$).

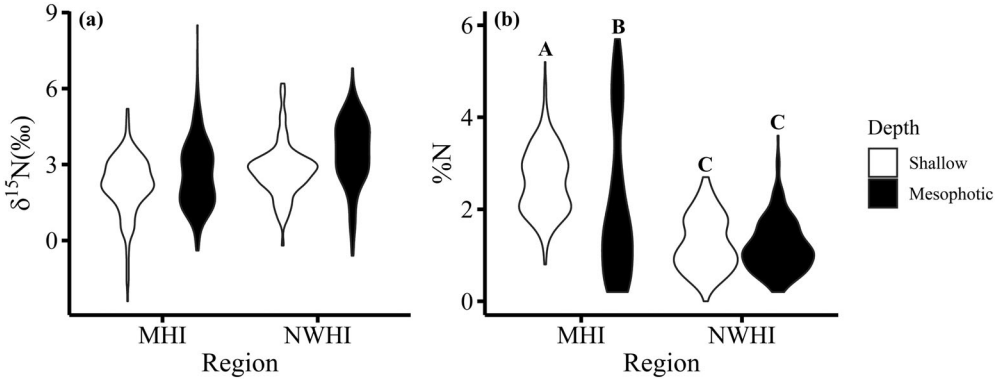

Fig. 3. Violin plot of (**a**) δ^{15}N and (**b**) %N from macroalgae samples collected from the Main Hawaiian Islands (MHI) (shallow $n = 275$; mesophotic $n = 258$) and Northwestern Hawaiian Islands (NWHI) (shallow $n = 97$; mesophotic $n = 183$) at shallow and mesophotic depths. Letters indicate significant differences from (**b**) a randomization test of a two-way interaction ANOVA ($\alpha = 0.0125$, $p = 0.0032$) and Tukey's post hoc test ($p < 0.0001$).

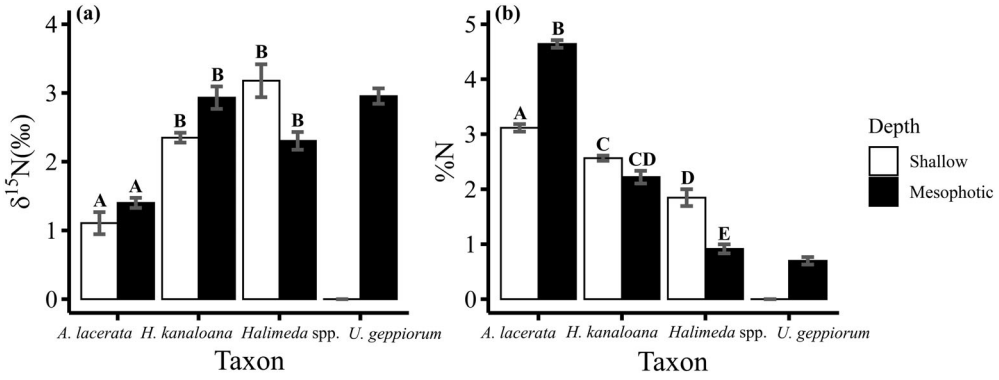

Fig. 4. Average (**a**) δ^{15}N and (**b**) %N from tissues of macroalgal genera with the highest sample sizes from both shallow and mesophotic depths in the Main Hawaiian Islands. *A. lacerata* (shallow $n = 41$; mesophotic $n = 61$), *H. kanaloana* (shallow $n = 222$; mesophotic $n = 22$), *Halimeda* spp. (shallow $n = 12$; mesophotic $n = 71$), *U. geppiorum* (shallow $n = 0$; mesophotic $n = 34$). Error bars are standard error. Letters indicate significant differences from randomization tests of two-way interaction ANOVAs ($\alpha = 0.0125$, $p = 0.0001$) and Tukey post hoc tests ($p < 0.0001$). *U. geppiorum* was selected for comparison with other genera but excluded from analyses because it is only found in the mesophotic.

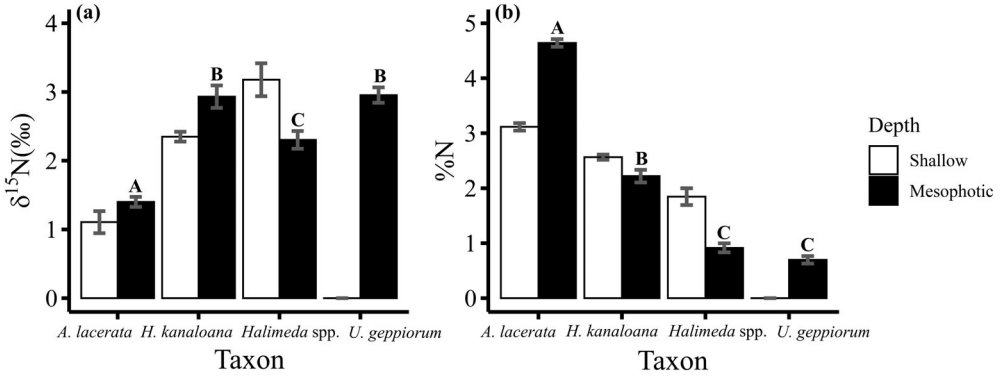

Fig. 5. Average (**a**) δ^{15}N and (**b**) %N from mesophotic macroalgal taxon tissue with differing holdfast structures. *A. lacerata* ($n = 61$) is saxicolous/psammophytic, *H. kanaloana* ($n = 22$) and *U. gepporium* ($n = 34$) are psammophytic, and *Halimeda* spp. ($n = 71$) is saxicolous. Error bars are standard error. Letters indicate significant differences from randomization tests of one-way ANOVAs ($\alpha = 0.025$, $p = 0.0001$) and a Tukey's post hoc test ($p < 0.0001$).

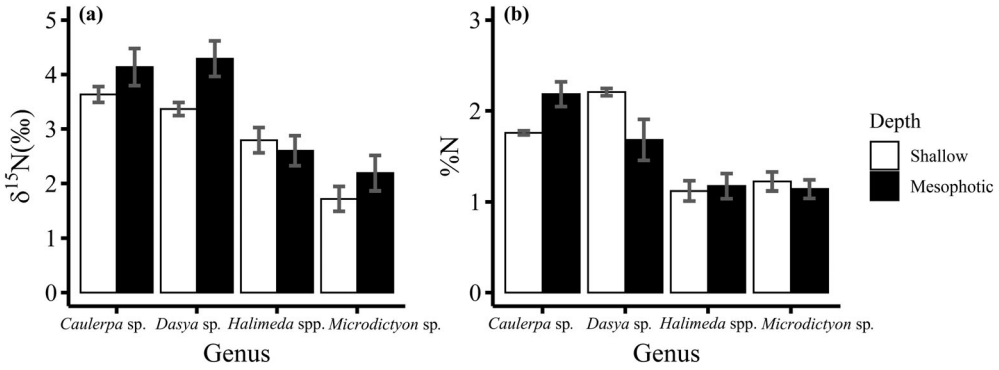

Fig. 6. Average (**a**) δ^{15}N and (**b**) %N from macroalgal genera tissues with greatest sample sizes in both shallow and mesophotic depths in the Northwestern Hawaiian Islands. *Caulerpa* sp. (shallow $n = 3$; mesophotic $n = 8$), *Dasya* sp. (shallow $n = 3$; mesophotic $n = 12$), *Halimeda* spp. (shallow $n = 30$; mesophotic $n = 20$), *Microdictyon* sp. (shallow $n = 15$; mesophotic $n = 21$). Error bars are standard error.

df = 2, $\alpha = 0.0125$, $p < 0.0001$) and depth ($F = 18.5$, df = 1, $\alpha = 0.0125$, $p < 0.0001$); along with an interaction between the two ($F = 7039$, df = 2, $\alpha = 0.0125$, $p = 0.0001$). *A. lacerata* (Tukey's post hoc test, $p < 0.0001$; Fig. 4b) and *Halimeda* spp. (Tukey's post hoc test, $p < 0.0001$; Fig. 4b), %N values differed between shallow and mesophotic depths. *A. lacerata* %N increased with depth (3.1% ± 0.07% to 4.6% ± 0.07%), while %N values for *Halimeda* spp. decreased with depth (1.9% ± 0.2% to 0.9% ± 0.1%; Fig. 4b).

There was a significant difference in mesophotic *A. lacerata*, *H. kanaloana*, *Halimeda* spp., and *U. geppiorum* δ^{15}N among genera ($F = 34.2$, df = 3, $\alpha = 0.025$, $p = 0.0001$). *A. lacerata* differed from all other genera (Tukey's post hoc, $p < 0.0001$; Fig. 5a), while only *H. kanaloana* and *U. geppiorum* did not differ (Tukey's post hoc, $p = 0.9998$; Fig. 5a). Mesophotic algal tissues yielded %N with significant differences between genera as well ($F = 542.2$, df = 3, $\alpha = 0.025$, $p = 0.0001$). *A. lacerata* had significantly higher %N than both psammophytic green algal genera (*U. gepporium* and *H. kanaloana*) and *Halimeda* spp. (Tukey's post hoc, $p < 0.0001$; Fig. 5b); however, *Halimeda* spp. and *U. geppiorum* did not differ (Tukey's post hoc, $p = 0.2874$; Fig. 5b).

Caulerpa sp., *Dasya* sp., *Halimeda* spp., and *Microdictyon* sp. were the most sampled genera across the photic zone from the Northwestern Hawaiian Islands. A two-way ANOVA with an interaction term (genus × depth) revealed a significant difference between the genera from the Northwestern Hawaiian Islands ($F = 14.9$, df = 3, $p < 0.0001$) and no interaction for δ^{15}N ($F = 0.9$, df = 3, $p = 0.4420$; Fig. 6a). A randomization test of a two-way ANOVA with an interaction term (genus × depth) revealed a significant difference in %N among genera ($F = 12.6$, df = 3, $\alpha = 0.0125$, $p = 0.0001$) but not for depth ($F = 0.04$, df = 2, $\alpha = 0.0125$, $p = 0.8293$), and no significant interaction ($F = 1.2$, df = 3, $\alpha = 0.0125$ $p = 0.3139$; Fig. 6b).

δ^{15}N increased, on average, from Maui to Hōlanikū (Kure Atoll) at both shallow and mesophotic depths (Table 3). One-way ANOVAs and randomization tests of one-way ANOVAs showed that island/atoll of collection was important ($p < 0.0001$). O'ahu (1.5‰ ± 0.2‰) and Kapou (Lisianski) (3.5‰ ± 0.3‰) were the only islands/atolls that significantly

Table 3. Average δ^{15}N and %N from sampled islands/atolls. Islands/atolls are listed in order from the north to the south within each depth. The names for the Northwestern Hawaiian Islands are Hōlanikū (Kure Atoll), Kuaihelani (Midway Atoll), Manawai (Pearl and Hermes Atoll), Kapou (Lisianski), Kamole (Laysan), and Lalo (French Frigate Shoals).

Island/Atoll	*n*	δ^{15}N ± SE (‰)	Min (‰)	Max (‰)	%N ± SE (%)	Min (%)	Max (%)
Shallow							
Manawai	54	2.6±0.1	0.8	4.8	1.4±0.1	0.6	2.7
Kapou	18	3.5±0.3	1.9	6.2	1.1±0.2	0.0	2.3
Lalo	25	2.6±0.2	−0.2	5.0	1.1±0.1	0.4	2.4
Niʻihau	1	1.5±0.0	1.5	1.5	0.8±0.0	0.8	0.8
Oʻahu	49	1.5±0.2	−1.7	4.4	2.9±0.1	1.5	4.7
Maui	225	2.4±0.1	−2.4	5.2	2.6±0.0	0.9	5.2
Mesophotic							
Hōlanikū	29	4.1±0.2	1.8	5.5	1.2±0.1	0.4	3.0
Kuaihelani	7	3.3±0.6	0.7	4.9	1.3±0.2	0.7	2.3
Manawai	40	4.1±0.2	1.8	6.3	1.1±0.1	0.2	3.6
Salmon	7	4.1±0.4	2.7	5.1	1.4±0.2	0.7	2.2
Kapou	27	3.3±0.2	1.5	5.2	1.3±0.1	0.7	2.3
Pioneer	18	3.7±0.5	−0.3	6.8	1.5±0.2	0.7	3.1
Kamole	12	2.7±0.4	0.4	4.1	0.9±0.1	0.3	1.9
Lalo	37	2.7±0.2	−0.6	5.2	1.3±0.1	0.2	2.9
Nihoa	6	3.0±0.6	0.8	4.9	0.7±0.2	0.3	1.3
Niʻihau	2	3.2±0.2	3.0	3.3	1.9±0.6	1.3	2.4
Oʻahu	135	2.1±0.1	−0.1	4.7	2.6±0.2	0.2	5.7
Molokaʻi	36	4.0±0.3	0.9	8.5	1.2±0.1	0.2	2.3
Maui	85	2.9±0.1	−0.4	5.9	1.6±0.1	0.2	3.5

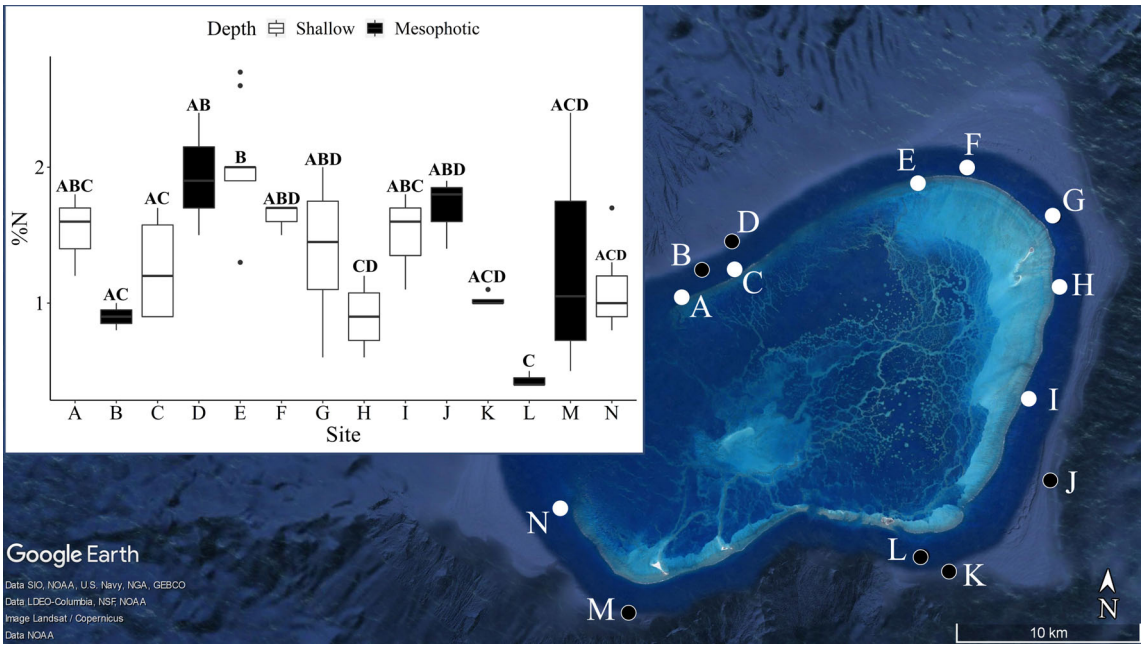

Fig. 7. Map of collection sites from Manawai (Pearl and Hermes Atoll), Hawaiʻi, in August 2019. White circles indicate shallow-water sites and black indicate mesophotic sites. Inset boxplot displays macroalgal tissue %N collected at each site. Different letters indicate significant differences from a randomization test of a one-way ANOVA and a Tukey's post hoc test ($p < 0.05$).

differed from other islands/atolls in shallow-water δ^{15}N (Tukey's post hoc test, $p < 0.0001$). Mesophotic locations (log-transformed data), had more significant differences in δ^{15}N between islands/atolls than shallow-water samples. Differences arose when comparing Oʻahu (2.1‰ ± 0.1‰), Maui (2.9‰ ± 0.1‰), and Lalo (French Frigate Shoals) (2.7‰ ± 0.2‰) to other islands/atolls (Tukey's post hoc test, $p < 0.02$). Spatially, %N displayed the opposite average trend, decreasing from Maui to Hōlanikū (Table 3). Oʻahu (2.9% ± 0.1%) and Maui (2.4% ± 0.1%) were the only two islands that produced significant differences when compared to other islands' shallow-water %N samples (Tukey's post hoc test, $p < 0.03$). Mesophotic samples differed significantly when comparing Oʻahu (2.6% ± 0.2%), Maui (1.6% ± 0.9%), and Kamole (Laysan) (0.9% ± 0.1%) to other island/atolls (Tukey's post hoc test, $p < 0.02$).

Averages are not an accurate representation of these highly variable data (e.g., although Oʻahu had an average shallow % N of 2.9%, it had several samples > 4.0%). To address site- and depth-specific variability during a single time period, samples collected during August 2019 from Manawai were analyzed. Shallow-water δ^{15}N ranged from 0.8‰ to 4.8‰ and mesophotic δ^{15}N ranged from 3.4‰ to 5.3‰. Sites at Manawai differed significantly in %N ($F = 5.3$, df = 13, $\alpha = 0.025$, $p = 0.0001$; Fig. 7). No significant differences were found between shallow and mesophotic sites in close proximity (< 4 km). While most sites at Manawai had < 2.0% N, sites A, D, E, and F averages were elevated and close to 2.0% N (Fig. 7).

Discussion

Samples from coastal regions of urbanized Maui and Oʻahu had values of δ^{15}N and %N that suggest the possible influence of anthropogenic nitrogen (Gartner et al. 2002; Dailer et al. 2010, 2012b; Amato et al. 2016, 2018), which was expected because these islands have higher human population densities and municipal to on-site sewage disposal systems for waste management. Shallow-water samples from west Maui produced δ^{15}N values from −2.4‰ to 5.2‰ with an average of 2.4‰ ± 0.1‰, and %N values from 0.9% to 5.2% with an average of 2.6% ± 0.0%. Shallow-water samples from south Oʻahu produced δ^{15}N values from −1.7‰ to 4.4‰ with an average of 1.5‰ ± 0.2‰, and %N values from 1.5% to 4.7% with an average of 2.9% ± 0.1%. While Dailer et al. (2010) found elevated δ^{15}N (> 50‰) in areas adjacent to wastewater treatment plants around Maui, our δ^{15}N values reflected possible fertilizer use with high %N. This variability in potential source may be due to the spatial and possible temporal heterogeneity of SGD sources (Dailer et al. 2012b) depending on spring location, proximity to point and nonpoint sources of nutrient pollution, as well local currents and water motion (Amato et al. 2016; Dulai et al. 2021). A previous study from coastal waters of Oʻahu near a stormwater outflow in a highly populated development that included cesspools and/or septic systems found δ^{15}N and %N values of 15.1‰ and 3.5%, respectively (Lapointe and Bedford 2011). Although samples from our study were not collected near stormwater drains, we found higher %N values from Oʻahu and Maui, suggesting possible anthropogenic nitrogen sources.

Elevated δ^{15}N data are commonly associated with highly denitrified wastewater input, such as the injection well wastewater plumes that have been documented around Maui (Dailer et al. 2010, 2012a; Glenn et al. 2013). Our study found elevated δ^{15}N levels (Figs. 2a, 3a) at 70–95 m depths, suggesting the possibility that novel inputs of sewage or products from other biogeochemical processes may reach mesophotic depths (Dailer et al. 2010, 2012a). Recent studies of groundwater flow in and around Hawaiʻi island describe a newly found transport mechanism of fresh groundwater from onshore to offshore depths in volcanic islands with multilayer basaltic formations (Attias et al. 2020, 2021). It is conceivable that somewhat older, adjacent high islands of Oʻahu and Maui may have similar processes underway (Attias et al. 2020).

Negative δ^{15}N values, which can indicate biogeochemical cycling (Kendall and McDonnell 1998) and possibly synthetic fertilizer inputs (McClelland et al. 1997; Gartner et al. 2002; Costanzo et al. 2005), were present in greatest abundance at shallow-water sites around Maui and Oʻahu. The influence of fertilizers appears to dissipate before reaching mesophotic depths (Dailer et al. 2010). We also found elevated nitrogen levels around the Main Hawaiian Islands that suggest the presence of anthropogenic nitrogen (Fig. 2b), likely from agriculture fields (Bishop et al. 2017). Values exceeding 2.0% are commonly associated with anthropogenic nitrogen (Dailer et al. 2010; Amato et al. 2016), and the majority of Main Hawaiian Island samples exceeded 2.0% with several over 4.0% N.

Anthropogenic influences in the Main Hawaiian Islands should generally be absent from the Northwestern Hawaiian Islands because the Northwestern Hawaiian Islands lack human presence and infrastructure, are isolated, and have few urban nitrogen sources. Surprisingly, a few δ^{15}N values from the Northwestern Hawaiian Islands (3.2‰ ± 0.1‰) were > 6‰ or < 0‰ (Fig. 2a), outside the typical range for non-anthropogenically derived "natural" levels (0–4‰) (Gartner et al. 2002; Amato et al. 2016). The higher abundance of marine mammals, sea turtles, fish, and nesting shorebirds, and their resulting guano deposits in the Northwestern Hawaiian Islands may cause some of this variation as compared to the overfished Main Hawaiian Islands (Friedlander and DeMartini 2002; Tiwari et al. 2010; Carretta et al. 2014; Rapp et al. 2017). Modest quantities of organismal excrement from native populations over small scales could increase δ^{15}N values in shallow-water algal tissue, similar to the effect of human wastewater. Given that these are natural processes in a nearly pristine environment with little anthropogenic input, we have adopted the use of "non-anthropogenic" vs. "natural"

to avoid confusion regarding nitrogen sources. Negative values could be influenced by biogeochemical processes that increase the abundance of lighter nitrogen (^{14}N) in nitrogen pools, which causes a decrease in the final δ^{15}N value (Kendall and McDonnell 1998). Given that the %N within Northwestern Hawaiian Islands samples was found to reflect nonelevated levels, it is most likely that non-anthropogenic sources of nitrogen are causing the variation in the δ^{15}N values. Most samples were < 2.0% and none exceeded 4.0% (Fig. 2b). The samples exceeding 2.0% were few and likely associated with animal fecal contributions and/or the localized upwelling of nutrient-rich water (Merrifield et al. 2001; Seki et al. 2001).

Previous studies have reported that mesophotic coral ecosystems share similar characteristics and species with shallow-water reefs (Brokovich et al. 2008; Hinderstein et al. 2010), and mesophotic coral ecosystems might act as refugia during unfavorable conditions (Bongaerts et al. 2010). However, in recent years, studies have determined that mesophotic coral ecosystems have high levels of endemism (Slattery et al. 2011; Kane et al. 2014; Hurley et al. 2016, Kosaki et al. 2017) and focus has been directed toward understanding the connectivity of species with shallow-water reefs (Lesser et al. 2010; Slattery et al. 2011). We found that this connectivity between shallow-water reefs and mesophotic coral ecosystems could potentially extend to nitrogen sources. Nitrogen sources with similar isotopic signatures appear to be generally consistent between these two ecosystems when undisturbed (i.e., Northwestern Hawaiian Islands; Fig. 3b). Within the developed and populated Main Hawaiian Islands, there are greater differences in nitrogen values between shallow and mesophotic depths, suggesting the influence of anthropogenic nitrogen and degradation on shallow-water reefs. However, the δ^{15}N and %N values presented here suggest that anthropogenic nitrogen or a deeper water source may be present at mesophotic depths around the Main Hawaiian Islands. When narrowing the scope of focus from regional patterns to genera, the results are consistent. Macroalgal genera from the Main Hawaiian Islands had higher %N at shallow depths, except for the invasive *A. lacerata* (Fig. 4b). In the Northwestern Hawaiian Islands, %N in the tissue of macroalgal genera did not differ between shallow-water reefs and mesophotic coral ecosystems, further suggesting possible connectivity of nitrogen between depths (Fig. 5b), as suggested by trophic studies in this region (Papastamatiou et al. 2015).

Coastal waters around the Main Hawaiian Islands can be influenced by SGD (Paytan et al. 2006; Johnson et al. 2008; Glenn et al. 2013). Influx of SGD has been found to be 2–4 times greater than surface inputs (Dulai et al. 2016) and provide an important source of nutrients, particularly nitrogen, to coral reef ecosystems (Paytan et al. 2006). SGD can also transport nitrogen from land-based agriculture (Bishop et al. 2017), golf courses (Knee et al. 2010), septic systems, cesspools, and nearshore wastewater injection wells (Dailer et al. 2010, 2012a; Bishop et al. 2017). SGD can influence benthic community structure (Amato et al. 2016, 2018; Richardson et al. 2017); reefs with greater SGD adjacent to areas with high human activity have higher abundances of macroalgae and lower species diversity (Amato et al. 2016, 2018). It is likely that shallow-water samples in this study were collected from areas influenced by SGD resulting in an increase in tissue %N.

Papastamatiou et al. (2015) found that sharks are important transporters of nutrients from shallow-water to mesophotic coral ecosystems in Hawai‘i. Sharks feed in shallow-water and then retreat to mesophotic depths in the evening where they excrete waste. This creates an important connection for nutrients between mesophotic coral ecosystems and shallow-water communities. Other studies have addressed the transportation of nutrients by various species among shallow-water ecosystems (McCauley et al. 2014; Williams et al. 2018), but more research is needed to understand the flow of nutrients into mesophotic coral ecosystems. Some locations within the Northwestern Hawaiian Islands have strong currents (H. Spalding, pers. observation) and strong seasonal variability in nutrients and chlorophyll (Polovina et al. 2001), suggesting the potential for upwelling of nutrient-rich water (Merrifield et al. 2001; Seki et al. 2001) and a possible explanation for elevated δ^{15}N nitrogen patterns. It is likely that both nearshore/shallow-water processes and upwelling can influence the nutrients found within mesophotic coral ecosystems depending on regional oceanographic processes.

Species and habitat types are not equally distributed across the Hawaiian Archipelago. It is possible for areas to be “hotspots,” whether in terms of biodiversity or nitrogen enrichment. Because of this, we examined site-specific differences at the level of island/atoll. Manawai was the most extensively surveyed atoll in August 2019 due to the discovery of the cryptogenic red alga *C. tumulosa* that displayed invasive traits across the reefs (Sherwood et al. 2020). Understanding whether algal tissue nitrogen concentrations differ at various sites at Manawai could provide insight into processes that may increase the growth or occurrence of this cryptogenic alga. Elevated levels of nitrogen in macroalgae (> 2.0%) from four sites around Manawai correlated with areas of high abundance of *C. tumulosa* (Fig. 7). Episodic upwelling could potentially be the cause of the high %N (Merrifield et al. 2001; Seki et al. 2001) and could be contributing to the growth of the cryptogenic alga. Additional studies focusing on the oceanographic conditions and potential for upwelling at Manawai, and the physiological preferences of *C. tumulosa*, are needed to better evaluate the influence of nitrogen on its success at this location.

A. lacerata (formerly referred to as *Avrainvillea amadelpha* in Hawai‘i), like many bryopsidalean macroalgae, is fast growing, low-nutrient tolerant (Smith et al. 2004), and able to reproduce by fragmentation (Vroom et al. 2003). This alga is commonly known as “mudweed” because of its mound-building ability (Littler et al. 2004), and was the first *Avrainvillea* species noted in Hawai‘i (Brostoff 1989). These abilities have made

A. lacerata a successful invader and ecosystem engineer, altering biotic and abiotic components of seagrass beds and some reefs (Gribben et al. 2013) around O‘ahu and transforming the environment into muddy habitats (Wade et al. 2018; Foster et al. 2019). An interesting finding was the extremely high %N found in *A. lacerata*. Shallow and mesophotic samples exceeded 4.0% N, yet typical percentages of anthropogenic N range from 2% to 4% (Lapointe and Bedford 2011; Dailer et al. 2012a,b; Amato et al. 2016). Its mound-building ability may influence the accumulation of nutrient-rich sediments around the holdfast structure, creating an environment conducive to increased nitrogen loading. Mesophotic samples had significantly higher %N (Fig. 4b), which could be due to the formation of larger mounds because of limited wave motion (Littler et al. 2004). These mounds collect soft sediments (Foster et al. 2019) that are hypothesized to form a suboxic environment. Suboxic areas are commonly associated with denitrification (Gaye et al. 2013), which lowers nitrate concentrations and increases δ^{15}N (Kellman and Hillaire-Marcel 1998; Fry 2006). Interestingly, the δ^{15}N values of *A. lacerata* were significantly lower than other genera (Fig. 4a), suggesting that denitrification is not a factor in these mounds.

A. lacerata’s mound-building tendency allows it to be psammophytic and sequester nitrogen from interstitial waters (Williams 1984). We compared mesophotic samples of psammophytic species (*A. lacerata*, *H. kanaloana*, and *U. gepporium*) and saxicolous *Halimeda* spp. from the Main Hawaiian Islands. The holdfast structures of *A. lacerata*, *H. kanaloana*, *Halimeda* spp., and *U. geppiorum* differ substantially. *A. lacerata* was collected from O‘ahu and has a saxicolous/psammophytic holdfast that sequesters sediments (Littler et al. 2004). *H. kanaloana* was collected from Maui and has a deep psammophytic holdfast (Spalding 2012). *Halimeda* spp. were collected from Maui, O‘ahu, and Moloka‘i and are saxicolous. *Udotea gepporium* was collected from O‘ahu and has a shallow psammophytic holdfast (Sansone et al. 2017; Sauvage et al. 2019). Previous studies have found that deeply penetrating, psammophytic macroalgae have higher nitrogen concentrations than saxicolous individuals (Williams 1984, 1988; Sansone et al. 2017). Our data support these findings as *A. lacerata* and *H. kanaloana* had the highest %N (Fig. 5b), suggesting mound-building and deep psammophytic holdfasts can increase nitrogen acquisition. Further research is needed to better understand how *A. lacerata*’s mound-building characteristics impact its nitrogen sequestration. Additionally, *U. gepporium* and *Halimeda* spp. were found to have similar % N content, suggesting that *U. gepporium* sequesters nitrogen similarly to saxicolous macroalgae due to its shallow psammophytic holdfast.

Nutrient regimes in coral reefs across the world have been successfully described using macroalgal stable isotopes and tissue nutrients (Dailer et al. 2010; Adam et al. 2021; Lapointe et al. 2021b; Vaughn et al. 2021). Knowledge of the nutrient regimes from shallow to mesophotic reefs throughout the Hawaiian Archipelago, informed by known land-use practices and their recent changes, could be used to identify areas that are likely to be influenced by point and nonpoint pollution around the Main Hawaiian Islands via increased nitrogen loading in macroalgal tissue (Lapointe and Bedford 2011; Dailer et al. 2012a,b; Amato et al. 2016). SGD clearly plays a role in supplying enhanced nutrient loads to shallow-water depths around the Main Hawaiian Islands (Dailer et al. 2012a, b; Amato et al. 2018; Dulai et al. 2021), and may also fuel the growth of mesophotic invasive or bloom-forming macroalgae in volcanic islands via submarine vents (Attias et al. 2020, 2021). Conversely, upwelling or internal waves from deeper water (200 to 350+ m depths) may introduce nitrogen inputs with altered nitrate δ^{15}N in the range of 6–7‰ (Casciotti et al. 2008; Knapp et al. 2011; Wilson et al. 2019). Extensive meadows of the invasive alga *A. lacerata* that occur to 90 m depths off O‘ahu (Spalding et al. 2019b) may be fueled by SGD, deeper water, and/or changes in sediment biogeochemistry due to its mound-building holdfast system. The high abundance of mesophotic Ulvacean species (Spalding et al. 2016, 2019a,b), which are typically associated with eutrophied shallow-water, also suggests a nitrogen source at mesophotic depths. Additional research is needed to determine the possible influence of anthropogenic nitrogen connectivity between shallow and mesophotic depths, the possible role of SGD vs. deep-water input, and their cumulative influence on the distribution and abundance of invasive and bloom-forming mesophotic macroalgae.

Data availability statement

Data and code can be accessed at the open access repository https://github.com/nickstrait/Hawaiian-Archipelago-Macroalgae-Nitrogen.

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Conflict of Interest

None declared.

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