$\delta^{15}N$ in Macroalgae as a Proxy for Sewage Pollution in Puakō, HI

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Abstract

Sewage pollution from nearshore communities can impact coastal ecosystems and is hazardous to human health. Macroalgae and water samples were collected at sixteen nearshore sites along the Puakō coast, HI, USA, two separate times during morning low tides to evaluate if sewage was present. The $\delta^{15}N$ values in macroalgae were analyzed and compared to different nitrogen sources to determine the source of nitrogen in the water. Also, $\delta^{15}N$ values in the macroalgae and *C. perfringens* concentrations were compared between high and low groundwater sites. High $\delta^{15}N$ values in the macroalgae, high levels of *C. perfringens,* and high nutrient concentrations confirmed the presence of sewage in the nearshore waters at Puakō at some locations. However, there was no significant difference in $\delta^{15}N$ values in the macroalgae and water *C. perfringens* concentrations between high and low groundwater sites. These results suggest sewage is entering not only with groundwater but may enter through other pathways or be thoroughly mixed by nearshore currents along the coast. The Puakō community, with this data, can decide whether to implement more effective wastewater management practices.

Introduction

Nearly 60% of the world's coral reefs are permanently lost or at risk of being permanently lost (Cesar et al. 2003). Sewage pollution is among the many factors contributing to this degradation (Cesar et al. 2003). Sources of sewage pollution include discharge from outfalls and injection wells, cesspool and septic tank leakage, and runoff (Anderson et al. 2002, Heisler et al. 2008, Dailer et al. 2010). As the sewage from cesspools, septic tanks and injection wells seeps and leaches through the ground, it can contaminate groundwater that discharges into the ocean, polluting nearshore waters (Taniguchi et al. 2002, EPA 2013).

Sewage discharge can have detrimental effects on coral reef systems and pose threats to human health (GESAMP 2001, Fong & Lipp 2005, Littler et al. 2006, Dailer et al. 2010). Submarine groundwater discharge is not only a major source of nutrients and elements, but can also be a major transporter of contaminants such as sewage (Taniguchi et al. 2002, Street et al. 2008). Sewage entering into the marine environment contain not only high concentrations of nutrients such as nitrogen and phosphates but bacteria and viruses (GESAMP 2001, Koop et al. 2001). Consequences of sewage pollution include algal blooms, coral reef degradation, and potential threats to human health including gastrointestinal diseases, diarrhea, and hepatitis (GESAMP 2001, Koop et al. 2001, Fong & Lipp 2005, Littler et al. 2006, Dailer et al. 2010).

Monitoring sewage inputs is a critical step to ensuring the health of our coastal ecosystems and recreational water users. There are multiple methods for investigating sewage contamination.

One method uses $\delta^{15}N$ in macroalgae, an effective proxy for determining the presence and source of anthropogenically introduced nitrogen, including sewage, into marine waters (Heaton 1986). Many studies have used $\delta^{15}N$ in macroalgae to understand the spatial distribution of sewage (Umezawa et al. 2002, Lapointe et al. 2005, Mutchler et al. 2007, Dailer et al. 2010, Orlandi et al. 2014). The reason $\delta^{15}N$ in macroalgae can be used is because the $\delta^{15}N$ in macroalgae will reflect the $\delta^{15}N$ of the nitrogen source in the waters the macroalgae is growing because they minimally fractionate the two stable isotopes of nitrogen: ^{14}N and ^{15}N (Heaton 1986, Fogel & Cifuentes 1993, Umezawa et al. 2002, Mutchler et al. 2007, Dailer et al. 2010). Different nitrogen sources have different isotopic nitrogen ratios that distinguish them from one another. Sewage $\delta^{15}N$ signature ranges between +10‰ to over +20‰, whereas atmospheric N_2 has a signature of 0‰, fertilizers range between -4‰ and +4‰, and soils range between +4‰ and +9‰ (Heaton 1986).

Another method of detecting sewage in waters is through measurements of fecal indicator bacteria (FIB). One FIB used is *Clostridium perfringens* (*C. perfringens*) (Fujioka & Shizumura 1985, Fung et al. 2007). *C. perfringens* is a bacterium found in human waste, and it is a reliable indicator as its concentrations are correlated with fecal pollution (Fujioka & Shizumura 1985).

Sewage pollution is a worldwide problem, and as nearshore communities develop, sewage contamination of coastal waters and ecosystems becomes a pressing issue (Friedlander et al. 2008). Many coastal areas in Hawaii are affected by sewage pollution (Dailer et al. 2010). Hawai`i has the highest number of cesspools in the United States (Friedlander et al. 2008, EPA 2013). The Hawai`i State Department of Health lists sewage from cesspools and septic tanks as one of the top twelve contaminants of concern in groundwater (EPA 2013).

The Hawai'i Coral Reef Program has identified Puakō, Hawaii as a top priority site to minimize anthropogenic threats due to land-based pollution inputs (Gombos et al. 2010). In a comparison study between 1971–1981 and 2007–2008 data, the Hawai`i Division of Aquatic Resources (HDAR) reported a 35% decrease in coral cover and 38% increase in turf and macroalgae at Puakō (HDAR 2013). Sewage has been detected in Puakō's coastal waters, and sewage leakages from cesspools are of particular concern (Dailer et al. 2013, Kim et al. 2014). Cesspools are primarily used for waste disposal in residential houses and their leakage is suspected to be linked to the decline of coral and increase in algal growth observed at Puakō (Hawaii Coral Reef Network 2005, HDAR 2013). For effective wastewater management practices to be put into place, a clear understanding of the spatial distribution of sewage pollution along Puakō's coastal waters is crucial.

The objectives for this study were: (1) determine if sewage was present in the coastal waters along Puakō's shoreline and (2) compare high groundwater sites versus low groundwater sites for $\delta^{15}N$ values in macroalgae and *C. perfringens* concentrations in Puakō's coastal waters. To address the first objective, this study hypothesized sewage is present in the coastal waters along Puakō's shoreline. High $\delta^{15}N$ in macroalgae and high *C. perfringens* concentrations in the coastal waters would indicate presence of sewage pollution. Higher macroalgal $\delta^{15}N$ values would also be associated with higher nutrient concentrations and higher *C. Perfringens* concentrations. To address the second objective, this study hypothesized higher macroalgal $\delta^{15}N$ values, higher *C. perfringens* concentrations and higher algal ranks at high groundwater sites.

These higher concentrations found at high groundwater sites would be due to the greater amount of contaminated water discharged than at low groundwater sites.

Methods

Site Description

Puakō is located on the northwest (leeward) side of Hawai`i Island, USA (19.9675°N, 155.8467°W) (Fig. 1). The open bay houses a fringing coral reef in relatively protected waters and an offshore basalt bench. The coastline primarily consists of basalt hard substrate and sand. The average rainfall for the area is roughly 0.25 m/year, and the average groundwater discharge flux is 0.4 to 0.7m^3/m^2d (Street et al. 2008). There are over 163 residential homes along the Puakō coast with 47 homes relying on cesspools for wastewater treatment, many of which are situated close to the water table.

Sample Collection

To detect the presence and determine the spatial distribution of sewage in the coastal waters of Puakō, $\delta^{15}N$ in macroalgae, *C. perfringens* concentrations, and nutrient concentrations were measured. Field sampling was conducted at 16 sampling stations along the Puakō coastline in the intertidal zone at water depth less than 0.5m (Fig. 2). These stations were chosen based on public shoreline access points and their close proximity to residential houses. Each station was sampled during two sampling events (November 2014 and March 2015) during low tide mornings. Macroalgae is exposed during low tide, making macroalgal tissue collection and algal cover observation easier. In addition, bacteria concentrations fluctuated throughout the day and morning sampling provided conservative water quality estimates (EPA 2010). Percent algae cover for each site was recorded based on an algae rank (1: 0–15%; 2: 15–25%; 3: 25–50%; 4: 50–75%; 5: 75–100%). Nine of the sites were classified as high groundwater (salinity < 25‰) and seven as low groundwater (salinity> 25‰). Using a cutoff of 25‰ divided the sites into two fairly even groups. A total of 32 algae samples consisting of a composite of algae found in each area, as well as water samples, were collected and analyzed for *C. perfringens* and nutrient concentrations. A composite of the macroalgae found at each site was used as no single species was found at all sites.

Analytical Methods

A) $\delta^{15}N$ in macroalgae

$\delta^{15}N$ in macroalgae was measured using methods from an earlier study in Maui (Dailer et al. 2010). Immediately after algae were collected, samples were placed into a cooler with site water for return to the laboratory. At the laboratory, macroalgae were rinsed with deionized water and left to dry at 50°C until a constant weight was achieved. The algal samples were then ground (wiggle bugged), weighed, placed into tin capsules, and analyzed for $\delta^{15}N$ using a Costek elemental analyzer and a Thermo-Finnigan Delta Plus isotopic ratio mass spectrometer (IRMS) at UH Hilo Analytical Laboratory. $\delta^{15}N$ in macroalgae is reported in parts per thousand (‰) and

calculated using the following equation: $\delta^{15}N(‰) = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{15}N/{}^{14}N$.

B) Clostridium perfringens

A membrane filtration technique was used to quantify *C. perfringens* (Bisson & Cabelli 1979). Water samples were filtered through a sterile membrane filter and washed with sterile 0.1% Peptone water before being placed on media plates. The media plates were put into an anaerobic jar in an incubator at 45°C for 18 to 24 hours. After incubation, the plates were exposed to ammonium hydroxide for 30 seconds, and the number of pink colonies was recorded.

C) Nutrients and Physiochemical Parameters

Water temperature, salinity, and dissolved oxygen were measured on site using a YSI. Water samples were transported on ice to University of Hawai`i Analytical Laboratory for nutrient analysis on a Pulse Technicon™ II autoanalyzer using standard methods. Water samples from each station were filtered (Whatman™ GF/F) and analyzed for total dissolved nitrogen (TDN), total dissolved phosphorous (TDP), nitrate+nitrite ($NO_3^-+NO_2$), ammonium (NH_4^+), silica (H_4SiO_4), and phosphate (PO_4^{3-}) using a TOC/TN Analyzer and Lachat Quikchem 8500.

Statistical Methods

To determine relationships between $\delta^{15}N$ macroalgal tissue values, *C. perfringens,* nutrients, and physiochemical values, linear correlations were used. Two sample t-tests were used to investigate differences in $\delta^{15}N$ values, *C. perfringens* concentrations, and algal rank between high and low groundwater discharge sites. $\delta^{15}N$ values and *C. perfringens* concentrations were transformed using a square root transformation. Minitab17 was used for all statistical analyses ($\alpha = 0.05$).

Results

$\delta^{15}N$ in macroalgae

The average (±SE) $\delta^{15}N$ values in macroalgae ranged between 3.6 ± 0.0‰ and 11.5 ± 0.21‰. Stations 3 and 4 had the highest average $\delta^{15}N$ values and measured values greater than 10‰, 2 – 8 ‰ higher than other stations. (Fig. 3). Variability between sampling events for each site was low with the highest standard error being 1.5‰.

The nitrate+nitrite ($NO_3^-+NO_2$) concentrations among sites had a wide range and all exceeded 15µM. The site with the highest macroalgae $\delta^{15}N$ values (site 4) also had the highest $NO_3^-+NO_2$, PO_4^{3-}, H_4SiO_4, TDP, and TDN water concentrations (Table 1). Higher macroalgae $\delta^{15}N$ values were associated with higher nutrient concentrations. Positive correlations existed between $NO_3^-+NO_2$ ($r = 0.687$, $p < 0.001$) (Fig. 4) and PO_4^{3-} ($r = 0.791$, $p < 0.001$) (Fig. 5) concentrations in the water and $\delta^{15}N$ values in macroalgae.

$\delta^{15}N$ values in macroalgae were similar between high groundwater discharge (salinity < 25ppt) and low groundwater discharge (salinity > 25ppt) stations ($t = 0.33$, $p = 0.742$) (Fig. 6). The

average (±SE) $\delta^{15}N$ values in macroalgae were 6.50 ± 1.99‰ at high groundwater discharge sites and 6.09 ± 1.2‰ at low groundwater discharge sites. No significant correlation was found between salinity and $\delta^{15}N$ values in macroalgae ($r = -0.289$, $p = 0.109$). In addition, algal rank did not differ between high and low groundwater discharge sites ($F = 1.27$, $p = 0.318$).

Clostridium perfringens

All sites measured detectable concentrations of *C. perfringens* ranging between 1.17 ± 0.83 CFU/100ml at site 13 to 12.00 ± 8.33 CFU/100ml at site 14. Half of the sites exceeded the recommended 5 CFU/100ml *C. perfringens* concentrations, the standard recommended to the Hawaii Department of Health for marine recreational waters (Fig. 7).

C. perfringens concentrations were similar between high (salinity < 25ppt) and low groundwater (salinity > 25ppt) stations ($t = 1.26$, $p = 0.219$) (Fig. 8). The relationship between *C. perfringens* concentrations and $\delta^{15}N$ values was near significant ($r = 0.600$, $p = 0.071$) (Fig. 9). There was no significant correlation between salinity and *C. perfringens* concentrations ($r = 0.699$, $p = 0.071$). No significant correlation was found between *C. perfringens* concentrations and $\delta^{15}N$ values in macroalgae ($r = 0.699$, $p = 0.071$).

Discussion

It is important to understand how coastal communities affect nearshore environments. Many components of this study suggest there is sewage present in the nearshore waters of Puakō. $\delta^{15}N$ values in macroalgae higher than 10‰ indicate sewage present in the surrounding water the macroalgae grows in (Hunt 2010). Two sites had detectable sewage. However, it is likely the $\delta^{15}N$ values are underestimated and therefore, more sites are affected by sewage pollution. A study conducted by Swart et al. (2014) found that in environments with nitrate concentrations of 5 to 10 µM, the $\delta^{15}N$ values in macroalgae are underestimated by 4 to 6‰ as macroalgae no longer minimally fractionate and preferentially consume ^{14}N, resulting in lower $\delta^{15}N$ values in the macroalgae. Since the nitrate+nitrite concentrations exceeded 5 µM at all sites, it is likely that $\delta^{15}N$ values are underestimated. After correcting the $\delta^{15}N$ values by adding 6‰ to $\delta^{15}N$ values for each site, the majority of sites had $\delta^{15}N$ values greater than 10‰. So, it is likely sewage is present all along Puakō coast.

Some limitations exist in the study design. A factor that may have influenced the results is the sampling of a composite of macroalgae species found at each site rather than comparing the $\delta^{15}N$ values of a single species of macroalgae across sites. Although it is ideal to sample the same species at each site when comparing $\delta^{15}N$ macroalgae values, it is not always feasible or realistic due to macroalgae species distribution. *Pterocladiella spp., Ulva spp., and Cladophora spp.* were among various macroalgae species growing at some sites but there was no common algae species found at every site, so a composite of different species found at each site was used for $\delta^{15}N$ analysis. Previous studies indicate slight differences in $\delta^{15}N$ values among different macroalgae species (Umezawa et al. 2002, Carballeira et al. 2012, Viana & Bode 2012). To address this potential confounding factor, a composite macroalgae sample was collected from each site.

C. perfringens concentrations of 10 to 100 CFU/100ml indicates non-point contamination (Fung et al. 2007). The recommended water quality standard for *C. perfringens* is less than 5 CFU/100ml for coastal beaches (Fung et al. 2007). Nine of the 16 sites exceeded the recommended standards for *C. perfringens* concentrations and one site was classified to have non-point contamination. There was a near significant positive correlational relationship between *C. perfringens* concentration and $\delta^{15}N$ values in macroalgae. This trend can be attributed to sewage having both high *C. perfringens* concentrations and high $\delta^{15}N$ values.

Sewage contains a lot of nutrients, especially nitrate and phosphate. Areas affected by sewage would also be expected to have high nutrient concentrations, as were found. This further reinforces the presence of sewage in the nearshore waters of Puakō.

The similarity in *C. perfringens* concentrations and $\delta^{15}N$ values between high and low groundwater discharge sites suggests high groundwater discharge sites do not necessarily equate to sewage pollution. However, submarine groundwater discharge can significantly affect the coastal water environments, and multiple studies have connected submarine groundwater discharge with sewage pollution (Taniguchi et al. 2002, Umezawa et al. 2002, Slomp & Cappelen 2004, Dailer et al. 2010). Also, although this study found no relationship between salinity and $\delta^{15}N$ in macroalgae, other studies found that lower salinity sites were associated with higher $\delta^{15}N$ macroalgae values (Mutchler et al. 2007). The hydrodynamics of the Puakō area is unknown and is likely the reason why a distinct spilt in *C. perfringens* concentrations and $\delta^{15}N$ values in macroalgae between high and low groundwater discharge sites was not observed. It is possible sewage is entering the water through other pathways or is being mixed along the coastline by nearshore currents. Understanding the hydrology of an area is necessary to determine how and where sewage is entering the ocean (Taniguchi et al. 2002, Umezawa et al. 2002).

Examining $\delta^{15}N$ in macroalgae is an effective way to measure and track sewage pollution in marine waters. The issue of sewage pollution is not specific to Puakō. Close monitoring of nutrients is necessary to address the problem of coral reef nutrient enrichment (Koop et al. 2001, Cesar et al. 2003). Many studies have used $\delta^{15}N$ in macroalgae to determine location and source of sewage pollution (Umezawa et al. 2002, Lapointe et al. 2005, Mutchler et al. 2007, Dailer et al. 2010, Orlandi et al. 2014). It is difficult to address the consequences associated with sewage pollution and eutrophication once they are set into motion. It is advantageous to take a preventative approach by closely monitoring nutrients and implementing effective waste management plans (Wu et al. 1999, Heisler et al. 2008).

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References

Anderson DM, Gilbert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25: 704–726

Bisson JW, Cabelli VJ (1979) Membrane filter enumeration method for *Clostridium perfringens*. Applied Environ Micro Bio 37: 55–66

Carballeira C, Viana IG, Carballeira A (2012) δ^{15}N values of macroalgae as an indicator of the potential presence of waste disposal from land-based marine fish farms. J App Phycol 25: 97–107

Cesar H, Burke L, Pet-Soede L (2003) The economics of worldwide coral reef degradation. Technical report, Cesar Environmental Consulting, Arnhem, The Netherlands

Costanzo SD, O'Donohue MJ, Dennison WC, Loneragan NR, Thomas M (2001) A new approach for detecting and mapping sewage impacts. Mar Pollut Bull 42: 149–156

Dailer ML, Knox RS, Smith JE, Napier M, Smith CM (2010) Using δ^{15}N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai`i, USA. Mar Pollut Bull 60: 655–671

Dailer M, Smith C, Glenn C (2013) Preventing the introduction and spread of nutrient driven invasive algal blooms and coral reef degradation in West Hawai`i. Hawai`i Coral Reef Initiative Research Program. http://www.hcri.ssri.hawaii.edu/files/research/pdf/Dailer_HCRI_NOAA_Final_Report.pdf (accessed 3 March 2014)

Derse D, Knee KL, Wankel SD, Kendal C, Berg CJ, Paytan A (2007) Identifying sources of nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae. Environ Sci 41: 5217–5223

EPA (2010) Sampling and consideration for variability (temporal and spatial) for monitoring of recreational waters. http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/P12-MonRept-final_508.pdf (accessed 3 May 2015)

EPA (2013) Cesspools in Hawai`i. http://www.epa.gov (accessed 15 Jan 2015)

Fogel ML, Cifuentes LA (1993) Organic geochemistry. Plenum Press, New York, NY

Fong T, Lipp EK (2005) Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiol Molec Bio Rev 69: 357–371

Friedlander A, Aeby G, Brown E, Clark A, Coles S, Dollar S, Hunter C, Jokiel P, Smith J, Walsh B, Williams I, Wendy Wiltse W (2008) The state of coral reef ecosystems of the main Hawaiian islands. In: Anonymous, p. 222-269

Fujioka RS, Shizumura LK (1985) *Clostridium perfringens*, a reliable indicator of stream water quality. J Water Pollut 15: 217–229

Fung DYC, Fujioka R, Vijayavel K, Sato D, Bishop D (2007) Evaluation of Fung double tube test for *C. perfringens* and easyphase test for f-specific RNA coliphages as rapid screening tests for fecal contamination in recreational waters of Hawaii. Journal of Rapid Methods Automation in Microbiology 15:217–229

GESAMP (2001) Protecting the oceans from land-based activities. http://www.gesamp.org/ (accessed 15 Jan 2015)

Gombos M, Komoto J, Lowry K, MacGowan P (2010) Hawai'i coral reef strategy: priorities for management in the main Hawai`ian islands 2010-2020. http://coralreef.noaa.gov/ (accessed 15 Jan 2015)

Hawai`i Coral Reef Network (2005) Puakō, Hawai`i. www.coralreefnetwork.com (accessed 15 Jan 2015)

Hawai`i Department of Health (2006) 2006 state of Hawai`i water quality monitoring and assessment report. http://health.Hawai`i.gov/cwb/files/2013/05/Integrated _2006_StateOfHawai`i.pdf (accessed 15 Jan 2015)

Hawai`i Division of Aquatic Resources (2013) South Kohala reefs are in in dire straits. http://www.kaikuleana.net/DireStraits.pdf (accessed 15 Jan 2015)

Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem Geo Iso Geosci Sec 59: 87–102

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: a scientific consensus. Harm Algae, 8: 3–13

Kim CJS, Yoshioka RM, Harvel CD (2014) Linking sewage pollution and water quality to spatial patterns for *Porites* growth anomalies in Puakō , Hawai`i

Koop K, Booth D, Broadbent A, Brodie J, Bucher D, Capone D, Coll, J, Dennison W, Erdmann M, Harrison P, Hoegh-Guldberg O, Hutchings P, Jones GB, Larkum ADW, O'Neil J, Steven A, Tentori E, Ward S, Williamson J, Yellowlees D (2001) ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Mar Pollut Bull 42: 91–120

Lapointe BE, Barile PJ, Littler MM, Littler DS (2005) Macroalgal blooms on southeast Florida coral reefs: II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen. Harm Algae 4: 1106–1122

Littler MM, Littler DS, Brooks BL (2006) Harmful algae on tropical coral reefs: bottom-up eutrophication and top-down herbivory. Harm Algae 5: 565–585

McClelland JW, Valiela I (1998) Linking nitrogen in estuarine producers to land-derived sources. Limnol Oceanogr 43: 577–585

Mutchler T, Dunton KH, Townsend-Small A, Fredriksen S, Rasser MK (2007) Isotopic and elemental indicators of nutrient sources and status of coastal habitats in the Caribbean Sea, Yucatan Peninsula, Mexico. Est Coast Shelf Sci 74: 449–457

Orlandi L, Bentivoglio F, Carlino P, Calizza E, Rossi D, Costantini ML, Rossi L (2014) $\delta^{15}N$ variation in *Ulva lactuca* as a proxy for anthropogenic nitrogen inputs in coastal areas of Gulf of Gaeta (Mediterranean Sea). Mar Pollut Bull 84: 76–82

Slomp CP, Cappelen PV (2003) Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impacts. J of Hydrology 295: 64–86

Street JH, Knee KL, Grossman EE, Paytan A (2007) Submarine groundwater discharge and nutrient addition to the coastal zone and coral reefs of leeward Hawaii. Mar Chem 109: 355–376

Swart PK, Evans S, Capo T, Altabet MA (2014) The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate. Bio Geo Sci 11: 6147–6157

Taniguchi M, Burnett WC, Cable JE, Turner JV (2002) Investigation of submarine groundwater discharge. Hydrological Processes 16: 2115–2129

Umezawa Y, Miyajima T, Yamamuro M, Kayanne H, Koike I (2002) Fine-scale mapping of land-derived nitrogen in coral reefs by δ15N in macroalgae. Limnol Oceanogr 47: 1204–1416

Viana IG, Bode A (2012) Stable nitrogen isotopes in coastal macroalgae: geographic and anthropogenic variability. Sci of Total Environ 443: 887–895

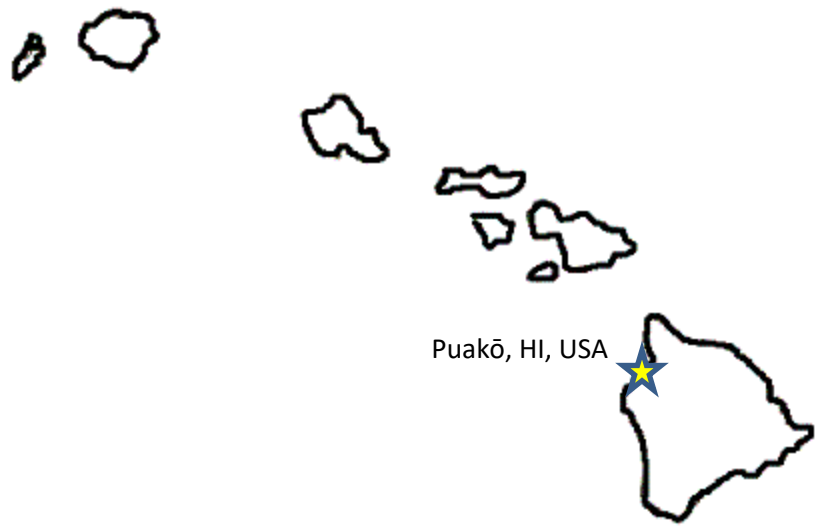

Figure 1: Location of study site, Puako, HI, USA.

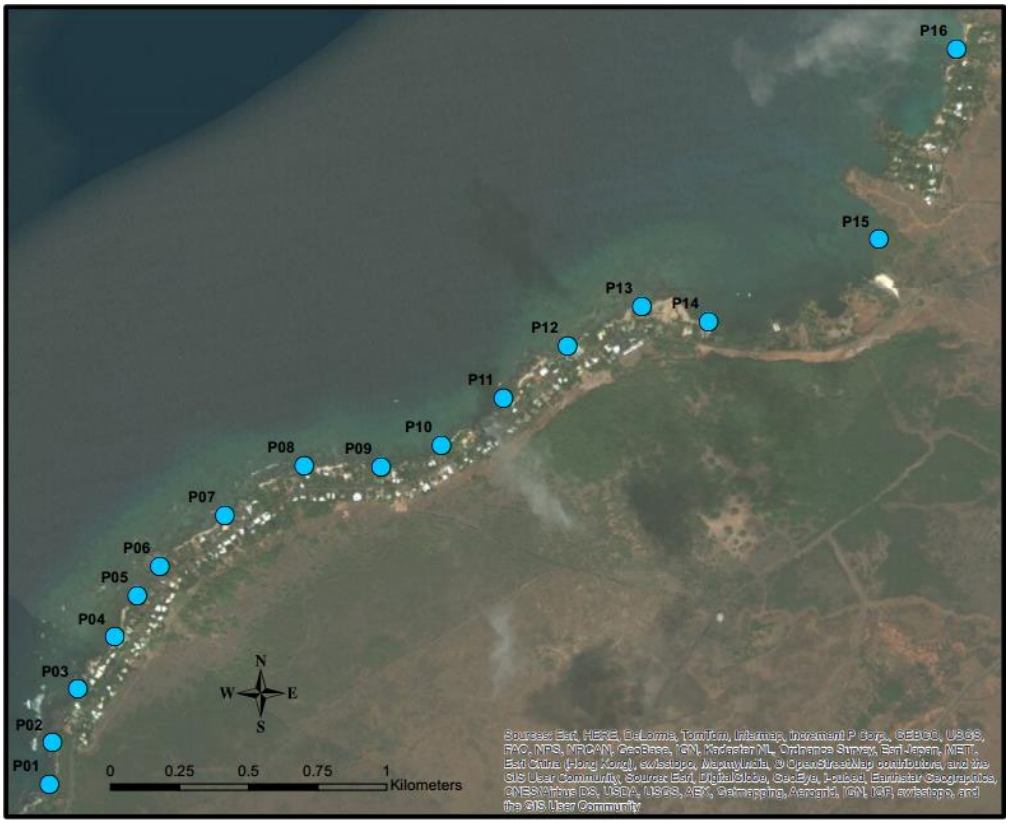

Figure 2. Map of 16 stations sampled along coastline in Puakō.

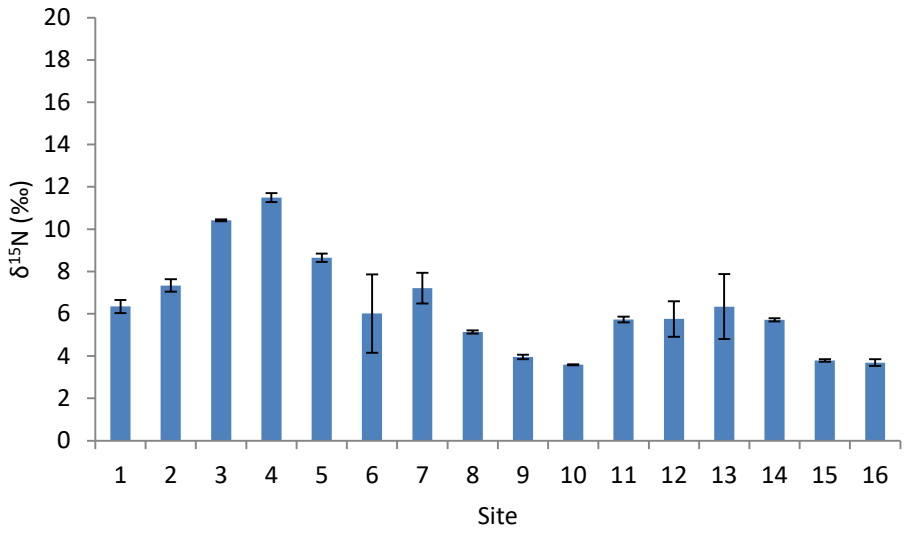

Figure 3. Comparison of $\delta^{15}N$ (‰) in macroalgae (mean ± SE) among 16 stations.

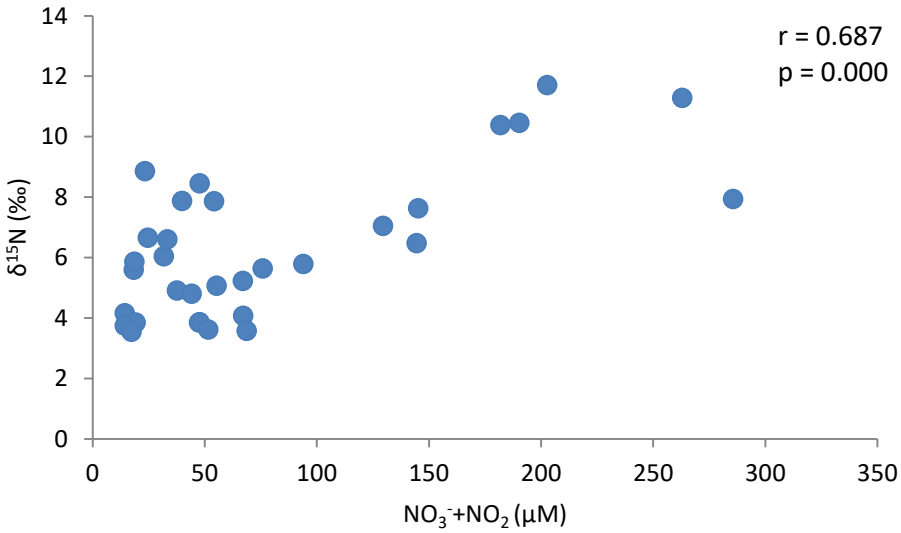

Figure 4. $NO_3^-+NO_2$ concentration vs. $\delta^{15}N$ in macroalgae.

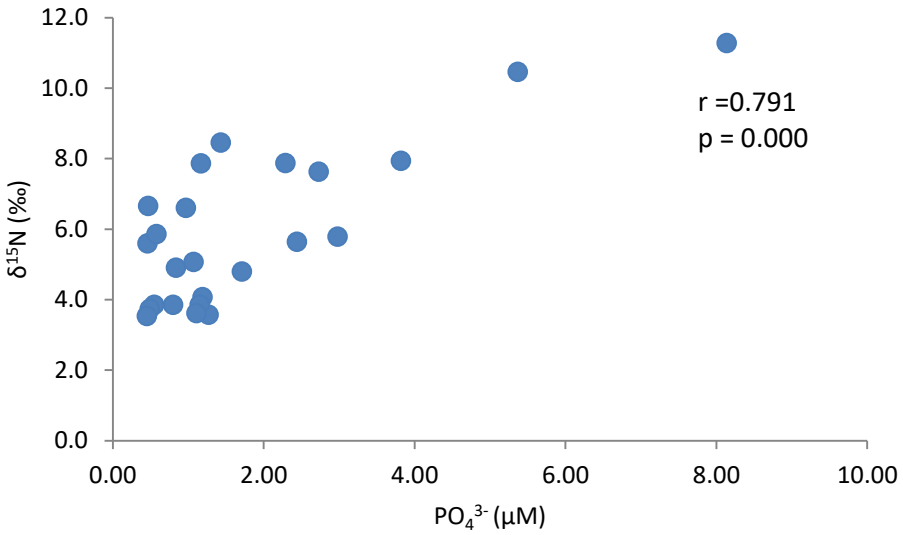

Figure 5. PO_4^{3-} concentration vs. $\delta^{15}N$ in macroalgae.

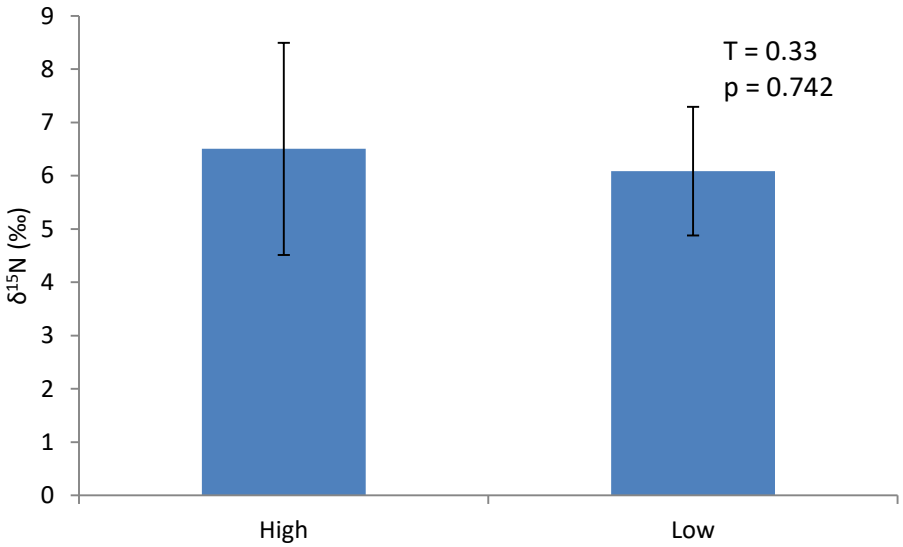

Figure 6. $\delta^{15}N$ values at high and low groundwater sites (mean ± SE).

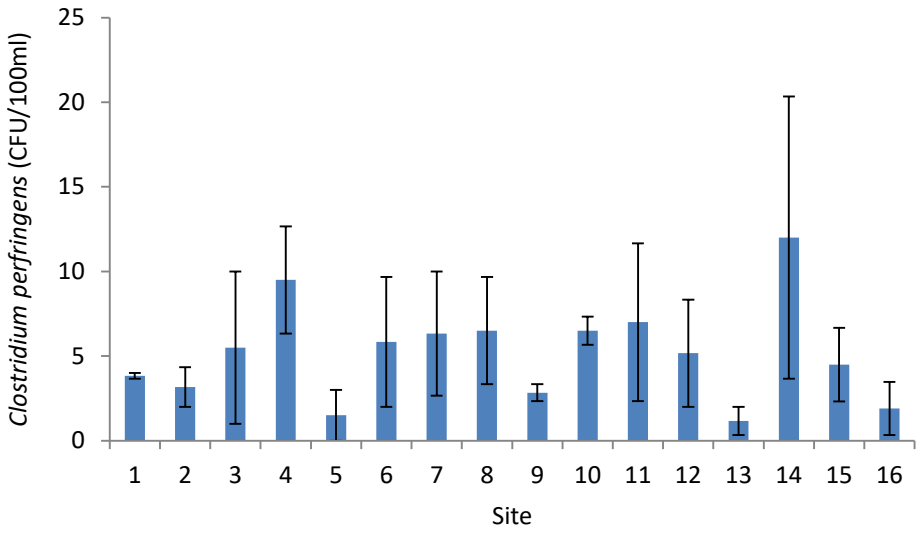

Figure 7. *Clostridium perfringens* concentrations in water among 16 stations (mean ± SE).

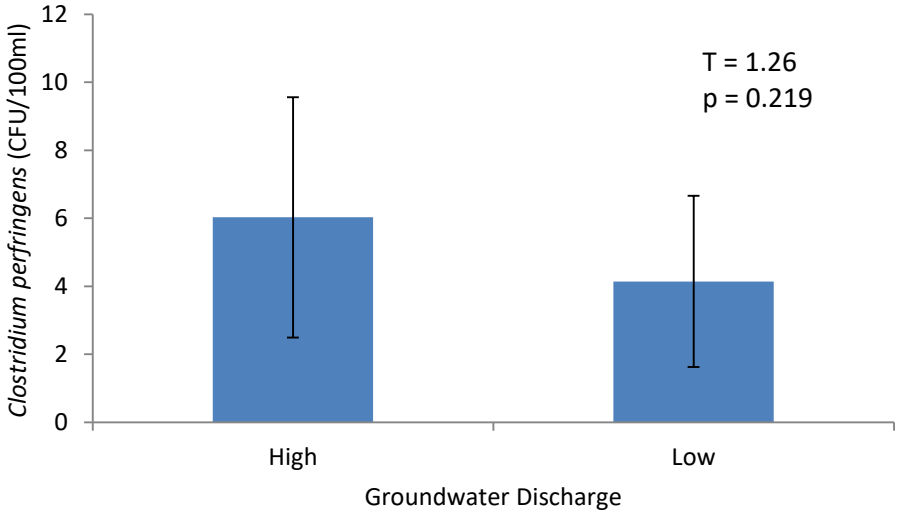

Figure 8. *Clostridium perfringens* in water at high and low groundwater sites (mean ± SE).

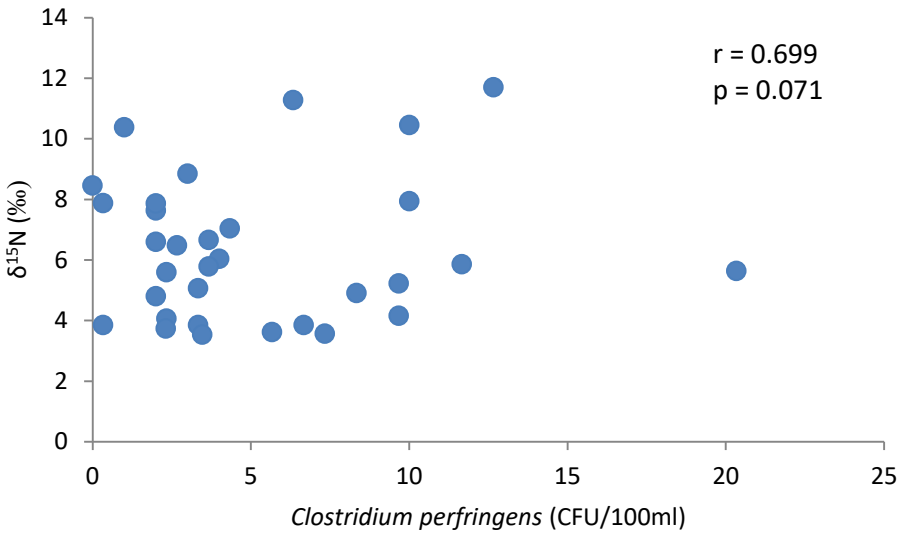

Figure 9. *Clostridium perfringens* water concentration vs. $\delta^{15}N$ in macroalgae.

Table 1: Average (± SE) nutrient concentrations and macroalgal $\delta^{15}N$ values for 16 sites.

Station	Latitude (°N)	Longitude (°W)	Salinity (‰)	Temperature (°C)	$NO_2^-+NO_3^-$ (µM)	PO_4^{3-} (µM)	H_4SiO_4 (µM)	NH_4 (µM)	TDP* (µM)	TDN* (µM)	$\delta^{15}N$ (‰)
1	19.95770	155.85831	28.2±0.7	26.1±0.6	28.29±3.58	0.49±0.02	156.98±38.38	1.01±0.23	0.57±0.06	41.69±2.21	6.3±0.3
2	19.95905	155.85822	7.9±0.8	26.8±0.2	137.43±7.81	2.43±0.30	630.16±142.61	0.65±0.07	2.50±0.29	149.12±9.92	7.3±0.3
3	19.96080	155.85738	9.7±0.2	26.5±0.4	186.13±4.25	5.19±0.18	582.83±63.35	1.62±0.58	5.40±0.31	207.18±9.88	10.4±0.0
4	19.96250	155.85619	12.3±3.2	26.2±0.6	232.92±30.16	8.28±0.14	614.40±68.73	1.32±0.04	8.85±0.36	237.88±29.18	11.5±0.2
5	19.96386	155.85545	27.4±3.6	26.2±0.8	35.61±12.17	1.17±0.27	143.79±58.41	1.17±0.31	1.47±0.57	66.83±25.37	8.6±0.2
6	19.96480	155.85472	28.4±3.9	25.9±0.4	34.25±19.91	0.79±0.38	142.07±77.09	1.37±0.09	1.26±0.00	54.41±31.96	6.0±1.9
7	19.96645	155.85260	20.4±0.5	26.4±0.6	215.15±70.59	3.83±0.01	601.36±202.25	0.58±0.12	4.17±0.34	197.17±43.67	7.2±0.7
8	19.96805	155.85002	15.8±1.7	27.1±1.1	61.21±5.89	1.07±0.01	375.43±40.87	2.00±0.40	1.31±0.24	86.94±11.60	5.1±0.1
9	19.96803	155.84750	17.0±0.9	26.9±0.7	57.49±9.67	1.17±0.02	248.04±28.87	1.33±0.44	1.58±0.10	80.87±5.31	4.0±0.1
10	19.96874	155.84554	16.7±0.5	26.8±0.5	60.17±8.56	1.19±0.08	423.74±21.01	0.91±0.42	1.45±0.20	83.29±9.70	3.6±0.0
11	19.97025	155.84352	27.3±1.2	26.4±1.1	18.59±0.14	0.52±0.06	90.93±37.99	1.13±0.33	0.73±0.09	34.26±6.27	5.7±0.1
12	19.97195	155.84143	25.7±1.4	26.2±0.5	35.50±2.16	0.91±0.07	161.36±49.84	1.33±0.55	1.02±0.10	50.00±5.55	5.7±0.8
13	19.97325	155.83902	27.3±1.0	26.4±0.4	42.08±2.10	2.00±0.29	217.09±50.39	1.15±0.42	2.05±0.33	56.73±10.15	6.3±1.5
14	19.97275	155.83685	7.4±0.7	26.4±0.0	84.99±9.05	2.71±0.27	565.2±149.87	1.06±0.42	2.84±0.18	100.40±16.71	5.7±0.1
15	19.97542	155.83130	28.7±0.1	26.9±0.6	16.79±2.45	0.52±0.03	135.53±17.20	1.02±0.27	0.65±0.00	25.39±2.04	3.8±0.1
16	19.98161	155.82877	18.0±1.0	27.2±0.4	32.40±15.05	0.63±0.18	200.79±59.16	1.09±0.43	0.84±0.24	42.75±8.95	3.7±0.2

*TDP, total dissolved phosphorous; TDN, total dissolved nitrogen