Assessment of Epiphytes as a Sewage Pollution Proxy in Guam

Stevia Morawski

Advisor: Dr. Kiho Kim

Spring 2012
University Honors in Environmental Studies

Assessment of Epiphytes as a Sewage Pollution Proxy in Guam

Morawski, Stevia; Pinkerton, Kate; Kim, Kiho
Department of Environmental Science, American University, Washington, DC 20016

Abstract

Seagrass beds are highly productive systems under threat from human activity. In Guam, a significant level of tourism and U.S military presence combined with low-quality sewage treatment plants may threaten seagrass beds. A recent study by Pinkerton et. al using stable isotope analysis indicated there was detectable nitrogen enrichment in coastal areas in Guam from sewage sources. The purpose of this study was to determine if algal epiphytic growth on seagrass could be used as an indicator of sewage pollution. Samples were collected in Guam analyzed with stable isotope mass spectrometry. Epiphyte load, determined by mg dry wt of epiphyte $^{-1}$ dry wt of seagrass shoot, was not related to $\delta^{15}N$ (p value = 0.1735). To test possible explanations for the lack of relationship, seagrass versus epiphyte metabolism of $\delta^{15}N$ was compared, and seagrass shoots were tested for accumulation of $\delta^{15}N$ along the length of the blade. There was not a significant difference in $\delta^{15}N$ values between epiphytes and seagrass (p value = 0.1848), but there may have been variation in epiphyte composition or grazer contamination. There was also no significant variation in δ^{15} N was found along the length of seagrass (one-way ANOVA, p value= 0.5141). Thus, in Guam, epiphytes are not a useful proxy for sewage pollution.

Introduction

Seagrasses and ecosystem services

Seagrasses are submerged plants that spend their life cycle marine environments. They can form vast meadows that grow on a variety of substrates in shallow coastal or shelf waters. Seagrasses mainly consist of a long shoot apical shoots, connected to rhizomes and roots. They grow clonally, meaning individuals originate from a single ancestor, vegetatively rather than sexually (Vermaat, 2009). Seagrasses, however, are flowering plants (angiosperms) and can reproduce sexually through pollination.

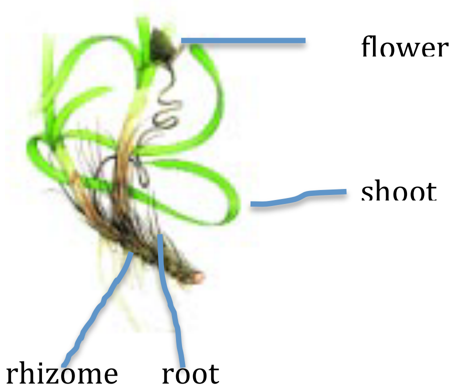


Figure 1: Diagram of *Enhalus acoroides.*
Picture obtained from Coles et al, 2004

Seagrasses take up nutrients both from their roots and their shoots, roughly in equal amounts, and distribute them throughout their system. There has been relatively little research on nutrient transport in seagrass, but there are many processes known to require nutrient transport. For example, relocation of nutrients from senescent leaves or nutrient transport from the leaf blade to the basal meristem both require the movement of nitrogen (Larkum, Orth et al. 2006). Seagrasses grow from the basal meristem and nitrogen travels preferentially to points of new growth

(Fry, 1983), which creates an accumulation of nitrogen at the base. Additionally, the rhizosphere is usually an area of intense nitrogen fixation, which can provide an important source of new nitrogen for the growth of seagrass (Welsh, 2000).

Seagrasses are one of the most important types of marine vegetation in terms of the ecosystem services they provide. They are a major source of food for large herbivores such as dugongs, manatees, and sea turtles, and provide habitat for a high number of commercially and recreationally important fishery species (Orth, 2006). Seagrass beds function as a critical buffer against erosion and wave energy due to their strong roots.

Additionally, seagrass beds play a critical role as a carbon sink in the world's oceans due to their disproportionately high sequestration rates. Seagrass growth accounts for only 1% of ocean productivity, but represents 12% of sequestered carbon (Vermaat, 2009). Thus, they play an important role in the global carbon cycle with clear implication for climate change.

Seagrass beds also have a positive impact on water quality by filtering nutrients and pollutants along coastlines. This filtration occurs through sedimentation in calm water, and also through active uptake by seagrass and associated communities of micro and macro-organisms (Vermaat, 2009).

The value of the ecosystem services provided by seagrass has been estimated at US$34,000 per hectare per year (Short et al. 2011). With its major contribution to fisheries, carbon sequestration, and water filtration, seagrass meadows should be considered ecosystems worth monitoring and protecting. However, many studies have found that seagrasses are declining worldwide. A synthesis of 215 studies indicated that seagrass habitat had disappeared globally at a rate of 110 km per year between 1980 and 2006 (Waycott et al. 2009). This has largely been attributed to human activity and shoreline development. Increases in waste outputs, associated with growing populations, impacts nutrient loading in seagrass beds and has a negative effect on seagrass health (Orth, Carruthers et al. 2006). As of 2011, ten species of seagrass (14% of all species) have been identified as having elevated risk of extinction. Three of those species qualify as Endangered under the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Short et al. 2011).

Epiphytes

An notworthy component of seagrass ecosystems are the epiphytic assemblages of microscopic algae that are attached to the seagrass leaf blades. Epiphytes are highly productive and diverse. The assemblages are usually dominated by species of diatoms and red, brown and blue-green algae (Moncreiff, 1992). Epiphytic communities have low biomass in comparison to seagrasses, but their primary productivity is often of the same magnitude (Terrados, 2008). Studies have indicated that epiphytes may be the primary food source within seagrass communities, as opposed to the seagrasses themselves (Moncreiff, 1992). Thus, epiphytic assemblages make an important contribution to the flow of energy, carbon, and nutrients through seagrass ecosystems.

Though epiphytes are an important component of seagrass meadows, a number of studies have shown that when the competitive balance shifts from seagrass to algal epiphytes, seagrass decline can occur (Borowizka, 2006). In excess abundance, epiphytes block the active leaf uptake sites for nutrients (Apostolakia, 2012), and shade seagrasses such that they do not receive adequate light (Ruiz, 2001). High levels of epiphytes can also attract herbivores and cause declines through grazing (Holmer et al., 2003; Ruiz, 2001; Prado et al., 2010).

Many studies have equated influxes of nitrogen and other nutrients with increased epiphyte loading (Frakovitch, 2009; Apostolakia, 2011; Balata, 2010; Duarte, 1995). Therefore, it is reasonable to test if the level of epiphyte load can be used as in indicator of eutrophication. However, the science is divided on this count. Some studies point to epiphytic overgrowth as an indicator of anthropogenic nutrient pollution and a cause of seagrass loss (Tomasko and Lapointe, 1991; Frankovich and Fourqurean, 1997), while other studies concluded that their respective study areas were not suitable for using epiphytes as a proxy for nutrient pollution (Fourqurean, 2010; Balenta, 2008; Terrados and Pons, 2008; Piazzi, 2004). These studies cite a number of confounding factors that prevent epiphytes load from indicating nutrient pollution, such as changes in epiphyte composition, seagrass depth, and increases in grazing.

Guam

This study aimed to assess the possibility of using epiphytes as an indicator of nitrogen pollution in Guam. Guam is a small island in the Mariana Archipelago, with a land mass of 544 km^2 and 125.5 km of coastline. It is a tropical environment, with little seasonal variation in temperature. The island is one of the most heavily inhabited islands in Micronesia, with a population of about 185,674 (CIA, 2012). Seagrasses in Guam cover about 2.8% of total reef area, with Enhalus acoroides as the dominant species (Lobban and Tsuda, 2003).

Nutrient pollution is a continuing problem in Guam's coastline. The Guam Waterworks Authority's (GWA) was recently taken to court for waste-water treatment plants that have been in violation of the Clean Water Act since 2003 or earlier (Hail, 2010). Lack of funding has been blamed for the slow progress in addressing water contamination. National Pollutant Discharge Elimination System (NPDES) monitoring reported that water samples taken around the coast exceeded nitrate-nitrogen standard of 0.10 mg/L half the time (Porter, Leberer et al. 2005).

As of 2005, there are 19 active NPDES on Guam, including treated wastewater from sewage plants. Nonpoint sources include nutrients from septic tank systems and agricultural runoff (Porter, Leberer et al. 2005). Six municipal wastewater treatment plants in particular are suspected to be operating below standards. In 2009, the EPA denied a permit to Agana Sewage Treatment Plant for ocean discharge. Since 1986 the plant had been granted a variance from secondary treatment, allowing wastewater to be discharged without using bacteria to remove organic matter (GWA, 2009). Agana Sewage Treatment Plant was denied the permit for variance given that the discharge did not meet standards of primary treatment and was in violation of minimum standards under the Clean Water Act (GWA, 2009).

To compound the problem of sewage pollution, Guam is expecting a population influx over the next few years, as the United States has promised to move 4,500 marines from bases in Okinawa to Guam. The move will cost over $20 billion and is expected to bring additional personnel and development to the island (Sakamaki, 2012). With an even greater population to support in the future, it is important to monitor and address sewage treatment issues before they worsen.

It is assumed that agriculture only plays a small role in nitrogen enrichment along the coast. Agriculture is not one of Guam's main economic activities, and only 3.6% of Guam is arable land under crop production (Oceania, 2012). Given its sewage pollution problems and its low amount of agricultural activity, Guam is an excellent candidate for monitoring nutrient pollution using stable isotope analysis of $\delta^{15}N$.

Stable Isotope Analysis and Ecosystem Monitoring

Nitrogen can occur naturally as one of two atomic forms. The lighter form contains seven protons and seven neutrons and is referred to as Nitrogen 14 (written as ^{14}N). The heavier isotope, nitrogen 15 (^{15}N), contains an extra neutron. ^{15}N is the rarer of the two, as only 4 are found out of every 1000 nitrogen atoms (Risk, 2009).

The weight difference between isotopes causes them to behave differently in biogeochemical reactions in nitrogen cycling. This leads to isotope fractionation, or the differential uptake of isotopes in chemical or physical processes. The result is a change in the isotope ratio (Risk, 2009). Stable isotope analysis uses mass spectrometry to determine the ratio of atmospheric nitrogen to heavier nitrogen, and is given in parts per thousand (per mille, ‰). Samples with high $\delta^{15}N$ values are referred to as "enriched."

Different sources of nitrogen carry distinct isotopic ratios, making it possible to identify sources of pollution. For example, nitrogen fertilizer is generally created by fixing atmospheric nitrogen, which has virtually no $\delta^{15}N$ signature. In contrast, human waste contains an enriched $\delta^{15}N$ ratio due to our higher trophic level, which causes N^{15} build up in tissue. The ~10‰ of treated sewage compared to the ~0‰ of fertilizer allows us to determine the source of nitrogen pollution (Constanzo, 2001). The fact that Guam has low levels of agriculture simplifies this process because studies do not have to account for interference sources rich in δN^{14}.

Stable isotope analysis is becoming an increasingly popular technique to monitor ecosystem health and identify sources of anthropogenic pollution (Kendall, 2007). Isotope signatures in seagrass in particular can be a biological indicator because seagrasses integrate water quality attributes into their tissues over a recordable period of time (Orth, 2006). Analyzing seagrass with stable isotope analysis provides a record of nutrients present in the water column.

Seagrass is also an ideal plant to monitor coastal pollution because it is sensitive to environmental changes. For example, seagrass has some of the highest light requirements of any plant in the world, which makes it extremely sensitive to turbidity that might result from increases in runoff or coastal nutrient enrichment (Orth, 2006).

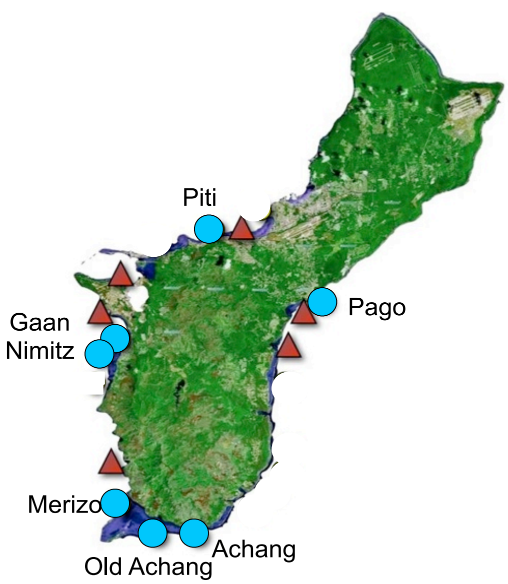
A recent unpublished study (Pinkerton et al., 2010) correlated high $\delta^{15}N$ values in seagrass with distance from sewage output sites in Guam. The study used parameters from Udy and Dennison (1997), where a $\delta^{15}N$ signature of 0-2 indicates an area unaffected by sewage output, and a signature of 5.1 indicates raw sewage. Isotope data in seagrass from Pinkerton's subsequent study in 2011 set a baseline for this study for identifying which sites had the most nitrogen pollution from sewage.

Pinkerton's study was used to determine the level of sewage pollution in each site. Then, average epiphyte loads at each site could be compared to the level of $\delta^{15}N$ pollution. Given that increases in nitrogen have been shown to stimulate epiphyte growth, it was hypothesized that sites with the highest level of $\delta^{15}N$ enrichment would have the highest average epiphyte loads.

Materials and Methods

Data used in this analysis were from samples of *Enhalus acoroides* ,collected by Pinkerton et. al.. Samples were collected from seven sites in July of 2011 (Figure 2). For our own study, seven sites were chosen from Pinkerton's data for range of expected $\delta^{15}N$ enrichment. Achang and Piti are located within marine protected areas the other four sites are not. Leon Guerros was the farthest site from a sewage treatment plant. Piti and Gaan were relatively close to a treatment plant and a sewage outfall, respectively. Samples were generally taken around midday and most sites were located using Burdick's Guam Coastal Atlas (Burdick, 2005) and cross-referenced with hand-held GPS unit.

Map of study sites



sewage sources

Sample sites

Figure 2: Map of study sites

Seagrass Sampling

Methods outlined in the Manual for Scientific Monitoring of Seagrass Habitat: Western Pacific Edition were used. At each site, 10 quadrats (50 cm x 50 cm) at random locations were created and used for sampling. At quadrats 1, 5, and 10, a shoot of average length was taken for N and C isotope analysis.

The sampled shoots were cleared of sediments, and the epiphytes were scraped off using a razor blade. The epiphytes were saved for N and C isotope analysis. All samples were wrapped in aluminum foil and dried at 40°C for 48 hours at the University of Guam Marine Lab. The samples were transported to American University, where they were ground to a powder and weighed to 2.5 mg ± 0.2. Samples were sent for stable isotope analysis at the Carnegie Institution for Science Geophysical Laboratory.

Statistical Analysis

Differences were assessed in Microsoft Excel and Stat Crunch using regression, paired t-test, and one-way ANOVA.

Results

$\delta\, ^{15}N$ 15 levels

$\delta^{15}N$ varied widely between sites. For seagrass, $\delta^{15}N$ values ranged 1.6- 9.1‰ and for epiphytes, $\delta^{15}N$ values ranged 2.0-5.0‰. Average standard deviation was 0.1158 for the seagrass and 0.1142 for the epiphytes. Both the lowest seagrass and epiphyte $\delta^{15}N$ values were documented at Achang. The highest epiphyte $\delta^{15}N$ value was documented in Merizo, and the highest seagrass $\delta^{15}N$ values occurred at Pago.

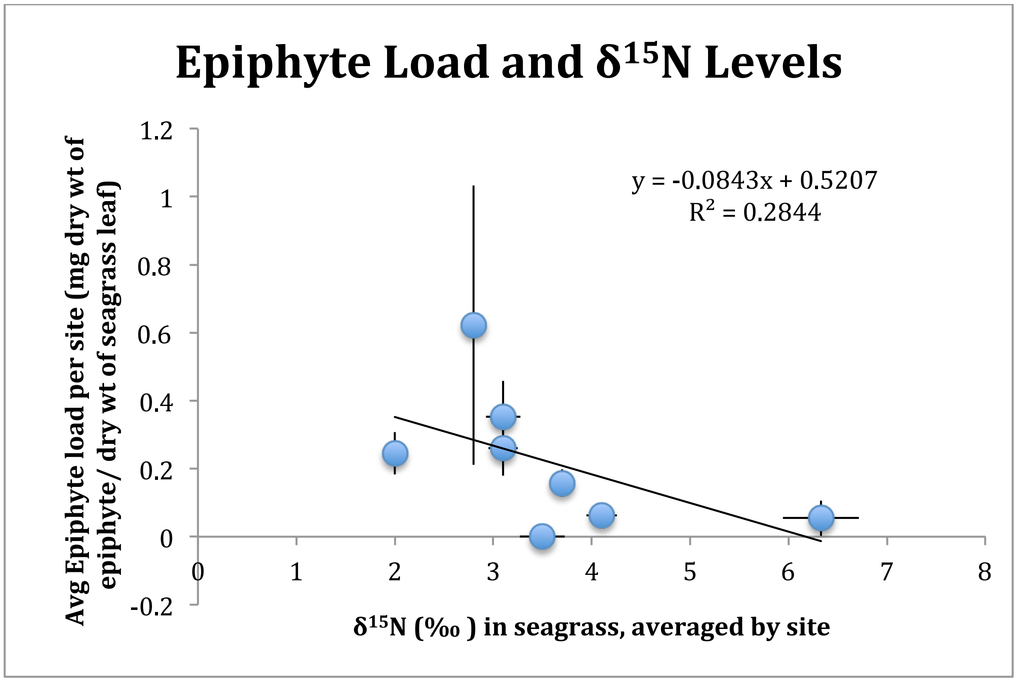


Figure 3: average δ ^{15}N values in seagrass were taken as a general indicator for amount of sewage pollution. The average δ ^{15}N value in sampled seagrasses at each site were compared to average epiphyte load at each site.

Average δ ^{15}N values of seagrass shoots at each site were taken as an indicator overall sewage pollution present at the site. Average epiphyte load for each site was plotted against each site's corresponding δ ^{15}N value. There was not a significant relationship between the epiphyte loads and the δ ^{15}N value by site (p value =0.1735).

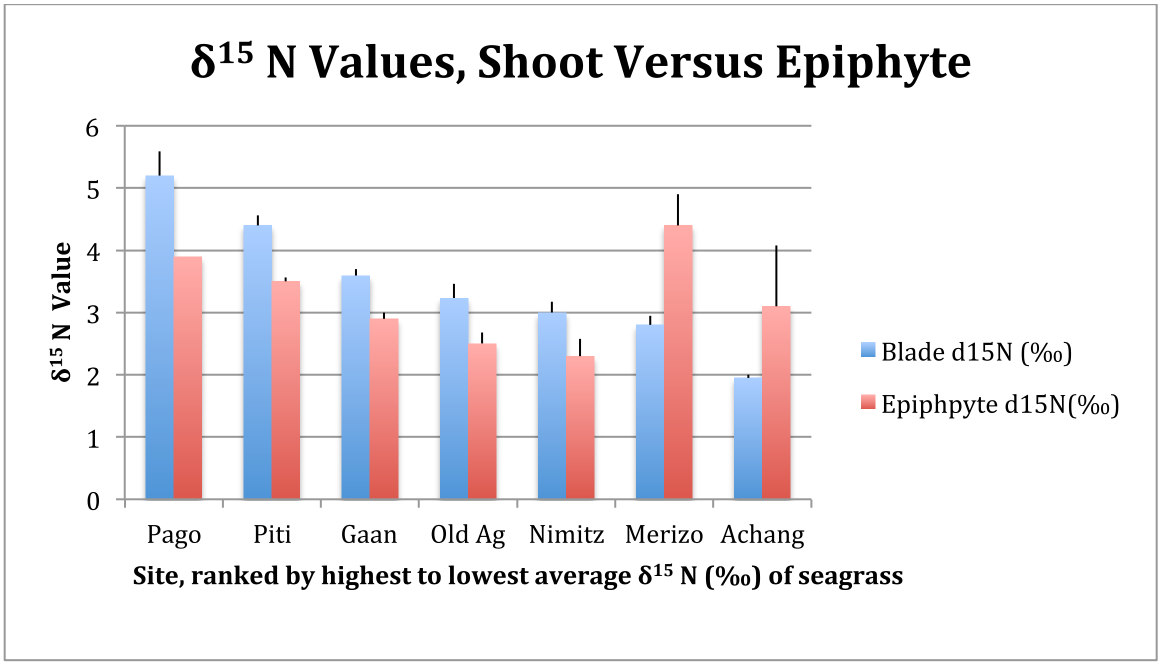


Figure 4: This chart compares δ ^{15}N values between seagrass and epiphytes. The sites are arranged in decreasing level of δ^{15}N in seagrass.

δ ^{15}N values were averaged for each site for epiphytes and seagrass and plotted on the histogram. Sites were plotted in order of decreasing values of δ ^{15}N in seagrass. δ ^{15}N levels in epiphytes tend to follow the decreasing trend except for Merizo and Achang, the two sites with the lowest seagrass δ ^{15}N values. Overall, there was no significant difference in isotope values between seagrasses and epiphytes (paired t-test, p value= 0.1848).

Significant Differences Between δ ^{15}N levels in Shoots versus Epiphytes

Site	**P value**
Pago	0.01320
Piti	0.01296
Gaan	0.01647
Nimitz	0.0492
Old Agat	0.0502
Achang	0.2170

Table 1: Difference between levels in shoots versus epiphytes were found using t-tests

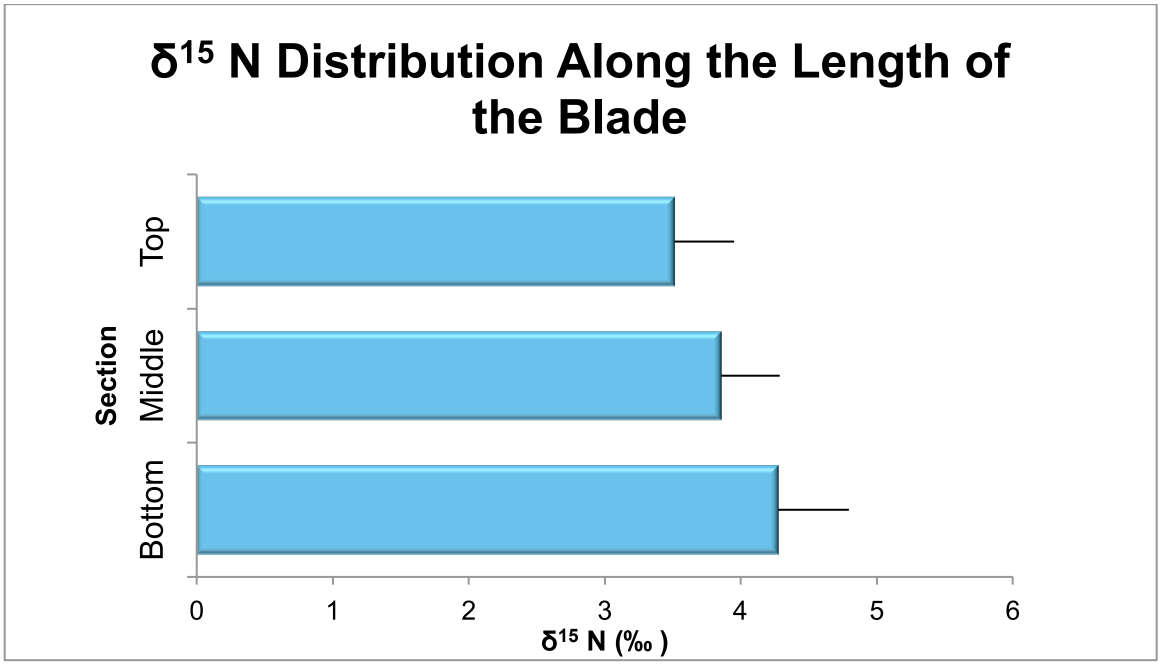


Figure 5: visualizes the $\delta^{15}N$ value in the top, middle, and bottom sections of the seagrass blade

Nitrogen Distribution Along the Seagrass Shoot

The sections of seagrass samples were divided into three equal sections: bottom, middle, and top. There is no significant variation in $\delta^{15}N$ along the length of seagrass (one-way ANOVA, p value= 0.5141).

Discussion

With poor sewage treatment practices in Guam and an expected influx of population in the coming years, it is important to continue monitoring sewage pollution in coastal areas. Due to the relatively low level of agriculture on Guam, it should be possible to identify sources of nitrogen pollution using stable isotope analysis. However, this technique may not be easily available to natural resource managers on the island. Therefore, efforts should be made to find other indicator of nitrogen pollution. This study assessed the degree to which epiphytes on *Enhalus acoroides*, the dominant species of seagrass in Guam, could be used to monitor nitrogen pollution from sewage.

It is clear that epiphyte load is not a usable proxy for nutrient pollution in Guam. Figure 3 shows that there is no significant relationship between the epiphyte loads and the $\delta^{15}N$ value by site (p value =0.1735). This is consistent with a number of studies that have shown similar results. For example, Frankovich and Fourqurean (1997) found that within 15 m of a point source of nutrients in Florida Bay, epiphyte loads are significantly higher, but also that nutrient availability only explains 14% of the variation of epiphyte loads across the bay as a whole. Other studies have also shown that epiphyte loads are not accurate indicators of nutrient availability due to a number of variables such as grazing, seagrass depth, and epiphyte composition (Fourqurean, 2010; Balenta, 2008; Terrados and Pons, 2008; Piazzi, 2004).

Despite many cases of nutrient enrichment stimulating epiphyte growth (Frakovitch, 2009; Apostolakia, 2011; Balata, 2010; Duarte, 1995), there was a weak negative trend between $\delta^{15}N$ and epiphyte load in Guam. Though this result was contrary to expectations, there has been a previous study that also found a decrease in epiphyte loading with increased inputs of animal waste, which was attributed to exceptionally high herbivore pressure in the study areas (Ruiz, 2001).

As an effort to explain the lack of a relationship, it was hypothesized that epiphytes and seagrass metabolize $\delta^{15}N$ differently, prohibiting epiphyte load from acting as a proxy for nitrogen pollution. To test this prediction, $\delta^{15}N$ levels were compared between seagrass and epiphytes. Figure 4 displays a noticeable trend in $\delta^{15}N$ values between seagrass and epiphytes as $\delta^{15}N$ availability decreases in the first five sites. As $\delta^{15}N$ levels decrease in seagrass, epiphyte levels decrease by a relatively comparable amount. However, the last two sites, Merizo and Achang, there is a spike in the $\delta^{15}N$ levels in the epiphytes even as the seagrasses have the lowest $\delta^{15}N$ values among all seven sites.

The higher isotope signature in epiphytes from Merizo and Achang may suggest herbivore contamination, or perhaps a different community structure than the other sites. The species of epiphytes collected in our study were not identified, making this distinction difficult. A study that compared epiphyte assemblage on the leaves of *Posidonia oceanica* exposed to different levels of nutrients from different anthropogenic sources showed there were differences in composition and abundance of epiphytic assemblages on leaves between disturbed and non-disturbed sites (Balata, 2008). In fact, the study concluded that variation in composition and abundance of epiphytes as an adequate detector of moderate nutrient increases. However, Balata also concluded that the presence of non-autotrophic organisms in epiphyte communities and contaminant grazers may prevent a direct relationship between nutrient availability and epiphyte load (Balata, 2008). In Guam, the case may be the same. With changes in the overall nitrogen availability and other factors, epiphyte community structure may change from site to site. Different epiphyte communities may also grow in different abundances on seagrass, thereby introducing a confounding factor that may be difficult for which to account when using epiphyte growth as a proxy for nutrient pollution.

A study that analyzed shifts in composition of epiphytic macroalgae during a period of seagrass loss in Cockburn Sound, Western Australia, also found that epiphyte load could not be used to indicate nutrient pollution. Patterns of nutrient loading and seagrass loss were correlated with shifts in epiphyte species composition, although it was not a predictor of future seagrass loss (Cambridge, 2007). Perhaps in future studies in Guam, species of epiphytes will be noted at each site and conclusions can be drawn about epiphyte assemblage in relation to sewage pollution.

Fourqurean et al. (2010) offer other possibilities as to why epiphytes cannot be used as a proxy for nitrogen pollution. Since algal growth is so much faster relative to seagrass, there may be some threshold defined by the inability of seagrass to use available nitrogen, above which increases in nitrogen availability would only stimulate increased biomass of algal competitors. If this were the case in Guam, Figure 4 would be characterized by a plateau in $\delta^{15}N$ levels in seagrass on the left of the graph (at the highest $\delta^{15}N$ values), while epiphyte $\delta^{15}N$ continued to increase. In Guam, there is indication that nitrogen is still a limiting factor to growth, keeping seagrass below this hypothetical nitrogen threshold.

The degree of epiphyte loading is determined both by bottom-up factors that control production and top-down factors that affect removal of epiphyte biomass. Top-down forces, such as grazing may be affecting epiphyte loads in a way that obscures the effects of nutrients (Fourqurean, 2010). In many cases, grazing has been noted to be as or more important to determining epiphyte biomass than nutrients (e.g. Hootsman and Vermaat, 1985; Neckles et al., 1993; Williams and Ruckelshaus, 1993; Short et al., 1995). Due to the plethora of factors and determinants of epiphyte load, aquatic systems where epiphyte load can act as in indicator of nitrogen pollution may be rare.

It was suspected that there was δ 15N accumulated at the base of the shoot. If this were the case, the basal portion of the blade may reflect a higher δ 15N value than would be representative of the site. To determine whether this affected our use of epiphytes as an indicator of pollution, the top, middle, and bottom sections of seagrass were analyzed separately to identify any accumulation of δ 15N. In the samples analyzed, there was no significant build-up of δ 15N in any particular section of the shoot. In Figure 3, there appears to be slightly more build up of δ 15N towards the bottom of the shoot, but this was not significant. This finding was contrary to expectations due to the basal growth of seagrass and the tendency of plants to transfer nitrogen to points of growth (Fry, 1983). While there may have been a significant accumulation of Wt.% N at the bottom of the shoot, δ 15N did not behave in a similar manner. As a recommendation for future studies, it will likely not matter which part of the shoot is sampled for stable isotope analysis, as any section will yield similar results. Furthermore, nitrogen accumulation can be ruled out as a factor affecting epiphytes as a pollution proxy in this study.

Conclusions

There are numerous factors that may prevent the use of epiphyte load as an indicator of nutrient pollution in Guam. It is likely that changes in epiphyte composition or variation in herbivory introduced confounding factors into the study. It is also possible that in Guam's coastal waters, seagrass may be below the threshold at which the competitive balance shifts from slow-growing rooted macrophytes, to faster-growing primary producers such as epiphytes. Other methods, though possibly more expensive, will have to be used to monitor sewage pollution in Guam in the future. With the current substandard sewage treatment in Guam coupled with the imminent influx of U.S military personnel, some form of monitoring will be necessary to ensure Guam's valuable seagrass meadows are unharmed.

Acknowledgements

Special thanks to Kate Pinkerton (American University) for use of data and improvements made to this paper. Thanks also goes to Kiho Kim (American University) for his comments and guidance. Data collection and analysis were made possible in part by Laurie Raymundo (University of Guam), and David Baker (Carnegie Institute for Science).

References

Apostolakia, E. T., M. Holmer, et al. (2011). "Epiphyte dynamics and carbon metabolism in a nutrient enriched Mediterranean seagrass (Posidonia oceanica) ecosystem." Journal of Sea Research **66**(2): 135-142.

Apostolakia, E. T., S. Vizzinic, et al. (2012). "Leaf vs. Epiphyte Nitrogen Uptake in a Nutrient Enriched Mediterranean Seagrass (Posidonia oceanica) Meadow." Aquatic Botany **96**: 58-62.

Balata, D., I. Bertoccib, et al. (2008). "Comparison Between Epiphyte Assemblages of Leaves and Rhizomes of the Seagrass Posidonia oceanica Subjected to Different Levels of Anthropogenic Eutrophication." Estuarine, Coastal and Shelf Science **79**(3): 533-540.

Balata, D., L. Piazzi, et al. (2010). "Effects of enhanced loads of nutrients on epiphytes on leaves and rhizomes of Posidonia oceanica." Journal of Sea Research **63**(3-4): 173-179.

Borowizka, M. A., P. S. Lavery, et al. (2006). Epiphytes of seagrasses. The Netherlands, Springer.

Burdick, D. M. (2005). Guam Coastal Atlas. N. O. A. A. Administration. **149**.

Cambridge, M. L., J. R. How, et al. (2007). "Retrospective Analysis of Epiphyte Assemblages in Relation to Seagrass Loss in a Eutrophic Coastal Embayment." Marine Ecology Progress Series **346**: 97-107.

CIA. (2012). "World Factbook: Guam." Retrieved April 28, 2004, from https://www.cia.gov/library/publications/the-world-factbook/geos/gq.html.

Coles, R., L. McKenzie, et al. (2004). Seagrasses in Queensland waters. Queensland, Australia, Reef Research Centre.

Constanzo, S. D., M. J. O'Donohue, et al. (2001). "A new appraoch for detecting and mapping sewage impacts." Marine Pollution Bulletin **42**(2): 149-156.

Duarte, C. (1995). "Submerged aquatic vegetation in relation to different nutrient regimes." Ophelia **41**(87–112).

E.Vermaat, J. (2009). "Linking clonal growth patterns and ecophysiology allows the prediction of meadow-scale dynamics of seagrass beds." Perspectives in Plant Ecology, Evolution and Systematics **11**: 137-155.

Frankovich, T. A., A. R. Armitage, et al. (2009). "Nutrient Effects on Seagrass Epipyte Community Structure in Florida Bay." Journal of Phycolology **45**: 1010–1020.

Frankovich, T. A. and J. W. Fourqureane (1997). "Seagrass Epiphyte Loads Along a Nutrient Availability Gradient, Florida Bay, USA." Marine Ecology Progress Series **159**: 37-50.

Frederick T. Short et al (2011). "Extinction risk assessment of the world's seagrass species." Biological Conservation **144**(7): 1961-1971.

Fry, B. (1983). "Leaf Growth in the Seagrass Syringodium Filiforme." Aquatic Botany **16**: 361-368.

GWA (2009). Fact Sheet on EPA's Final Decision on the application for renewal of a CWA 301(h) variance for the Agana Sewage Treatment plant. U. S. E. P. Agency.

Hail, J. (2010). GWA violating Clean Water Act for 7 years. Kuam News. Guam.

Holmer, M., M. Pérez, et al. (2003). "Benthic primary producers — a neglected environmental problem in Mediterranean maricultures?" Marine Pollution Bulletin **46**: 1372-1376.

Hootsman, M. J. M. and J. E. Vermaat (1985). "The Effects of Periphyton-Grazing by Three Epifaunal Species on the Growth of Zostera marina L. Under Experimental Conditions." Aquatic Botany **22**: 83-88.

Larkum, A., R. Orth, et al. (2006). Seagrasses: Biology, Ecology and Conservation. The Netherlands, Springer.

Lobban, C. S. and R. T. Tsuda (2003). "Revised checklist of benthic marine macroalgae and seagrasses of Guam and Micronesia." Micronesica **35**: 54-99.

Moncreiff, C. A., M. J. Sullivan, et al. (1992). "Primary production dynamics in seagrass beds of Mississippi Sound: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton." Marine Ecology Progress Series **87**: 161-171.

Neckles, H. A., R. L. Wetzel, et al. (1993). "Relative Effects of Nutrient Enrichment and Grazing on Epiphyte-Macrophyte (Zostera marina L.) Dynamics." Oecologia **93**: 285-295.

Oceania. (2012). "Guam Agriculture."   Retrieved April 22, 2012.

Orth, R., T. Carruthers, et al. (2006). "A Global Crisis for Seagrass Ecosystems."

Piazzi, L., D. Balata, et al. (2004). "Patterns of spatial variability in epiphytes of Posidonia oceanica: Differences between a disturbed and two reference locations." Aquatic Botany **79**(4): 345-356.

Prado, P., J. Romero, et al. (2010). "Nutrient status, plant availability and seasonal forcing mediate fish herbivory in temperate seagrass beds." Marine Ecology Progress Series **409**: 229-239.

Risk, M. J., B. E. Lapointe, et al. (2009). "The use of d15N in assessing sewage stress on coral reefs." Marine Pollution Bulletin **58**: 793-802.

Ruiz, J. M., M. Pérez, et al. (2001). "Effects of Fish Farm Loadings on Seagrass (Posidonia oceanica) Distribution, Growth and Photosynthesis." Marine Pollution Bulletin **42**(9): 749–760.

Sakamaki, S. (2012). U.S. Troop Shift to Guam May Ease Tensions With Japan Over

Okinawa Bases. Bloomberg News. Tokyo.

Short, F. T., D. M. Burdick, et al. (1995). "Mesocosm Experiments Quantify the Effects of Eutrophication on Eelgrass, Zostera marina." Limnology and Oceanography **40**: 740-749.

Terrados, J. and F. Javier (2008). "Epiphyte Load on the Seagrass Posidonia oceanica (L.) Delile Does Not Indicate Anthropogenic Nutrient Loading in Cabrera Archipelago National Park (Balearic Islands, Western Mediterranean)." Scientia Marina **72**(3).

Tomasko, D. A. and B. E. Lapointe (1991). "Productivity and biomass of Thalassia testudinum as related to water column nutrient availability and epiphyte levels: field observations and experimental studies." Marine Ecology Progress Series **75**: 9-17.

Vermaat, J. E. (2009). "Linking clonal growth patterns and ecophysiology allows the prediction of meadow-scale dynamics of seagrass beds." Perspectives in Plant Ecology, Evolution and Systematics **11**(2): 137–155.

Waycott, M., et al. (2009). "Accelerating loss of seagrass across the globe threatens coastal ecosystems." Proceedings of the National Academy of Sciences **106**: 12377-12381.

Welsh, D. T. (2000). "Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria interactions and significance to primary productivity " Ecology Letters **3**: 57-71.

Williams, S. L. and M. H. Ruckelshaus (1993). "Effects of Nitrogen Availability and Herbivory on Eelgrass (Zostera marina) and Epiphytes. ." Ecology **74**: 904-918.