The impact of NH_4^+ loading on the scleractinian coral *Acropora*

by

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Abstract

Coral reefs are one of the most diverse and complex ecosystems in the world supporting an estimated 25-38% of all marine life. Unfortunately, coral reef ecosystems and their keystone organisms, scleractinian coral, are declining at an alarming rate due to anthropogenic activities. Some species such as those within the genus *Acropora*, are imperative to reef ecosystems on a global scale; however, *Acropora* are considered to be the most sensitive to anthropogenic stressors. How these crucial reef builders respond to stressors such as bleaching and acidification is widely known; however, how *Acropora* respond to eutrophic conditions is greatly understudied in comparison as the ocean has historically been nitrogen (N) limited. Scleractinian coral rely upon associated microbes, *Symbiodinium*, and heterotrophic feeding to supplement their N demand. Under high N conditions, the balance between the coral and *Symbiodinium* is disrupted with uncontrollable *Symbiodinium* division and the retention of nutrient-rich photosynthates. The balance between metabolism (photosynthesis and respiration) and N cycling rates allow for a better understanding of coral physiological responses to environmental stressors. Here I am interested in determining how varying ammonium (NH_4^+) levels impact 1.) metabolism measurements in aquacultured and wild *Acropora* and 2.) remineralization, nitrification, and uptake rates in aquacultured and wild *Acropora* and their surrounding environment. These experiments seek to improve the understanding of how scleractinian coral respond to eutrophic conditions, specifically NH_4^+ loading.

Surface area measurements of each *Acropora* fragment were required to properly scale metabolism and N cycling rates. Methods to determine surface area measurements are ubiquitous throughout scientific literature and the accuracy of these methods are often debated upon. Two of the most cost-effective and cited surface area methods are foil wrapping and wax dipping.

Neither method produced statistically significant differences in the aquacultured *Acropora* surface area estimations and displayed similar results using calibrated objects. I determined the foil wrapping method best suited our study.

Metabolism measurements conducted on scleractinian coral can estimate the energy produced and consumed by the coral and its associated *Symbiodinium* and microbes. I measured respiration and primary production in *Acropora* exposed to varying levels of NH_4^+ under light and dark conditions. I found a significant, positive relationship between aquacultured *Acropora* primary production and the level of NH_4^+ treatment; however, there was not a significant relationship between NH_4^+ treatment levels and respiration in both the aquacultured and wild *Acropora*. Respiration rates were significantly different between wild *Acropora* fragments collected from two different sites surrounding the island of Guam. These findings indicate that factors other than NH_4^+ influence respiration rates while primary production is limited by N.

Rates of remineralization, nitrification, and uptake are not well established for corals and their associated microbial communities. However, these rates, similarly to metabolism rates, can determine how scleractinian coral respond to increasing levels of NH_4^+. I quantified rates of remineralization, nitrification, and uptake for wild and aquacultured *Acropora* subjected to elevated NH_4^+ treatments using an isotopic tracer method. Rates of remineralization, nitrification, and uptake in aquacultured and wild *Acropora* were not statistically different among NH_4^+ treatment levels. Lastly, rates of remineralization, uptake, and nitrification of both aquacultured and wild *Acropora* under varying NH_4^+ treatments are presented and compared to rates from previous studies. *Acropora* uptake and nitrification rates were consistent with rates presented in previous studies with scleractinian coral under ambient seawater. These rates of N

cycling in *Acropora* are some of the first to be quantified for scleractinian coral under elevated NH_4^+ treatments.

Table of Contents

List of Figures

List of Tables

List of Equations

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Chapter 1 - Introduction

Coral reefs are one of the most diverse and complex ecosystems on Earth supporting an estimated 25-38% of all marine life (McAllister 1995, Fisher et al. 2015). In general, one to nine million species can be found living on or within warm water coral reefs (Reaka-Kudla 1997) with more species yet to be described (Fisher 2015). This diversity and richness is comparable to the diversity and richness found in tropical rainforests (Sebens 1994). Coral reefs provide a wide range of ecosystem services to humans that include but are not limited to fisheries, coastal protection, biogeochemical cycling, and cultural services (Woodhead et al. 2019). These ecosystem services are conservatively estimated to be valued at $9.9 trillion per year (Costanza et al. 2014). Scleractinian coral are keystone organisms in which coral reef biodiversity, richness, and ecosystem services rely upon. These keystone ecosystem engineers are facing anthropogenic threats that could place 75% of all reefs under an 'extreme' threat level by 2050 (IPCC 2014). Anthropogenic activities are accelerating the deterioration of coral reefs, including scleractinian coral. Eutrophication, overfishing, and ocean acidification have caused reefs to deteriorate by leading to increased disease susceptibility and bleaching in scleractinian coral (Hoegh-Guldberg 1999, Hughes et al. 2003, Goldberg et al. 2004).

Eutrophication, in particular, is often an overlooked anthropogenic stressor in reef systems despite an estimated 24% of global anthropogenic nitrogen (N) released in coastal watersheds reaching coastal ecosystems (Malone & Newton 2020). N loading to an otherwise N limited environment can lead to biogeochemical imbalances in marine organisms such as scleractinian coral (Moffat 1998). Scleractinian coral have adapted to low nutrient conditions not only through their ability to heterotrophically feed, but also through symbiotic relationships with *Symbiodinium* and other microbes that live within or on the coral (Rädecker et al. 2015). In

limited N environments, microbes living within and on the coral can directly fix N for coral usage; the *Symbiodinium* also produce and send carbon (C) and N rich photosynthates to the coral host (Falkowski et al. 1984, Glaze et al. 2022). Both the coral host, *Symbiodinium,* and other microbes can use external sources of ammonium (NH_4^+) (Wafar et al. 1990). Often external NH_4^+ is brought into reef ecosystems by fish excretion, but the levels of NH_4^+ are low enough in the water column to maintain a N balance throughout the coral (Shantz et al. 2015). However, increased levels of NH_4^+ in the water column surrounding the coral host can lead to uncontrollable rates of *Symbiodinium* cell division and the retention of photosynthates (Stimson & Kinzie 1991). This disruption can lead to increased bleaching susceptibility and overall deterioration of the coral host health (Wooldridge 2013).

In my thesis, I attempt to determine how scleractinian coral responds to increased NH_4^+ loading by quantifying portions of the N cycle and measuring metabolic rates of species within the genus *Acropora*. I chose this coral genus as *Acropora* are not only one of the most widespread and important reef builders in the world, but they are one of the most threatened by anthropogenic activities. Two species of coral were studied, the first being aquacultured *Acropora*, and the second being *Acropora pulchra* collected from two reef sites surrounding the island of Guam.

The N cycling and metabolism calculations conducted throughout this research depended upon knowing the surface area of each *Acropora* fragment. Previous literature has investigated many methods into measuring the surface area of coral with foil wrapping and wax dipping being most commonly used (Marsh 1970, Hoegh-Guldberg 1988, Holmes 2008, Conley & Hollander 2021). Foil wrapping and wax dipping can produce results similarly to advanced technologies such as CT scans but are generally cheaper and more portable than advanced

technological methods (Holmes 2008, Raz-Bahat et al. 2009, Veal et al. 2010$_a$). In Chapter 2, I compare the foil wrapping and wax dipping methods using calibrated objects and aquacultured *Acropora* fragments to determine which method would not only provide reliable surface area measurements of *Acropora* but also to find which method was easiest to replicate in a field setting.

Metabolism measurements conducted on the *Acropora* fragments were used as an indicator to estimate the overall health of each coral fragment in addition to determining the overall energy consumed and produced by the coral and its associated organisms. The balance between coral respiration and primary production (net production) is thought to be reliant on low levels of N in the surrounding water column. The coral host controls the retention or release of N to the *Symbiodinium*; however, under high external N conditions the *Symbiodinium* is able to rapidly assimilate N (Rädecker et al. 2015). This rapid N assimilation leads to high cell division and often results in rates of primary production that are greater than those under ambient N conditions (Snidvongs and Kinzie 1994, Fagoonee et al. 1999, Ferrier-Pagès et al. 2000, Sakami 2000, Ferrier-Pagès et al. 2010, Beraud et al. 2013). I designed and constructed a metabolism chamber to measure primary production and respiration in coral fragments. This design is presented alongside metabolism measurements in Chapter 3.

In low nutrient waters, coral are able to support its N demand not only through heterotrophic feeding but also alongside organisms that live within and on the coral host (Risk & Muller 1983, Wafar et al. 1990, Lesser et al. 2004, Siboni et al. 2008, Houlbrèque & Ferrier-Pagès 2009). Cycling and transformation of N is crucial to maintain N requirements of both the host and associated organisms. Quantifying and comparing rates of N processes could provide an insight into the alleviation of nutrient limitation on coral reefs. Few studies have provided rates

of remineralization, nitrification, and uptake of scleractinian coral and its associated microbial communities under ambient conditions yet alone rates under varying NH_4^+ loading conditions. In Chapter 4, I provide rates for remineralization, nitrification, and uptake for two species of *Acropora* under varying NH_4^+ treatments.

Chapter 2 - Allometric relationships of *Acropora* fragments: determining the surface area and relationships to coral mass

Abstract

Techniques measuring scleractinian coral fragment surface area are numerous with foil wrapping and wax dipping methods being most common. While both methods are cost-effective and easily replicated, the wax dipping method relies upon the removal of the coral tissue and destruction of the remaining coral skeleton, and the foil method could have more potential to over or under-estimate the skeleton surface area. In this study, we compare the foil and wax methods with calibrated objects and aquacultured *Acropora* skeletons. Other methods including geometric and projection-based approaches are additionally compared. The wax and foil method did not produce statistically significant differences in the aquacultured *Acropora* surface area estimations ($p=0.7642$, ANCOVA) and displayed similar results using the calibrated objects: foil ($R^2=0.9887$); wax ($R^2=0.9847$). Our results most likely apply to corals with a morphology similar to *Acropora*, though could apply to others, particularly those with simpler, smaller morphologies. Additionally, our approach to compare methods could be used on any morphology of coral.

Introduction

Estimating the active surface area of coral is necessary to compare metabolic rates per unit area of active biomass. Scientific literature has highlighted many methods to analyze the surface area of scleractinian coral. (Marsh 1970, Hoegh-Guldberg 1988, Holmes 2008, Conley & Hollander 2021). Two of the most commonly used methods that are low cost and easily

replicated are the foil wrapping and wax dipping methods (Marsh 1970, Stimson & Kinzie 1991).

Despite the advancement of technologies such as 3D scanning that have the potential to more accurately determine the total exposed surface area of a coral, both the foil wrapping and wax dipping methods continue to be used in research. Recent publications use the foil method (e.g. Babbin et al. 2021, Manikandan et al. 2021, Wang et al. 2021, Plafcan & Stallings 2022, Schlecker et al. 2022) as well as the wax method (Leinbach et al. 2021, Mickael et al. 2021, van der Zande et al. 2021, Ferrier-Pagès et al. 2022, Jacquemont et al. 2022).

Since the wax and the foil methods are inexpensive and easy to conduct in remote field sites or where expensive scanning equipment may not be available, we focus on comparing those two methods using calibration objects of known total surface areas and aquacultured *Acropora* skeletons. We additionally compare these two methods to a geometric approximation approach as well as an estimation based on total mass of the skeleton.

Additionally, the total surface area of coral is different from the planar (projectional) surface area; thus, we also compared our methods to a projectional based approach. Planar or projectional surface area are the measurements frequently used to scale metabolic rates of coral in whole reef ecosystems.

We were particularly interested in determining the accuracy of foil wrapping as that method can be quickly and gently applied to living coral, whereas the wax method relies upon sacrificing the coral tissue and skeleton.

Methods

Calibration objects

We assembled 33 plastic calibration objects (ETA Hand2Mind) in various lengths of cubes (Figure 2.1) to create surface area calibration curves using the foil and the wax methods. These calibrated objects ranged in size from 6 cm^2 to 32 cm^2.

Coral fragments

Living *Acropora* (ORA® Aquacultured Scripp's Green Tip *Acropora*) fragments were purchased from LiveAquaria (Rhinelander, WI). The coral tissue was airbrushed off of 24 fragments and the remaining skeletons were soaked in freshwater for 24 hours to remove any residual organic matter and salt. After the freshwater soak, the skeletons were rinsed with DI water and then placed in a 100 ºC drying oven for 24 hours. The calibration objects and coral were kept at 25 ºC prior to foil wrapping or wax dipping.

Foil method

We wrapped a single piece of aluminum foil around the entirety of each calibration object and coral skeleton, careful not to overlap the foil. We removed each foil piece from its associated object and weighed it using a Sartorius Entris 124-1SUS (accuracy 0.1 mg). The mass of the foil used to cover each skeleton was converted to surface area by using the mass of the same foil used to cover the calibration objects with object area (cm^2) divided by foil mass (g).

Wax method

A single 2 mm hole was drilled in the middle of each calibrated object after the foil was removed prior to wax dipping. A 2 mm diameter bamboo skewer was placed in each hole to hold the object while dipping in melted wax. The coral skeletons were already glued to a coral plug so drilling and the placement of a bamboo skewer on or within the coral were not required. We weighed each calibrated object and associated bamboo skewer alongside each coral skeleton and its plug using a Sartorius Entris 124-1SUS (accuracy 0.1 mg). We melted paraffin wax at 65 ºC in a beaker placed in a water bath. The temperature of the water bath was continuously

monitored using a thermometer. After the wax was fully melted and stabilized at a temperature of 65 ºC, we proceeded to dip each object carefully in the paraffin wax for two seconds to ensure minimal wax coverage on the skewer or plug. We removed each object from the wax and quickly rotated it in the air to promote even and full coverage of the wax on the object. After dipping, each object was allowed to dry at room temperature (25 ºC) for 15 minutes prior to being reweighed. The mass of the wax used to cover each skeleton was converted to surface area by using the mass of the same wax used to cover the calibration objects with object area (cm^2) divided by wax mass (g).

Caliper method

Right circular cylinders were used to represent segments of aquacultured *Acropora* skeletons (Nauman et al. 2009, Conley & Hollander 2021). We considered each branch on each coral skeleton to be a cylinder and measured the radius and height of each one using a standard vernier caliper with an accuracy of 0.1 mm. See section on calculations for additional details.

Projection method and coral skeleton mass

An overhead image of each aquacultured *Acropora* skeleton was taken approximately 10 cm above each skeleton alongside a ruler. The images were taken above the *Acropora* as this is the way *Acropora* are typically positioned in the water column (vertically). After uploading the images to ImageJ (v1.53t, Rasband 2014) we determined the scale of each image using the number of pixels per 1 cm on the ruler. After each scale was set, we traced the projectional surface of each coral skeleton which expressed the projectional surface area value in cm^2.

We also directly measured mass of each coral skeleton as an additional proxy for fragment volume and to allow allometric comparisons of the various tested methods.

Calculations and statistics

We used R version 4.0.2 (R Core Team 2020) for all but one statistical analysis. We used STATISTICA version 13.3 (StatSoft 2017) to perform stepwise regressions to predict projectional surface areas.

Wax dipped and foil wrapped calibration curves were determined (Figure 2.2). We used the linear functions from both calibration curves alongside the mass of the wax and foil from each skeleton to calculate the surface area of the coral skeleton (tidyverse v1.3.2, Wickham et al. 2019).

Using the radius and height measurements, we calculated the lateral surface area (LSA) of each individual branch using the following cylinder equation:

Equation 2.1 Lateral surface area of a cylinder

$$LSA = 2\pi rh$$

Where r is radius and h is height. We then summed the LSA of each skeleton's branches to estimate the total surface area of the coral. We compared the surface area values of all methods to one another in addition to the mass of each coral skeleton. We determined the strength of each comparison by using results derived from each linear function.

We used ANCOVA to determine if there was a significant difference in surface area values between surface area methods, between each coral skeleton's mass, or between methods and skeleton mass combined where the surface area values determined from differing methods was the categorical variable and skeleton mass was the continuous variable. A post-hoc analysis was performed with a Bonferroni multiple testing correction to identify which surface area methods differed from one another (rstatix v0.7.0, Kassambara 2021 and tidyverse v1.3.2, Wickham et al. 2019).

A multiple linear regression analysis was conducted to see if the projection area was significantly associated with skeleton mass and surface area values determined by using the foil and LSA methods (rstatix v0.7, Kassambara 2021 and tidyverse v1.3.2, Wickham et al. 2019).

Results

Both linear functions from the wax and foil calibration curves report high R^2 values (R^2>0.9); however, a slightly higher R^2 value was observed for the foil wrapped curve compared to the wax dipped curve (Figure 2.2). Surface area values determined by LSA, foil, and wax methods were correlated to corresponding coral skeleton mass (Table 2.1, Figures 2.3, 2.4). When comparing surface area values calculated from each method to each other, R^2 values were above 0.9 for all comparisons (Table 2.2).

ANCOVA indicated mass of coral was significantly linearly related to surface area across methods. The method used to determine surface area also had a significant effect on predicted surface area. There was not a significant interaction between method and coral skeleton mass when predicting surface area (Table 2.1). Post-hoc analysis suggested the wax and the foil methods did not differ significantly from each other, but both were different from the LSA method (Table 2.3).

Forward stepwise multiple linear regression indicated that LSA was the only significant variable that could be used to predict projected surface area. However, the relationship between LSA and projected surface area was relatively weak (Figure 2.5, Table 2.4).

Discussion

When comparing the R^2 values from the calibration curves, we found that foil wrapping was slightly more explanatory than the wax method; however, further statistical tests found that the wax and foil method provided surface area estimates statistically similar to each other. Previous research provides conflicting claims that the wax method is more accurate than the foil method. Veal et al. (2010_a) found the foil method to be the least accurate out of X-Ray CT, structured light scanning, laser scanning, photogrammetric reconstruction, and wax dipping methods when determining the surface area of *Acropora intermedia*. Raz-Bahat et al. (2009) found methylene blue coating, wax dipping, and caliper measuring were all less accurate than foil wrapping in *Stylophora pistillata*.

Differences among studies may be due to the fact that there are two different wax dipping methods. Some studies use the double wax dipping method where the object is dipped twice. In our study we chose to follow the single wax dipping method where the object is only dipped once as Veal et al. (2010_b) found that that single is more accurate than double wax dipping but suggested that the double wax dipping method may be more appropriate for highly porous coral skeletons such as those within *Acropora*. Conversely, Holmes et al. (2008) found double wax dipping is more accurate than single wax dipping.

Overall, single wax dipping gives comparable results to advanced technological methods. The single wax dipping method generally has an accuracy higher or comparable to more advanced coral surface area methods such as CT scanning, desktop 3D laser scanning, photogrammetry, and handheld laser scanning (Holmes 2008, Raz-Bahat et al. 2009, Veal et al. 2010_a, Conley & Hollander 2021). Wax dipping still faces some methodological difficulties as

coral skeletons could retain wax particles differently than the wooden or plastic objects used to create the calibration curves leading to the over and under-estimation of coral surface area.

Support for the over and under-estimation of surface area via wax dipping varies. Conley & Hollander (2021) found wax dipping to overestimate the surface area of *S. pistillata* when compared to photogrammetry. In contrast, results from Naumann et al. (2009) showed that wax dipping underestimated the surface area of *L. pertusa, P. damicornis, G. fascicularis, Montipora sp.* and *Fungia spp.* when compared to CT scanning.

Wax or high-resolution optical scanning methods may account for each corallite within the coral skeleton as additional surface. The corallite once contained a living polyp and counting the corallite as additional surface area may overestimate the biologically active surface area of living coral. Surface area methods conducted while the coral polyp is still inhabiting the corallite could provide more accurate biologically active surface area estimates, but the wax method is not appropriate for use on living tissue.

The foil wrapping method generally overestimates the surface area of coral when compared to wax dipping, CT scanning, photogrammetric reconstruction, and wax-dye dipping (Hoegh-Guldberg 1988, Bythell et al. 2001, Veal et al. 2010_a). Despite this overestimation, the foil method allows for a surface area estimation without having to sacrifice the coral tissue, skeleton, or calibrated object. In a comparison to 3D imagery such as CT scans or laser scanning, previous research does highlight that both foil wrapping and wax dipping provide a similar degree of coral surface area accuracy (Holmes 2008, Raz-Bahat et al. 2009).

Methods using numerous 2D images taken around the coral and geometric based approaches also allow for coral surface area estimations without having to sacrifice the tissue or skeleton; however, these methods often result in surface area measurements that do not match

estimates provided by 3D imagery techniques (Naumann et al. 2009, Raz-Bahat et al. 2009, House et al. 2018). 2D methods achieved a 12-36% accuracy when estimating coral surface area compared to surface area estimates from CT scanning (Naumann et al. 2009). Geometric based approaches had an accuracy error of 31.74%, the highest accuracy error found between the foil, methylene blue, and wax methods (Raz-Bahat et al. 2009).

We ultimately adopted the foil wrapping method for our later experiments as our results indicated that the surface area estimates calculated from the foil and wax methods did not statistically differ from each other, and surface area values determined by LSA did statistically differ from both the foil and wax measurements. By foil wrapping coral skeletons, we could take surface area measurements without sacrificing the coral tissue or skeleton in addition to being a quick, cost effective, and accurate method. We stress that our results only apply to one coral morphology as we used small skeletons with little branching. Overall, our methods do outline surface area methodology that can possibly apply to other coral morphologies, especially small fragments commonly used in lab settings.

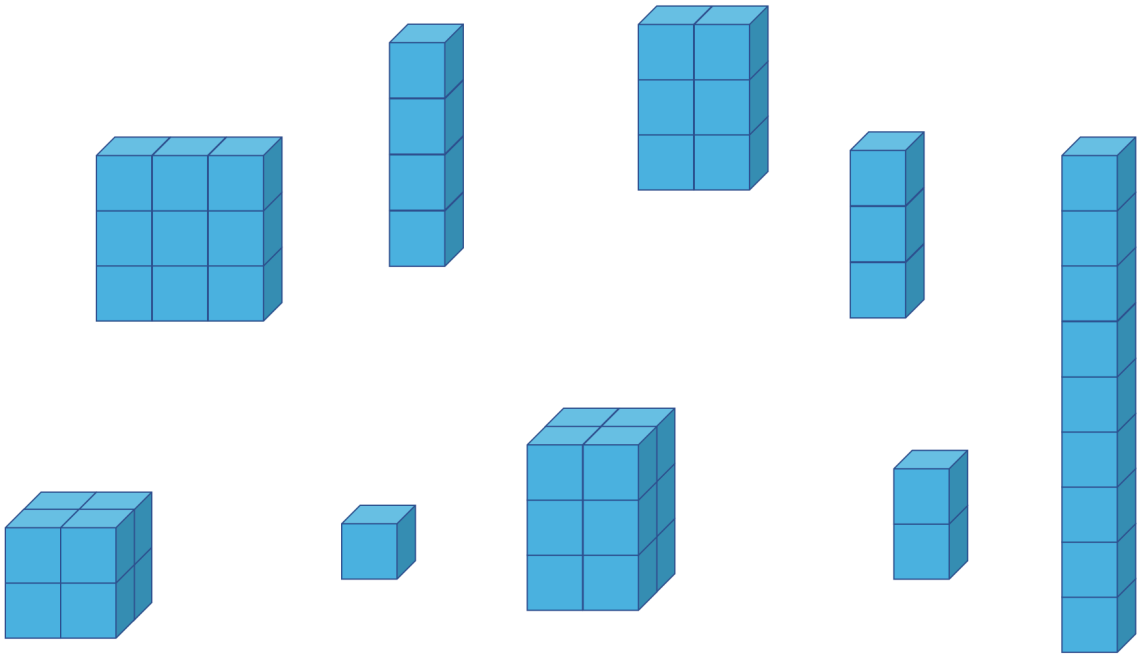

Figure 2.1 An illustration depicting the geometry of a selection (9 of 13) of cube shapes used as calibration objects.

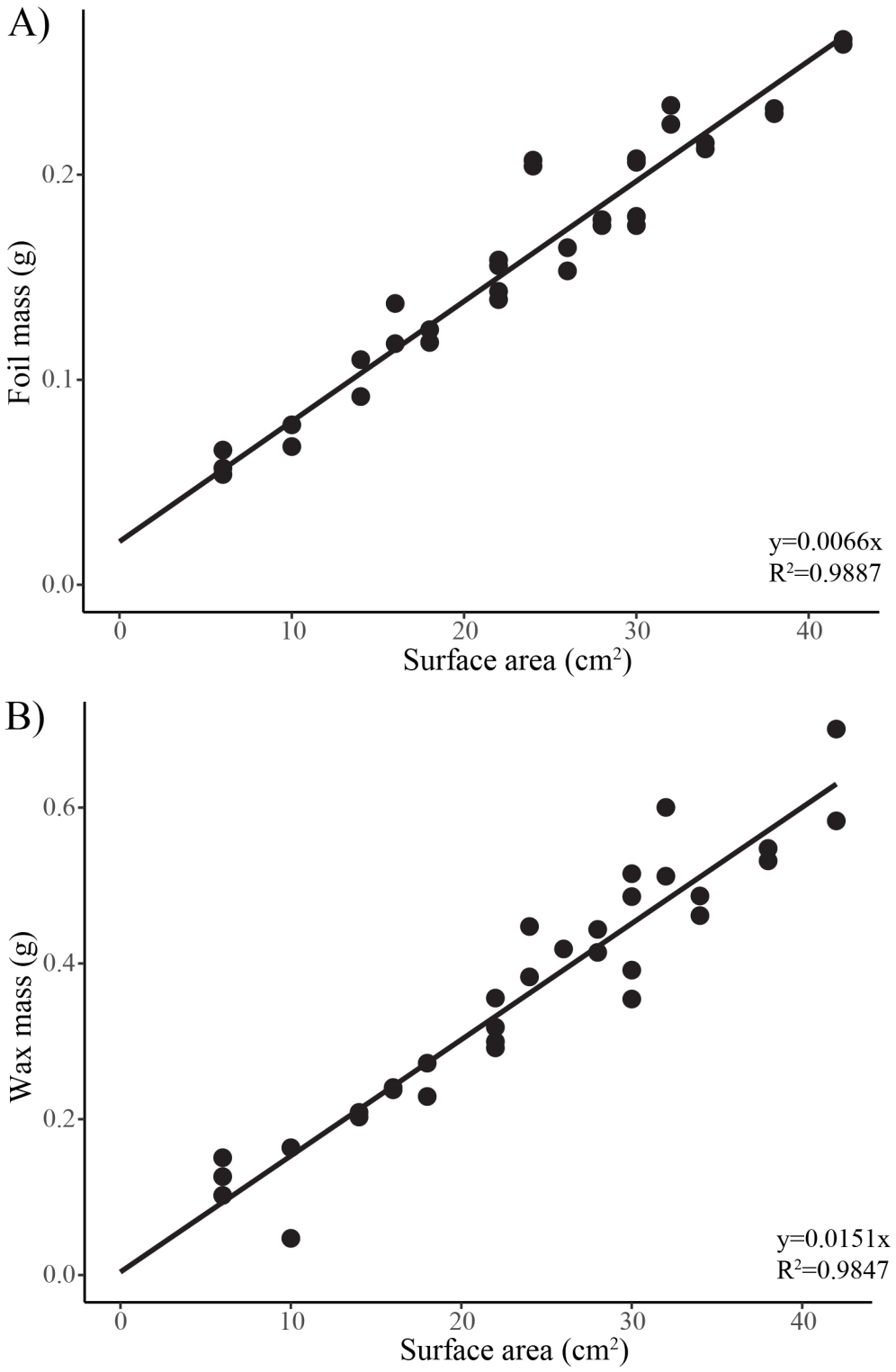

Figure 2.2 Graphs depict calibration curves for both the A) foil wrapped and B) wax dipped calibration objects with known surface areas. A slightly higher R^2 value is observed for the A) foil wrapped calibration curve compared to the B) wax dipped calibration curve.

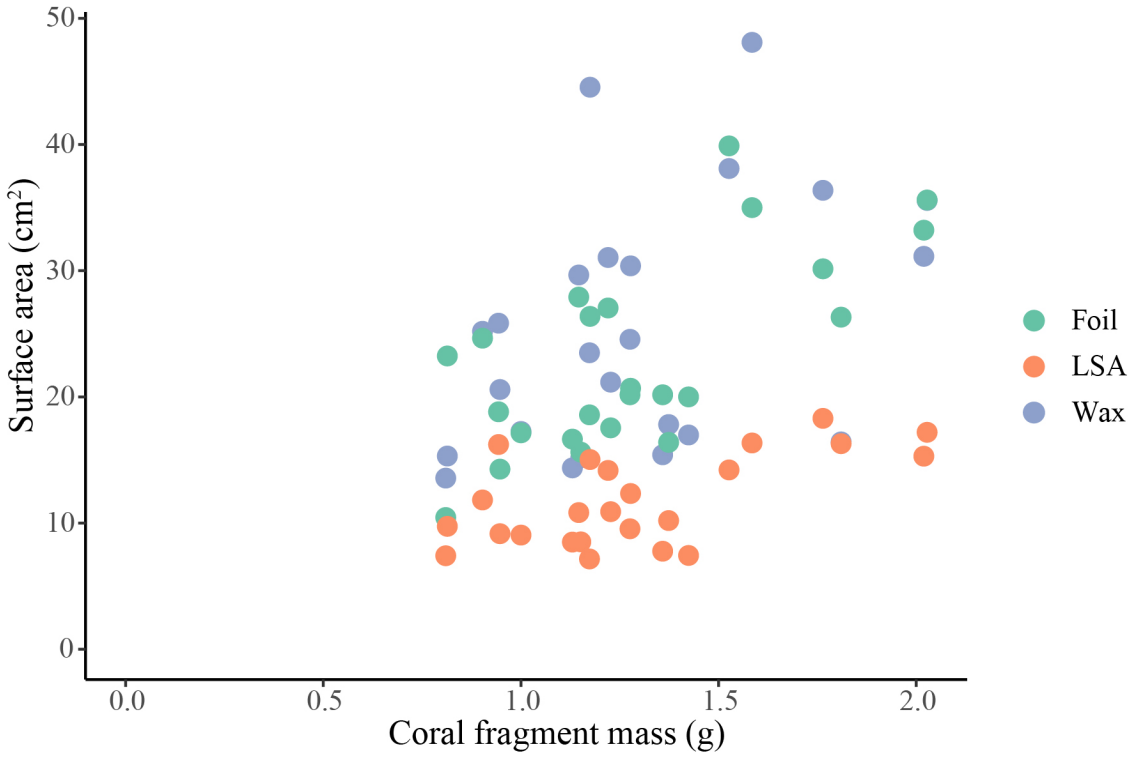

Figure 2.3 The relationship between aquacultured *Acropora* surface area and mass for three surface area methods conducted in this study. Data were analyzed by ANCOVA (Table 2.1).

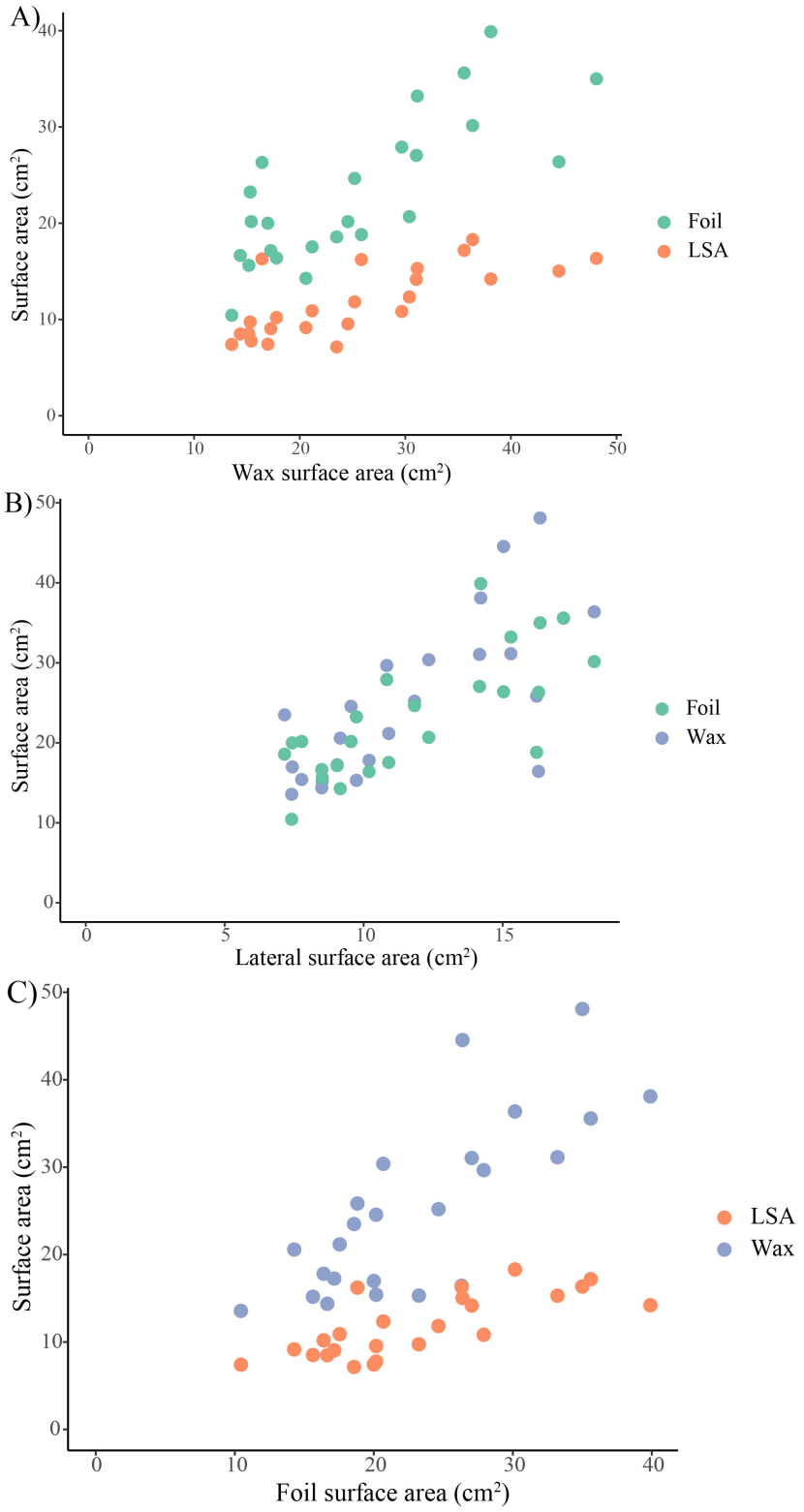

Figure 2.4 The relationship between coral surface area values estimated via differing methods. A) Wax method and foil or LSA method, B) LSA method and foil or wax method, C) Foil method and wax or LSA method.

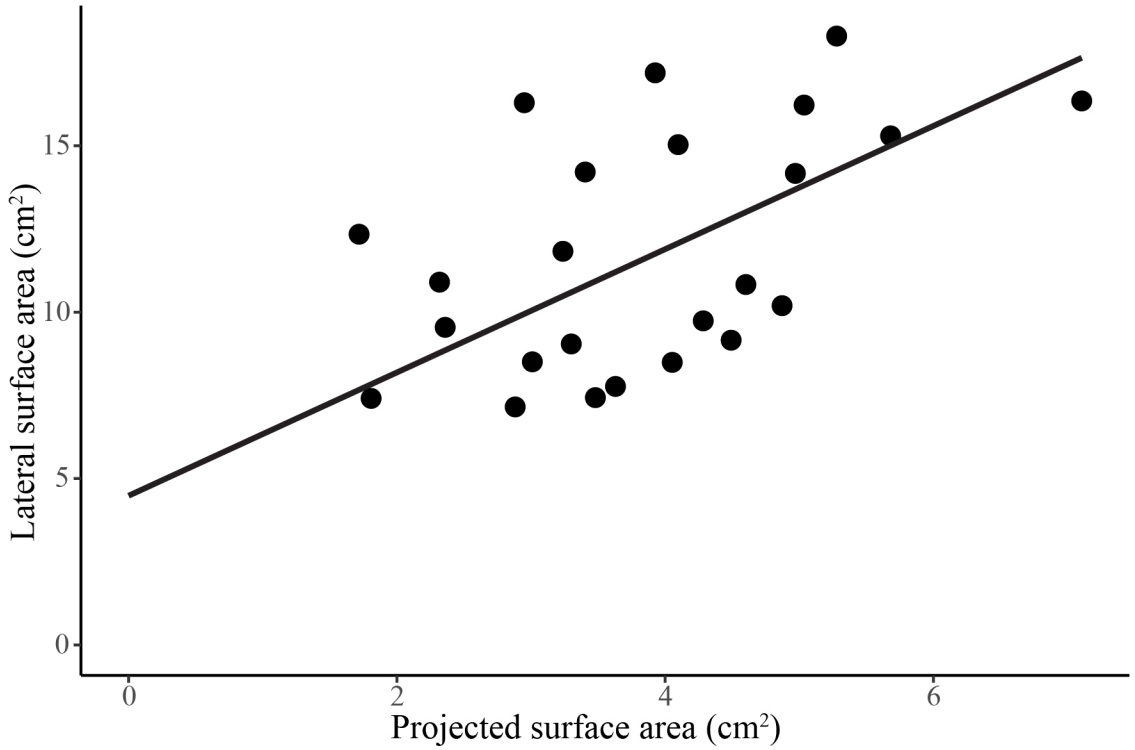

Figure 2.5 The relationship between projected surface area and LSA. Data were analyzed by a forward stepwise multiple linear regression (Table 2.4).

Table 2.1 Result table from the ANCOVA test comparing the surface area values estimated by various methods and coral skeleton masses.

Response: surface area					
	df	sum sq.	mean sq.	f-stat.	p-value
Methods	2	2531.81	1266.40	30.6966	3.759e-10
Mass	1	1036.16	1036.17	25.1157	4.298e-6
Methods: Mass	2	109.17	54.58	1.3231	0.2733
Residuals	66	2722.86	41.26		

Table 2.2 R^2 values and linear functions when comparing surface area values between methods. Each intercept was set to zero as surface area using any of the methods should be zero when there is no coral. High R^2 values are reported among all comparisons ($R^2>0.9$) in addition to statistically significant p-values ($p<0.001$).

Independent variable	Dependent variable	R^2	Linear function
Foil	Wax	0.9485	y=1.0869x
Foil	LSA	0.9563	y=0.4953x
Wax	Foil	0.9485	y=0.8727x
Wax	LSA	0.9408	y=0.4402x
LSA	Wax	0.9356	y=1.9129x
LSA	Foil	0.9520	y=1.7291x

Table 2.3 Results from the post-hoc analysis with a Bonferroni multiple testing correction. Methods compared were foil, LSA, and wax.

Term 1	Term 2	Variable 1	Variable 2	df	p-value	p adj.
Methods: Mass	Surface area	Foil	LSA	68	5.82E-8	1.75e-7
Methods: Mass	Surface area	Foil	Wax	68	2.47E-1	0.742
Methods: Mass	Surface area	LSA	Wax	68	4.79E-10	1.44e-9

Table 2.4 Significant results from a forward stepwise multiple linear regression analysis with b representing beta. No output was given if a significant association was not found. Surface area values determined from the LSA method were the only values significantly associated with the projected fragment surface area. R= 0.50116794, R^2= 0.25116930, Adjusted R^2= 0.21713154, F(1,22)=7.3791, and standard error of estimate= 1.1339.

	b*	Std. error of b*	b	Std. error of b	t-stat.	p-value
Intercept			1.72668	0.816627	2.114400	0.046046
LSA	0.501168	0.184493	0.180144	0.066315	2.716457	0.012604

Chapter 3 - *Acropora* metabolism: measuring primary production and respiration after NH_4^+ exposure

Abstract

Scleractinian coral metabolism measurements can estimate the overall health of a coral in addition to determining the overall energy consumed and produced by the coral and its associated microbes. We designed an incubation chamber to measure coral metabolic rates with minimal disturbance and continuous mixing. We exposed aquacultured and wild *Acropora* fragments to varying treatments of ammonium (NH_4^+) and determined their metabolic rates. In a laboratory experiment with aquacultured *Acropora*, we found a significant, positive relationship between NH_4^+ treatment levels and rates of primary production in aquacultured *Acropora* ($p=0.02$). However, we did not find a significant relationship between NH_4^+ treatment levels and respiration ($p=0.066$) or net production ($p=0.5668$). This finding provides support for the hypothesis that *Symbiodinium* photosynthesis is limited by nitrogen (N). We further conclude that respiration in aquacultured coral appears to be uninfluenced by N levels. In a field collection experiment with *Acropora pulchra*, respiration rates remain unchanged within the wild *Acropora* regardless of NH_4^+ treatment levels ($p=0.34$); however, we found a significant difference in respiration between collection origin for the wild coral ($p=0.012$). These results indicate that a factor outside of NH_4^+ exposure may influence respiration rates. We suggest that further research needs to be conducted on both aquacultured and wild coral to analyze factors contributing to respiration rates.

Introduction

Metabolism measurements can indicate the general health of an organism. In coral, primary production can indicate the energy produced through *Symbiodinium* photosynthesis whereas respiration reflects the energy consumed by the coral and its associated organisms (Muscatine et al. 1981). The connection between respiration and primary production is crucial to the overall wellbeing of the coral host with an estimated 51% of photosynthetically *Symbiodinium*-fixed energy is used in respiration associated with the whole coral (Davis 1983).

The balance between *Symbiodinium* photosynthesis and coral respiration is dependent upon low levels of nitrogen (N). While *Symbiodinium* can easily obtain carbon through carbon dioxide (CO_2) sequestration mechanisms, *Symbiodinium* heavily relies upon N from the coral host in nutrient poor water (Falkowski et al. 1984, Rahav et al. 1989, Grover et al. 2002). The coral host controls N retention or release to the *Symbiodinium* in quantities hypothesized to benefit the coral host (Falkowski et al. 1984). If the surrounding water column contains high levels of N, *Symbiodinium* rapidly assimilates N and increases their cell count in the coral tissue (Stimson & Kinzie 1991). The increased N allows *Symbiodinium* cells to increase photosynthetic rates (Snidvongs and Kinzie 1994, Fagoonee et al. 1999, Ferrier-Pagès et al. 2000, Sakami 2000, Ferrier-Pagès et al. 2010, Beraud et al. 2013). While primary production increases, respiration has been found to remain unchanged under high levels of N (Stambler et al. 1994, Reynaud et al. 2002, Beraud et al. 2013).

In our study we subject *Acropora* fragments to varying levels of ammonium (NH_4^+) over periods of five to seven days and measure their metabolic rates. We hypothesized, based on prior literature, that: 1.) *Acropora* primary production will increase as NH_4^+ exposure increases. 2.) *Acropora* respiration will remain unchanged regardless of the NH_4^+ treatment.

We additionally found in previous literature that there have been many methods and instruments used to gather metabolism measurements in coral (Carpenter et al. 1991, Yates & Halley 2003, Camp et al. 2015, Owen et al. 2020). Few of these instruments are easily transportable, low cost, and small enough to measure metabolism in coral fragments under artificial light and dark conditions. They also can disturb coral and do not allow easy control of water movement in the chambers. In addition to presenting metabolism rates of *Acropora* fragments under varying NH_4^+ treatment levels, we present a brief description of the metabolism chamber design and methods used in our study.

Methods

Metabolic chamber

We constructed our chamber material from clear, acrylic plastic (Interstate Plastics, Sacramento, CA). The metabolism chamber is a sealable, water-tight 8x10 cm^2 chamber with an internal volume of 690 cm^3 (Figure 3.1, Table 3.1). This acrylic chamber allowed for the promotion of water movement using a stir bar placed under a coral fragment holding platform. Dissolved oxygen (DO) and temperature measurements were taken through a port at the top using a proODO optical probe (YSI, Yellow Springs Instruments). Each coral fragment was placed inside the chamber within water in its respective tank to minimize stress associated with rapidly changing water conditions. The tank water surrounding the chamber ensured the coral fragment was being kept at a constant temperature to help minimize thermal stress.

Laboratory experiment with aquacultured Acropora

We placed 24 aquacultured *Acropora* (ORA® Aquacultured Scripp's Green Tip *Acropora*) in six, 57 L tanks (four fragments per tank). A heater set to 25 ºC, an air pump, and an

internal power filter with the biological, mechanical, and chemical filters removed were affixed to each tank. Two, 20-watt NICREW saltwater aquarium lights were placed above each tank on a 12-hour light and 12-hour dark cycle. After the acclimation period each tank was assigned one of six NH_4^+ treatments: 0, 2, 4, 8, 20, and 40 µmol NH_4^+ $tank^{-1}$ day^{-1}. Coral were exposed to their respective NH_4^+ treatment over the course of five days. 24 hours after the final NH_4^+ addition, we randomly placed one fragment from each tank in the metabolic chamber. We exposed each fragment to 30 minutes of darkness and 30 minutes of light from the two 20-watt NICREW saltwater aquarium lights (73 µmol quanta m^{-2} s^{-1}). During the periods of light and dark, we recorded temperature and DO every three minutes. Surface area of each fragment was determined by the foil wrapping method (Marsh 1974, Chapter 2).

Field collection experiment with Acropora pulchra

Acropora pulchra fragments were collected via snorkeling from two colonies at each of two sites. The sites were West Hagåtña Bay, a site with slightly higher NH_4^+ concentrations and potentially greater anthropogenic impacts (13.479650, 144.741750; 13.479833, 144.741733) and Luminao, a site with slightly lower NH_4^+ concentrations and lower anthropogenic impact (13.4652417, 144.6477483; 13.465467, 144.648050) off the coast of Guam. Six fragments were placed in each of the 12, 10 L tanks and were separated into each tank by colony. The fragments were allowed to acclimate for one week under continuously replenished seawater. After the acclimation period each tank was assigned one of three NH_4^+ treatments: 0, 9.8, and 98 µmol NH_4^+ $tank^{-1}$ day^{-1}. Flowthrough of the water was stopped, and coral were exposed to their respective NH_4^+ treatment over the course of seven days. One day after the final NH_4^+ addition, we randomly placed one fragment from each tank in the metabolic chamber. We exposed each fragment to 30 minutes of total darkness and recorded temperature and DO every three minutes.

Surface area of each fragment was determined by the foil wrapping method (Marsh 1974, Chapter 2).

Statistics

We used R version 4.0.2 (R Core Team, 2020) for all statistical analyses. To determine if there was a significant relationship between the level of NH_4^+ treatment and the three metabolism measurements: primary production, respiration, and net production, we ran regression analyses. We ran paired t-tests to determine if there was a significant difference in respiration values between *A. pulchra* fragments collected from two colonies at each site. ANCOVA was used to determine if there was a significant difference in respiration between collection sites, between NH_4^+ treatments, or if there was an interaction between site and NH_4^+ treatment.

Results

Laboratory experiment with aquacultured Acropora

We found a significant, positive relationship between NH_4^+ treatment levels and primary production rates in aquacultured *Acropora* (Figure 3.2.A). There was not a significant relationship between NH_4^+ treatments and aquacultured *Acropora* net production (Figure 3.2.B) nor between NH_4^+ treatments and aquacultured *Acropora* respiration (Figure 3.3) .

Field collection experiment with Acropora pulchra

We did not find a significant difference in respiration values between colonies at either collection site (W. Hagåtña Bay: 0.06±0.06; 0.09±0.10 µg DO^{-1} $minute^{-1}$ cm^{-2}) (Luminao: 0.16±0.06; 0.11±0.05 µg DO^{-1} $minute^{-1}$ cm^{-2}) (± 1 SD) (Figure 3.4). We then combined the respiration rates of coral fragments collected from different colonies at the same site that were

exposed to the same NH_4^+ treatment. We did not find a significant difference in *A. pulchra* respiration between NH_4^+ treatments. However, we did find a significant difference in respiration between collection sites. Fragments from Luminao (0.08±0.08 µg DO^{-1} $minute^{-1}$ cm^{-2}) generally had greater respiration than fragments from W. Hagåtña (0.14±0.06 µg DO^{-1} $minute^{-1}$ cm^{-2}) (± 1 SD). There was not a significant interaction of *A. pulchra* respiration between NH_4^+ treatment and coral origin (Figure 3.5).

Discussion

Our laboratory experiment results indicate that aquacultured *Acropora* primary production is positively correlated to the loading of NH_4^+ the fragment is exposed to supporting our first hypothesis. This finding supports previous research which suggests that *Symbiodinium* photosynthesis is limited by N, and with the introduction of external N primary production increases (Snidvongs & Kinzie 1994, Fagoonee et al. 1999, Ferrier-Pagès et al. 2000, Sakami 2000, Ferrier-Pagès et al. 2010, Beraud et al. 2013).

The average primary production rate in our aquacultured *Acropora* under no NH_4^+ loading was 0.17±0.05 µg DO^{-1} $minute^{-1}$ cm^{-2} whereas the average primary production rate under the highest NH_4^+ loading treatment, 40 µmol NH_4^+ $tank^{-1}$ day^{-1}, was 0.26±0.26 µg DO^{-1} $minute^{-1}$ cm^{-2} (± 1 SD). Stambler (1998) presents a similar average primary production rate of 0.17 µg DO^{-1} $minute^{-1}$ cm^{-2} under no NH_4^+ loading for *Stylophora pistillata*. Under an exposure rate of 8 mM $(NH_4)_2$ SO_4 $minute^{-1}$ for 35 days followed by a final exposure of 20 µmol NH_4^+ $tank^{-1}$, Stambler (1998) presents an average *S. pistillata* primary production rate of 0.74 µg DO^{-1} $minute^{-1}$ cm^{-2}. Stambler (1998) found primary production rates to be higher in *S. pistillata* exposed to NH_4^+ than the *S. pistillata* under no loading conditions similar to our findings;

however, the average primary production of *S. pistillata* exposed to NH_4^+ loading was higher than the any rates found in our study. Comparing primary production rates is often difficult as NH_4^+ loading periods and concentrations are quite variable; however, these rates despite being different often display the same trend of NH_4^+ loading increasing primary production in scleractinian coral.

Our second hypothesis was supported as respiration remained unchanged regardless of NH_4^+ treatment in both experiments. Other studies suggest similar hypothesis which correspond to our results that coral respiration rates remain uninfluenced by inorganic N (Stambler et al. 1994, Reynaud et al. 2002, Beraud et al. 2013). An increase in primary production and a lack of change in respiration are thought to be the result of *Symbiodinium* uncontrollably multiplying under increased levels of NH_4^+ in the water column leading to an overall increase in primary production and the retention of photosynthates (Suescún-Bolívar et al. 2016). The lack of photosynthates transferred to the coral host decreases host respiration and *Symbiodinium* respiration increases which leaves overall respiration unchanged (Stambler et al. 1994, Reynaud et al. 2002, Beraud et al. 2013). Other research contradicts our findings as respiration rate increased in nutrient enriched coral (Marubini and Davies 1996, Ferrier-Pagès et al. 2000).

We found that *A. pulchra* respiration only significantly differed between the collection origin despite being subjected to three differing levels of NH_4^+ indicating that another factor other than NH_4^+ is causing respiration values to differ. Ulstrup et al. (2011), found that respiration values were significantly different within two species located in varying regions on the Great Barrier Reef. Researchers highlight that small differences in environmental conditions alongside the species of *Symbiodinium* in coral tissue are thought to be the cause of differences in respiration values in coral collected from the wild (Ulstrup et al. 2011). In-situ salinity, depth,

and temperature are additional environmental factors that can influence coral respiration rates (McCloskey & Muscatine 1984, Muthiga & Szmant 1987, Edmunds 2005).

Specifically, Muthiga & Szmant (1987) reported average respiration rates of *Siderasterea siderea* under ambient conditions ranging from 0.15 to 0.33 μg DO^{-1} $minute^{-1}$ cm^{-2}. In comparison, our average respiration rates for aquacultured *Acropora* and *A. pulchra* (collected from Luminao, Guam) regardless of NH_4^+ treatment were slightly smaller in comparison, 0.13±0.02 and 0.14±0.06 μg DO^{-1} $minute^{-1}$ cm^{-2}, respectively (± 1 SD). Muthiga & Szmant (1987) then subjected *S. siderea* to differing salinity changes and reported an average respiration rate of 0.06 μg DO^{-1} $minute^{-1}$ cm^{-2} in coral exposed to the most drastic decrease in salinity (42% to 22%). The average respiration rate from *A. pulchra* collected from West Hagåtña Bay, Guam regardless of NH_4^+ treatment was 0.08±0.08 μg DO^{-1} $minute^{-1}$ cm^{-2} only slightly higher than that found by Muthiga & Szmant (1987) in coral exposed to drastic decreases in salinity (± 1 SD). Although we found very similar average respiration measurements to those presented by Muthiga & Szmant (1987), we are unable to conclude if salinity impacted our rates and compare our studies further. Due to this limitation, we highly recommend that future research collect additional water quality data such as salinity when measuring respiration especially if collecting coral surrounding the island of Guam.

We conclude that primary production in *Symbiodinium* appears to be limited by NH_4^+ in aquacultured coral while respiration appears to be uninfluenced in both wild and aquacultured coral. Respiration rates in wild *A. pulchra* only varied between coral origin with collection site nutrient data only showing a slightly higher level of NH_4^+ present at W. Hagåtña Bay (Table 3.2). This finding provides further support that factors other than nutrients impact respiration. We note that our NH_4^+ exposures were only for five to seven days, and longer exposures may lead to

different results. We stress that further research needs to be completed on both wild and aquacultured *Acropora* to determine the impacts of nutrient loading and other factors on metabolism.

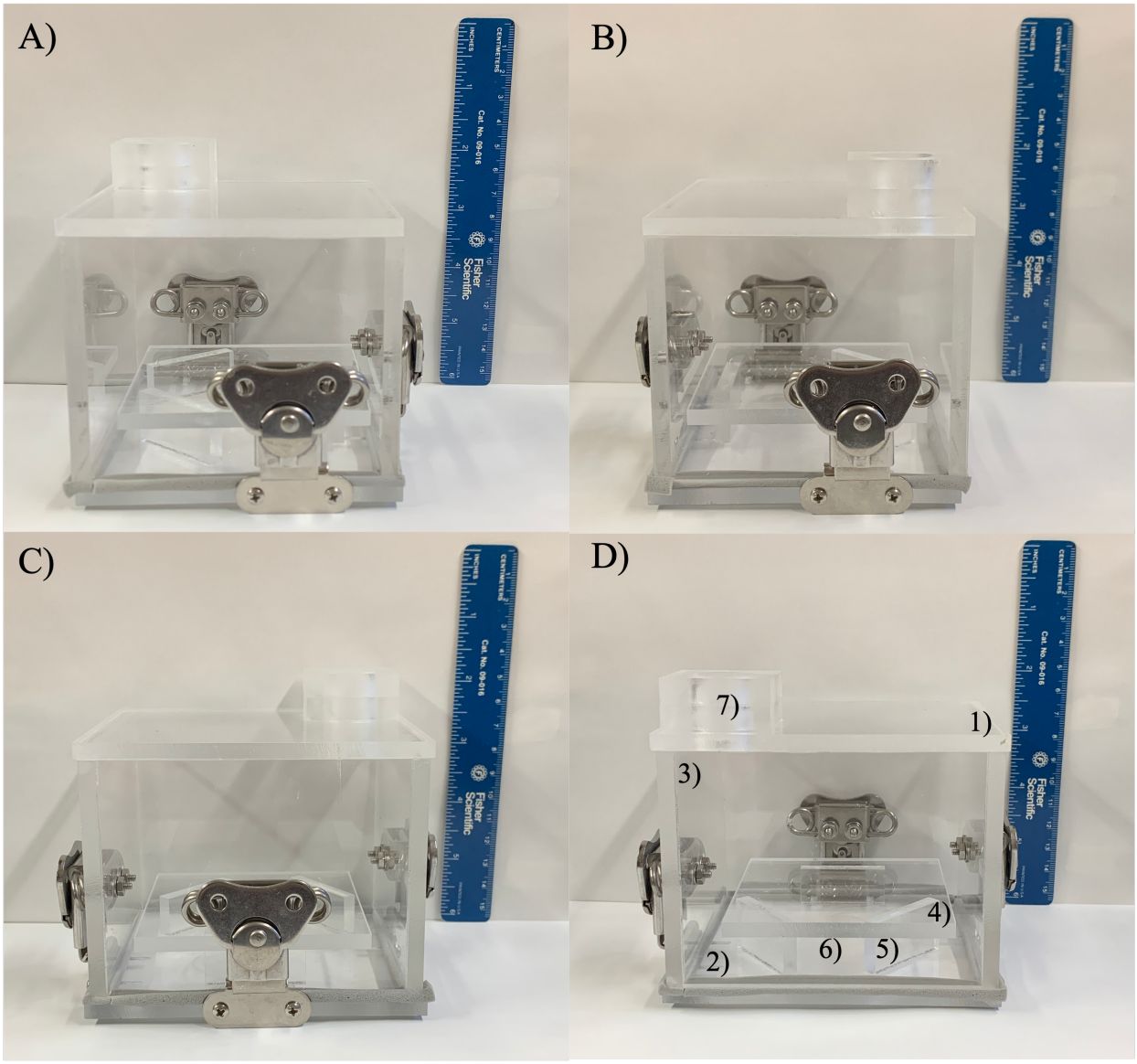

Figure 3.1 Metabolism chamber images. A) right side view, B) left site view, C) back view, D) front view and major components of the metabolism chamber. 1) Top piece, 2) Bottom piece, 3) Side pieces, 4) Coral table, 5) Table legs, 6) Space between table and bottom piece for stir bar placement, 7) Probe port. For exact measurements of these components see Table 3.1.

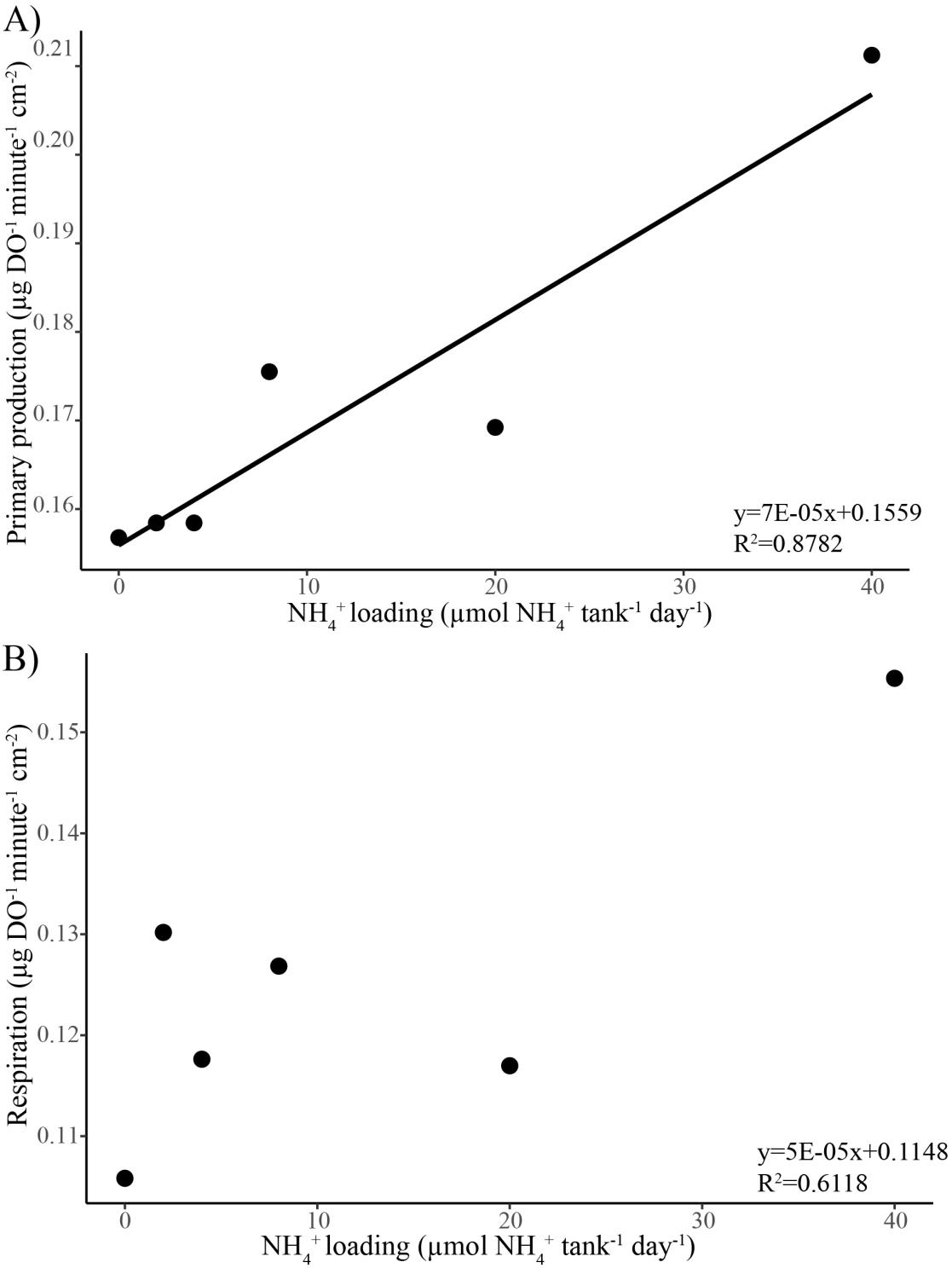

Figure 3.2 Average metabolic rates of aquacultured *Acropora* across six NH_4^+ treatments. A) A significant, positive relationship was found between coral exposed to varying NH_4^+ treatments and primary production (p=0.02). B) A marginally significant relationship was found between coral exposed to varying NH_4^+ treatments and respiration; however, the relationship is leveraged by the highest NH_4^+ treatment having the highest respiration rate (p=0.066).

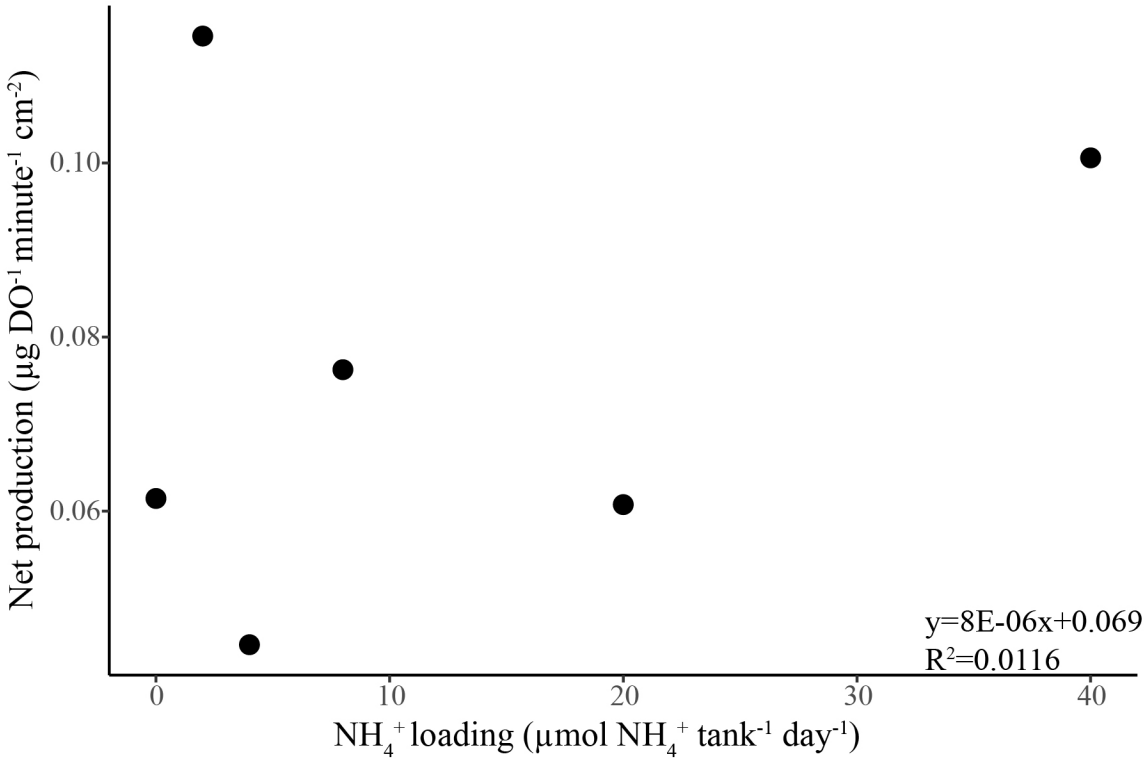

Figure 3.3 Average net production was found by subtracting respiration from primary production rates. There was not a significant relationship between coral exposed to varying NH_4^+ treatments and net production (p=0.5668).

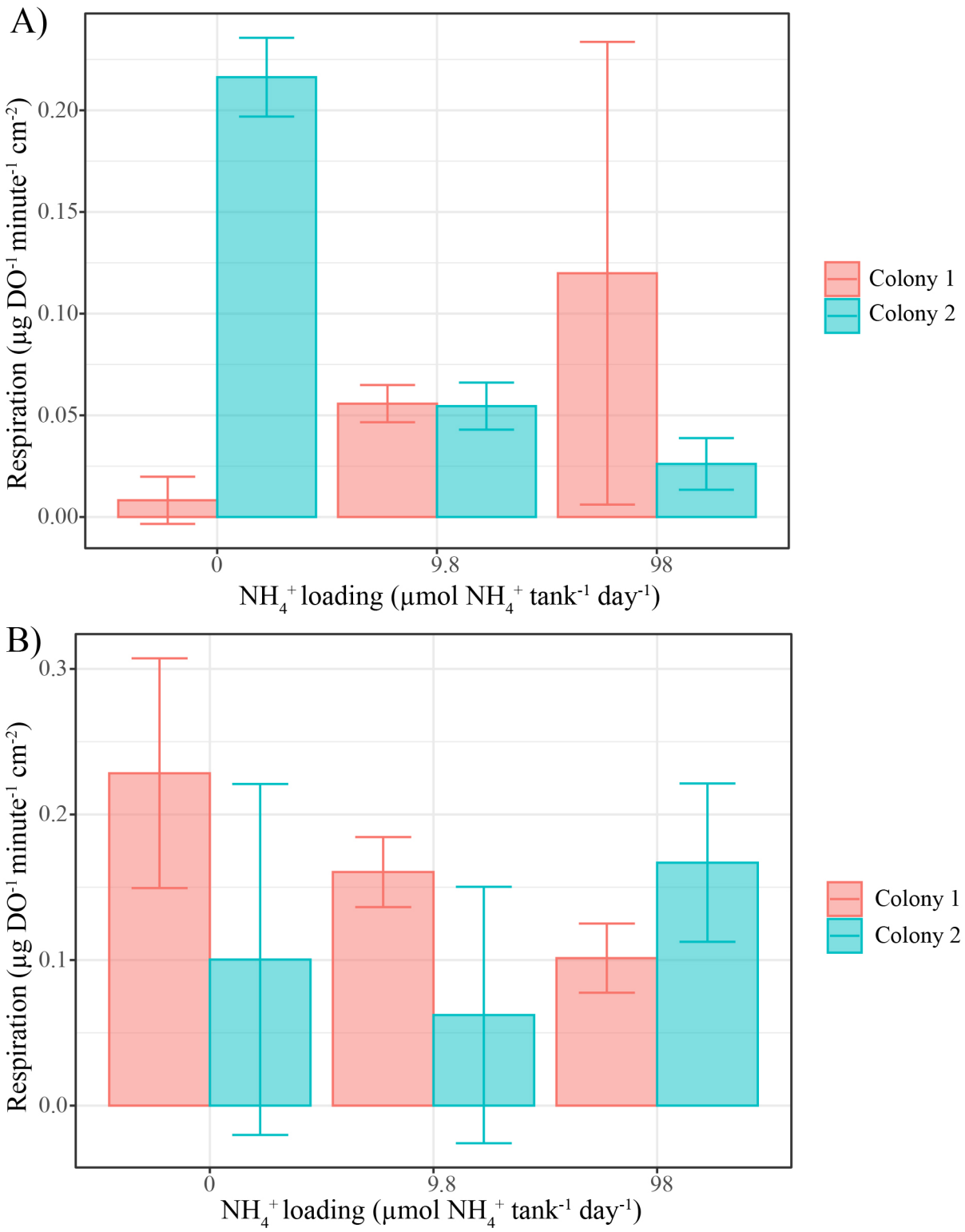

Figure 3.4 The average respiration rates of *A. pulchra* between colonies. There was not a statistical difference in respiration rates between *A. pulchra* colonies from A) W. Hagåtña (p=0.7139) or in colonies from B) Luminao (p=0.4677). Error bars represent standard deviation.

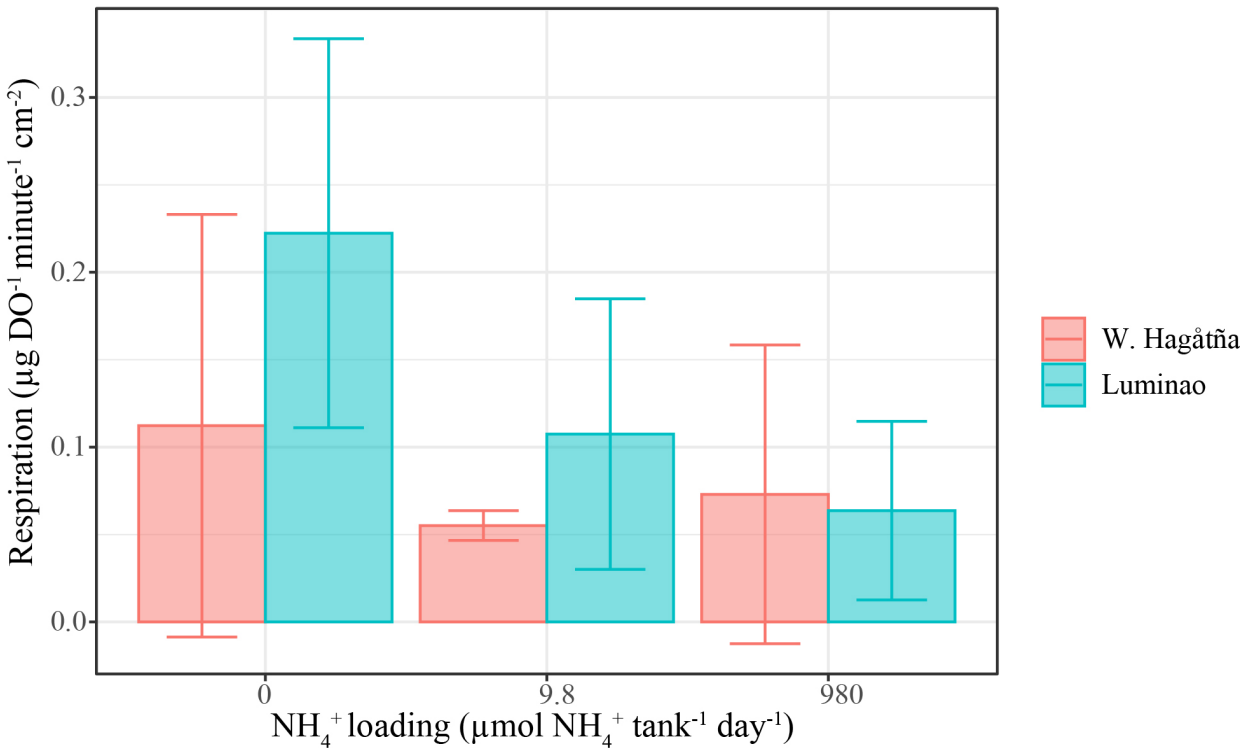

Figure 3.5 The average respiration values of *A. pulchra* between sites. Respiration rates were statistically significant between collection sites with *A. pulchra* collected from Luminao generally having higher respiration rates than *A. pulchra* collected from W. Hagåtña (p=0.01237). Respiration rates did not differ between NH_4^+ treatments (p=0.3404) nor was there a significant interaction between NH_4^+ treatments and collection sites (p=0.08354). Error bars represent standard deviation.

Table 3.1 Measurements of acrylic materials needed to construct one metabolism chamber. An image of each ID's corresponding part can be found in Figure 3.1.

ID	# of pieces needed	Description	Thickness (cm^2)	Length (cm^2)
1)	1	Top piece	0.54	10
2)	1	Bottom piece	0.54	10
3)	4	Side pieces	0.54	10
4)	1	Coral table	0.54	8
5)	3	Table legs	0.54	3.5

Table 3.2 Nutrient data for the water column surrounding each colony where *A. pulchra* was sampled. Total phosphorus (P) sampled at all colonies was found to be below the detectable limit (BDL) of 0.1 µmol L^{-1}.

Collection site	Colony #	Total N (µmol L^{-1})	Total P (µmol L^{-1})	NH_4^+ concentration (µmol L^{-1})
W. Hagåtña	1	16.7	BDL	0.48
W. Hagåtña	2	17	BDL	0.49
Luminao	1	12.1	BDL	0.46
Luminao	2	14.6	BDL	0.45

Chapter 4 - *Acropora* and ammonium: quantifying the nitrogen cycle surrounding *Acropora* under NH_4^+ loading

Abstract

Scleractinian coral thrive under nitrogen (N) limited conditions; thus, increased N fluxes from anthropogenic activities to these otherwise nutrient-depleted environments may threaten coral health. Here we quantify rates of remineralization, uptake, and nitrification for wild and aquacultured *Acropora* subjected to elevated ammonium (NH_4^+) loading. We found NH_4^+ loading did not appear to impact rates of remineralization and nitrification in tanks containing wild and aquacultured *Acropora*. Direct NH_4^+ uptake into wild and aquacultured *Acropora* was also not influenced by NH_4^+ levels. We further found that uptake, remineralization, and nitrification rates were generally similar across each tank. There were variations between rates calculated using two differing surface area methods and stress that the method to measure surface area is an important component of N cycling work. We present N cycling rates in this study to serve as an important resource for furthering the understanding of how scleractinian coral respond to increased N.

Introduction

Scleractinian coral are keystone organisms in which coral reef biodiversity, richness, and ecosystem services rely upon. These ecosystem engineers are facing anthropogenic threats that could place 75% of all reefs under an 'extreme' threat level by 2050 (IPCC 2014). Eutrophication, ocean warming, and ocean acidification have caused reefs to deteriorate by increasing disease susceptibility and bleaching rates in scleractinian coral (Hoegh-Guldberg

1999, Hughes et al. 2003, Goldberg et al. 2004). Eutrophication, in particular, is often an overlooked anthropogenic stressor in reef systems; however, an estimated 24% of global anthropogenic nitrogen (N) released in coastal watersheds reaches coastal ecosystems (Malone & Newton 2020). Increased N fluxes to an otherwise historically N limited ecosystem can cause biogeochemical nutrient cycling imbalances and even hypoxic conditions (Moffat 1998, Rabalais 2002).

All scleractinian coral and their *Symbiodinium* depend on carbon (C), N, and phosphorus (P) for survival, but the ratios at which C:N:P are required varies depending upon the surrounding environment, coral species, and *Symbiodinium* species (Blanckaert et al. 2020). C, N, and P cycling between the coral host and its associated organisms allow for coral to live in nutrient depleted environments. Scleractinian coral delivers dissolved inorganic carbon (DIC) from the water column to the *Symbiodinium* (Tansik et al. 2015). The *Symbiodinium* can further maximize C acquisition by concentrating carbon dioxide (CO_2) produced through coral respiration (Bradding et al. 2013). The overall availability of C for the *Symbiodinium* is high whereas N appears to be the limiting nutrient. Supplementary ammonium (NH_4^+) added to the surrounding water column can lead to increased *Symbiodinium* photosynthetic rates (Ferrier-Pagès et al. 2000).

Heterotrophic feeding by the coral host accounts for 15-35% of daily C demand in a healthy coral and up to 100% in a bleached coral (Houlbrèque & Ferrier-Pagès 2009). In high light environments heterotrophic feeding is unnecessary to supplement coral host C as up to 100% of a coral's daily C demand can be met through *Symbiodinium* photosynthesis (Falkowski et al. 1984). Heterotrophic feeding is often a common method to also supplement the coral host's N demand (Houlbrèque & Ferrier-Pagès 2009). Picoplankton in particular can account for 92%

of N removal from the water column suggesting that picoplankton is a major organic N source for coral (Ribes et al. 2003). Heterotrophic feeding can provide up to 3.7 μg N cm^{-2} day^{-1} or 70% of the whole coral N demand dependent upon environmental conditions (Bythell 1988, Houlbrèque & Ferrier-Pagès 2009). Dissolved N sources in the surrounding water including NO_3^-, NH_4^+, and free amino acids are also taken up into the coral during the act of heterotrophic feeding and are believed to contribute significantly to the whole coral N demand (Yamamuro et al. 1995, Hoegh-Guldberg 1999, Pupier et al. 2021). In addition to supplementing N through the act of heterotrophic feeding, coral have specialized microbes and symbiotic relationships that can contribute to the production and transformation of N (Risk & Muller 1983, Wafar et al. 1990, Lesser et al. 2004, Siboni et al. 2008). *Symbiodinium* can supplement nutrient demands of the coral host by translocating photosynthates which are composed of both C and N; however, photosynthates are comprised of a high C:N ratio contributing little to the N demand (Falkowski et al. 1984). Archaea and bacteria, including diazotrophs, are found all throughout the coral and are able to supplement additional N through N-fixation. The N supplied from the diazotrophs is estimated to contribute 6% of the whole coral's N demand (Moynihan et al. 2022).

N cycling within a coral reef ecosystem has always been balanced by and dependent upon low external N levels. *Symbiodinium*, in particular, rely upon recycled N with an estimated 90-98% of its N demand being met through cycling remineralized N with microbes in the surrounding water column, the coral host, and microbes associated with the coral host (Rahav et al. 1989, Atkinson et al. 1994). Internally, the coral host controls NO_3^- and NH_4^+ retention and release to the *Symbiodinium* in quantities hypothesized to benefit the coral host (Falkowski et al. 1984). Low available dissolved inorganic N (DIN) levels allow the coral to regulate the rates of *Symbiodinium* cell division and promote the translocation of photosynthates to the coral host

(Stimson & Kinzie 1991). Coral reef N loading can occur from terrestrial runoff, upwellings, migration of fish, atmospheric deposition, and N fixing microbes (D'Elia 1988, Barile & Lapointe 2005, Rädecker et al. 2015, Lesser 2021). With elevated DIN loading, high levels of NH_4^+ in the surrounding water column can result in uncontrollable rates of *Symbiodinium* cell division and the retention of photosynthates potentially increasing bleaching susceptibility (Wooldridge 2013, Suescún-Bolívar et al. 2016). Similarly, excess N can shift *Symbiodinium* from a N limited state to a P limited state causing a lipid compositional change in *Symbiodinium* cells which leads to increased bleaching sensitivity (Frentzen 2004, Tchernov et al. 2004). Furthermore, N increases can increase the severity and frequency of coral associated diseases (Baker et al. 2007). Bruno et al. (2003) found that increasing levels of inorganic N and phosphate (PO_4^{3-}) was correlated with the severity of aspergillosis and yellow blotch disease. Additionally, an increase in nitrate (NO_3^-) correlated positively with the frequency of black-band disease (Kuta & Richardson 2002).

Here, we focus on coral species within the genus *Acropora* as they are one of the most widespread and most vulnerable scleractinian coral to anthropogenic stressors (Ortiz et al. 2021). Previous research has highlighted *Acropora* responses to sedimentation, warming, and acidification, but the quantification of N cycling rates surrounding *Acropora* after exposure to NH_4^+ is less studied (Babcock et al. 1991, Loya et al. 2001, Albright et al. 2010). Here, we present the rates of nitrification, remineralization, and uptake for two different species of aquacultured and wild *Acropora* after exposure to various rates of NH_4^+ loading as few prior studies quantify multiple processes surrounding the scleractinian coral N cycle (Babbin et al. 2021, Glaze et al. 2022). We then estimated N cycling rates using changes in nutrient concentrations in addition to results derived from two ^{15}N tracer experiments. We hypothesized

that 1) aquacultured *Acropora* nitrification and uptake rates would increase and remineralization rates would decrease with increasing NH_4^+ loading, 2) wild *Acropora pulchra* nitrification and uptake rates would increase and remineralization rates would decrease with increasing NH_4^+ loading, and 3) N cycling rates would differ between *A. pulchra* collected from two sites possibly exposed to differing levels of anthropogenic activity surrounding the island of Guam.

Methods

Laboratory experiment with aquacultured Acropora

We filled six, 57 L tanks with 57 L of deionized water and 1,905 g of Instant Ocean Reef Crystals Reef Salt 24 hours prior to coral being introduced. A heater set to 25 ºC, an air pump, and an internal power filter with the biological, mechanical, and chemical filters removed were affixed to each tank. Two, 20-watt NICREW saltwater aquarium lights were placed above each tank. Combined, the lights emitted 73 µmol quanta m^{-2} s^{-1} within each tank and were placed on a 12-hour light and 12-dark cycle.

Our experiment used 24 *Acropora* (ORA® Aquacultured Scripp's Green Tip *Acropora*) fragments purchased from LiveAquaria (Rhinelander, WI). We decanted 75% of water in each received bag containing one fragment and slowly dripped tank water in to replace the water and allow acclimation. After the bags were filled, each coral was taken out, affixed on a 5.08 x 5.08 cm aragonite coral fragment tile using coral putty, and immediately placed in the respective tank. Coral were allowed to acclimate for seven days prior to NH_4^+ additions. During this acclimation period we photographed each fragment and monitored the tissue color. Over the acclimation period we did not see a change in the tissue color across all fragments.

Tanks containing the coral were randomly assigned an NH_4^+ treatment and were exposed to their respective NH_4^+ treatment for a total of five days following the seven-day acclimation period. To counter evaporation, we added double deionized water to tanks when the water level fell below 57 L and monitored the dissolved oxygen and temperature on a daily basis. We established a gradient of NH_4^+ exposure treatments resulting in a total of six NH_4^+ loading levels: 0, 2, 4, 8, 20, and 40 μmol NH_4^+ $tank^{-1}$ day^{-1} with exposure rates estimated from values from the National Atmospheric Deposition Program website for atmospheric deposition and fish excretion literature. We obtained fish NH_4^+ excretion values from Francis & Cotê (2018) (their Figure 3), which used both measurements and models to determine excretion rates from 16 migratory and residential Bahamian reef fish communities. We used WebPlotDigitizer to extract values from Francis & Cotê's Figure 3 and then averaged NH_4^+ excretion rates for May-August 2014 across 24-hour periods from both residential and migratory fish species. This resulted in an NH_4^+ exposure rate of 0.098 μmol NH_4^+ cm^{-2} day^{-1}. We used atmospheric deposition data from Puerto Rico, Hawai'i Volcanoes National Park, Mauna Loa, Samoa, and Virgin Islands from the National Atmospheric Deposition Program (NADP) website to estimate NH_4^+ deposition with data collected from 1980 through 2019 (data files downloaded May 17, 2021 from https://nadp.slh.wisc.edu/networks/national-trends-network). Average deposition rates across these sites were 0.003 μmol NH_4^+ cm^{-2} day^{-1}. Between fish excretion and atmospheric deposition alone, the rate of NH_4^+ exposure on a coral reef was estimated to be 0.1 μmol NH_4^+ cm^{-2} day^{-1}. Each fragment was estimated to have a surface area of 10.19 cm^2 leading to an estimated coral surface area per tank of 40.77 cm^2 which we then multiplied by our NH_4^+ exposure rate of 0.1 μmol $NH_4^+ cm^{-2}$ day^{-1} to predict a baseline NH_4^+ exposure rate of 4 μmol NH_4^+ $tank^{-1}$ day^{-1}. One coral bleached over the course of the NH_4^+ additions and was excluded from the study. The

remaining coral did not show any signs of bleaching and were included in the study. One coral from each tank was placed in an acrylic metabolism chamber to collect respiration and primary production 24 hours after the final NH_4^+ addition (Chapter 3). After coral fragments were removed from the metabolism chamber, we removed the coral tissue from each fragment with an airbrush containing double deionized water. The tissue was collected in a plastic bag, divided amongst test tubes, and frozen immediately. We used the remaining skeletons to conduct surface area measurements (Marsh 1970, Chapter 2).

After the metabolism measurement and removal of the first coral fragment in each tank, we enriched the seawater in each tank with ^{15}N by adding 7 mL of 0.0019 µmol ^{15}N-NH_4Cl stock solution (^{15}N-N 98% $^{15}NH_4Cl$, Sigma-Aldrich, St. Louis, MO) to each tank. Tanks were allowed to mix for 15 minutes, and 2 L of water were taken out of each tank, filtered with a 47-mm Whatman GF/F glass fiber filters (0.7 µm nominal retention, Whatman International Ltd., Maidstone, England), and frozen until analysis. The remaining $^{15}NH_4^+$ enriched water in each tank was allowed to incubate for 12 hours. After the $^{15}NH_4$ incubation period, metabolism measurements and tissue of the remaining coral in each tank were collected by following the same methods used on the fragment taken before $^{15}NH_4^+$ addition. Once the coral were removed, 5 L of water from each tank were removed, filtered, and frozen using the same methods conducted on the water sampled 15 minutes after $^{15}NH_4^+$ was added.

Coral tissue samples were used to estimate rates of gross N uptake within each coral fragment. We placed 150 µL of thawed coral tissue slurry into a tin capsule alongside 50 µL of 0.714 µmol N-NH_4Cl unlabeled stock solution to ensure a 35 µmol minimum of N for mass spectrometry analyses. The tin capsules were placed in a drying oven at 100 ºC for 24 hours. We shaped each individual capsule into a sphere after they were taken out of the drying oven and

placed them into a desiccator alongside an open vial containing 2.5 mol $KHSO_4$ to absorb any ammonium in the air until further analysis. We used a modified protocol from Holmes et al. (1998) in order to determine gross N remineralization rates using water collected at 15 minutes and 12 hours after $^{15}NH_4Cl$ addition. The NH_4^+ was collected with a diffusion method where 100 mL of sample was placed into 120 mL HDPE bottles with 0.012 mol MgO, 100 µL of 0.036 mol N-NH_4Cl unlabeled stock solution to ensure a 35 mmol minimum of N and an acidified teflon filter packet. The filter packet was made by sealing an acidified 1 cm diameter Whatman GF/D glass fiber filter within a folded piece of 2.5 cm teflon tape. The bottles were sealed with parafilm, capped, and then placed on a shaker table for three weeks to allow for diffusion of NH_4^+ onto the acidified filter. Each filter packet was removed from the HDPE bottles and the filter was extracted from the packet. The filter was packed inside a tin capsule and was shaped into a sphere. The tin capsules were placed into a desiccator alongside an open vial with 2.5 mol $KHSO_4$ until further analysis.

A modified protocol from Lotic Intersite Nitrogen eXperiment II (LINX II) (https://andrewsforest.oregonstate.edu/sites/default/files/lter/data/studies/an006/linxii_stream15n_exp_protocols_rev5.pdf) was used to determine gross nitrification rates using water samples from the beginning and end of the $^{15}NH_4^+$ period to estimate the amount of stable isotope entering NO_3^- that had already dissolved in the tank water. The samples were allowed to thaw, and 0.5 L of sample was placed into a beaker alongside 0.037 mol MgO and 5 mL of 0.07 mol N-KNO_3 unlabeled stock solution to ensure a 35 mmol minimum of N. The sample in the beaker was boiled, concentrating NO_3^- and eliminating NH_4^+. The concentrated sample was placed in 120 mL HDPE bottles alongside 0.012 mol of additional MgO, 0.003 mol of Devarda's alloy, and an acidified teflon filter packet. The HDPE bottles were sealed with parafilm and placed in a

drying oven at 60 ºC for 48-hours where NO_3^- was reduced to NH_4^+. The bottles were removed from the drying oven and placed on a shaker table for one week to allow for diffusion of NH_4^+ onto the acidified filter. The filter packet was removed, extracted, packed, and stored. The coral tissue and water samples packed into the tin capsules were sent to the Stable Isotope Mass Spectrometer Laboratory at Kansas State University to be analyzed for $\delta^{15}N$ on a mass spectrometer (ThermoFinnigan Delta Plus).

Background tank water NH_4^+ concentrations were determined by using a modified protocol from Holmes et al. (1999) (Protocol A) and analyzed using AquaFluor Handheld Fluorometer (Turner Designs) with a detection limit of 0.03 μmol NH_4^+ L^{-1}. For NO_3^- analysis, water samples were analyzed by the LTER Lab at Kansas State University using The Flow Solution (ALPKEM) on an autoanalyzer with a detection limit of 0.01 μmol NO_3^- L^{-1}.

Field collection experiment with Acropora pulchra

We established twelve, 10 L tanks supplied with continuously pumped, filtered seawater from Pago Bay, Guam (13.427459, 144.799001). Water flowed through and around the tanks one day before coral collection. Water tables were under shade cloth to ensure attenuation of sunlight to the coral and control temperature.

We collected 18 *A. pulchra* fragments via snorkeling from each of two colonies across two sites West Hagåtña Bay (13.479650, 144.741750; 13.479833, 144.741733) and Luminao (13.4652417, 144.6477483; 13.465467, 144.648050) off the coast of Guam (Guam Department of Agriculture collection permit: #SCR-22-001). Each coral fragment was placed in an individual bag with ambient seawater immediately after removing it from the host colony and transported to the laboratory in a cooler. 1 L of water was also collected near each colony at both sites which was immediately filtered and frozen upon return.

Six *A. pulchra* fragments were suspended with fishing line in each of the flow-through tanks and were separated into each tank by colony (Figure 4.1). *A. pulchra* were allowed to acclimate for one week prior to NH_4^+ loading additions. The two *Acropora* species we used in our study were morphologically similar; thus, we used the average total and projectional surface area of the aquacultured *Acropora* from our laboratory experiment to estimate total and projectional *A. pulchra* surface area per tank (96 cm^2 and 23.12 cm^2 respectively). The NH_4^+ loading rates were then calculated as in our laboratory experiment. We had a total of three NH_4^+ loading treatments: 0, 9.8, and 98 μmol NH_4^+ $tank^{-1}$ day^{-1}. Each NH_4^+ treatment was administered to four tanks over a period of seven days with ambient seawater flow from Pago Bay stopped prior to the start of NH_4^+ additions. Respiration measurements were taken on one coral fragment from each tank under dark conditions 24 hours after the last NH_4^+ addition (Chapter 3). After the metabolism measurements, each fragment was gently covered with a piece of foil (Marsh 1970, Chapter 2) to determine total surface area and divided into three test tubes. The test tubes were immediately frozen in dry ice and stored in -40ºC until further analysis. ^{15}N additions were conducted similarly to our laboratory experiment although different ^{15}N stock was added in addition to allowing an incubation time of 24 hours. We enriched the seawater in each tank by adding 5 ml of 0.008 μmol ^{15}N-NH_4Cl stock solution (^{15}N-N 98% $^{15}NH_4Cl$, Sigma-Aldrich, St. Louis, MO). Remaining $^{15}NH_4^+$ enriched water in each tank was allowed to incubate for 24 hours.

A. pulchra metabolism measurements conducted on fragments collected before and after the ^{15}N incubation were conducted in a separate tank with filtered, ambient seawater. This also diluted away any ^{15}N not assimilated into the coral. To determine N uptake within each coral, each coral fragment was thawed and placed in a 100 ºC drying oven for 14 hours. After drying,

we ground the fragment using a mortar and pestle and placed the dried, ground coral into tin capsules. Tin capsules were stored in a desiccator alongside an open vial with 2.5 mol $KHSO_4$ to absorb any ammonium in the air until further analysis. Water collected 15 minutes after the $^{15}NH_4Cl$ addition and after the 24-hour incubation period was used to determine N remineralization, net nitrification, and uptake rates. Methods to process these samples were identical to the laboratory experiment with aquacultured *Acropora*; however, we were unable to determine gross nitrification in *A. pulchra* due to processing limitations in the field.

Calculations and statistics

Gross uptake, remineralization, and nitrification were calculated following equations presented in Laws (1984). Gross remineralization was calculated using equation one. Gross nitrification and uptake calculations were determined using equations 11, 18 and a modified version of equation four that corrects for remineralization-related isotopic dilution as shown below.

Equation 4.1 Modified version of equation four from Laws (1984)

$$u=r-(S_T/S_0)/t$$

Where r is remineralization, S_T is the concentration of NH_4^+ at the end of the $^{15}NH_4Cl$ incubation, S_0 is the concentration of NH_4^+ 15 minutes after the $^{15}NH_4Cl$ addition and t is the total time of incubation. An additional slight modification to equation 18 was made to determine nitrification; the average tank NO_3^- concentration was substituted for the average tank NH_4^+ to account for ^{15}N entering the dissolved NO_3^- phase.

We subtracted each tank's concentration of NH_4^+ collected 15 minutes after the $^{15}NH_4Cl$ addition from the NH_4^+concentration collected at the end of the ^{15}N incubation period to

determine net uptake. Net nitrification was determined similarly, but NO_3^- concentrations were used instead of NH_4^+ concentrations.

We used R version 4.0.2 (R Core Team, 2020) for all statistical analyses. We first normalized all of our N cycling rates for both the aquacultured and wild *Acropora*. We then used ANOVAs to determine if the NH_4^+ treatment influenced the amount of N cycling throughout the tanks containing the *Acropora*.

We chose to focus on U_T, N^*, and R in the aquacultured coral; we believed these N cycling processes would give us the best insight into determining the influence of NH_4^+ on coral N cycling. U_T is the direct measurement of N uptake within the coral, N^* is the nitrification rate of the entire tank which includes the coral, and R is the remineralization of the entire tank which includes the coral (Table 4.1). We ran regression analysis on U_T, N^*, R in the aquacultured *Acropora* to determine if there was a significant relationship between NH_4^+ treatments and N cycling rates. A Bonferroni testing correction was performed to determine a corrected p-value of 0.0167 for these three tests.

Similar to the aquacultured *Acropora,* we chose to focus on analyses surrounding U_T and R in wild *A. pulchra*. We used net nitrification (N_N) as our estimate of the nitrification rate in each tank as we were unable to collect N^* data due to field limitations. We used t-tests to determine if there was a significant difference in U_T, N_N, and R between wild *A. pulchra* fragments collected from two colonies at two different sites. ANCOVAs were used to determine if there were significant differences in *A. pulchra* U_T, N_N, and R rates between collection sites, between NH_4^+ treatments, or if there was an interaction between collection sites and NH_4^+ treatments. We used the Bonferroni corrected p-value of 0.0167 in the analysis of our results. Finally, we normalized all the N cycling rates for both the aquacultured and wild *Acropora* by

dividing each rate by the overall mean rate for each experiment. We used regression analyses to determine if there was a significant relationship between the normalized rates from the NH_4^+ treatment level and aquacultured and wild *Acropora*.

Results

Laboratory experiment with aquacultured Acropora

We were able to estimate rates of remineralization, nitrification, and uptake in aquacultured *Acropora* (Table 4.2). We found that the normalized rates from the six NH_4^+ treatments significantly increased as the NH_4^+ treatment level increased indicating that as we added more NH_4^+ there was more N in the tanks cycling (Figure 4.2).

There was not a significant relationship between NH_4^+ treatment and U_T in *Acropora* ($p=0.655$) (Figure 4.3). There was not a significant relationship between NH_4^+ treatments and R ($p=0.75$) or N^* ($p=0.48$) in the tanks containing *Acropora* (Figure 4.4).

Field collection experiment with Acropora pulchra

We detected rates of remineralization, net nitrification, and uptake in wild *A. pulchra* (Table 4.3). We found that the rates surrounding the three NH_4^+ treatments slightly increased as the NH_4^+ treatment level increased but were not significant (Figure 4.5).

There was no significant difference in R ($p=0.04$), N_N ($p=0.61$), or U_T ($p=0.22$) between Luminao colony tanks ($p>0.04$) or R ($p=0.03$), N_N ($p=0.61$), or U_T ($p=0.55$) between W. Hagåtña colony tanks when comparing results to the corrected p-value of 0.0167. We then combined the N cycling rates from all *A. pulchra* that were collected from the same site that were exposed to the same NH_4^+ treatment. We did not find a significant difference in R ($p=0.11$), N_N ($p=0.08$), or U_T ($p= 0.30$) rates between Luminao and W. Hagåtña or in R ($p=0.21$), N_N ($p=0.04$), or U_T

(p=0.85) rates between NH_4^+ treatments when comparing results to the corrected p-value of 0.0167. There was not a significant interaction of *A. pulchra* N cycling rates between NH_4^+ treatment and collection site in R (p=0.13), N_N (p=0.30), or U_T(p=0.32) (Figure 4.6, Figure 4.7).

Discussion

Our research suggests that rates of nitrification, remineralization, and uptake remain uninfluenced by the level of five days of NH_4^+ exposure in tanks containing aquacultured *Acropora;* however, when rates were normalized, rates increased as the NH_4^+ treatment increased indicating that as we added more NH_4^+ there was more N in the tanks cycling. Additionally, our research suggests that tanks containing wild *A. pulchra* nitrification, remineralization, and uptake remains uninfluenced by the level of seven days of NH_4^+ exposure or the collection origin despite probable differing levels of anthropogenic activities. We were unable to support our hypotheses which stated that 1) aquacultured *Acropora* nitrification and uptake rates would increase and remineralization rates would decrease with increasing NH_4^+ treatments, 2) wild *Acropora pulchra* nitrification and uptake rates would increase and remineralization rates would decrease with increasing NH_4^+ treatments, and 3) N cycling rates would differ between *A. pulchra* collected from two sites exposed to probable differing anthropogenic activity surrounding the island of Guam. We averaged N rates across all NH_4^+ treatments for both aquacultured and wild *Acropora* and present a visual diagram in Figure 4.8. Note that Figure 4.8 contains some N fluxes that are associated with the microbes in the tank in addition to the microbes and *Symbiodinium* associated with the *Acropora* fragments. Only the direct uptake of N into the *Acropora* can be directly ascribed to the coral individual alongside the

microbes and *Symbiodinium* which can be located on the coral's surface or inside its tissue or skeleton.

Few studies have previously quantified coral nitrification and remineralization under ambient conditions and none we know of have compared rates under varying levels of NH_4^+ exposure. This is mainly because these experiments require ^{15}N tracer methods to fully characterize many N transformations. The importance of scleractinian coral N remineralization rates have not been documented until recently (McNally et al. 2017). Remineralization is an important source of NH_4^+ to the entire coral reef ecosystem, but previous studies have mostly focused on quantifying remineralization in benthic sediments, sponges, and reef fish (Williams et al. 1985, Jiménez & Ribes 2007, Wyatt et al. 2012, Francis & Cotê 2018). Although we did not have sediments, sponges, or fish contributing to remineralization rates, *Acropora* or other organisms within the tank could have contributed to the rates we detected as we were not able to separate remineralization rates from every organism within the tank (Hopkinson et al. 1987). These remineralization rates were not statistically different between *A. pulchra* collection site or NH_4^+ treatment in wild coral, nor between NH_4^+ treatments in aquacultured *Acropora.*

Nitrification occurs on and within all parts of scleractinian coral including the skeleton, tissue, mucus, and interstitial water between branches (Rädecker et al. 2015). We did not separate nitrification rates amongst all the individual parts of the coral or from other organisms in each tank, but we were able to generally support previous research which found that nitrification is occurring in areas surrounding coral (Rädecker et al. 2015). Glaze et al. (2022) reported an average nitrification rate of 2.70 μmol N m^{-2} day^{-1} across six scleractinian coral species including two species of *Acropora.* In comparison, we report average nitrification rates of 3.03±1 μmol m^{-2} day^{-1} using total surface area estimates and 18.24±4 μmol N m^{-2} day^{-1} using

projectional surface area estimates in aquacultured *Acropora* across six NH_4^+ treatments after excluding negative nitrification rates (± 1 SD). Our average nitrification rate calculated using total surface area is very similar to that found by Glaze et al. (2022); however, Glaze et al. (2022) fails to specify methods used to determine the coral surface area. Without that knowledge, comparison of nitrification rates should be viewed with caution. We can conclude that the rates at which gross nitrification is occurring within or on the coral does not appear to be influenced by NH_4^+ loading in aquacultured *Acropora.*

Although we found rates of N_N between NH_4^+ treatments in *A. pulchra,* we were unable to determine gross nitrification rates. Nitrification occurring on, within, or surrounding *A. pulchra* may have contributed to the net nitrification rates we found, but we cannot make that assumption as we were unable to separate rates amongst all the individual parts of the coral or from other organisms in each tank. N_N did not statistically differ between collection sites nor was there an interaction between site and NH_4^+ treatment. Specific nitrification measurements are needed in future studies to more accurately compare nitrification rates of wild *A. pulchra* under differing NH_4^+ treatments.

We were able to quantify NH_4^+ uptake rates within the coral; however, we did not see a significant difference in direct uptake rates between NH_4^+ treatment or collection site in wild *A. pulchra* nor between NH_4^+ levels in aquacultured *Acropora.* Glaze et al (2022), found an average uptake rate of 289.8 µmol N m^{-2} day^{-1} across six coral species. We found rates of 4.8±5 µmol N m^{-2} day^{-1} and 27.49±25 µmol N m^{-2} day^{-1} in aquacultured *Acropora* and 3.01±5 µmol N m^{-2} day^{-1} and 9.34±16 µmol N m^{-2} day^{-1} in wild *A. pulchra* calculated using total surface area and projected surface area, respectively (± 1 SD). Glaze et al. (2022) reported uptake to be 10x higher than our values. A comparison between these uptake rates needs to be viewed with

caution as we found our uptake rates to be higher when using projectional based surface area values compared to total surface area as Glaze et al. (2022) did not specify what surface area measurement method was used. Other literature reports smaller uptake rates ranging from 0.03 μmol N m^{-2} day^{-1} in in-situ *A. palmata* and even larger values of 1224-22032 μmol N m^{-2} day^{-1} in *P. damicornis* exposed to lagoon water with a background concentration of 180 μmol NH_4^+ (Bythell 1990, Hoegh-Guldberg & Williamson 1999). It appears that uptake rates of scleractinian coral are highly variable between and within species under ambient and NH_4^+ enriched conditions.

Despite a higher uptake rate, Glaze et al. (2022) found nitrification rates to be generally smaller than uptake rates similar to our findings. However, our data suggests that while nitrification rates were generally lower than uptake rates, the rates were not highly variable from each other similarly to findings presented by Wafar et al. (1990). Further studies should attempt to quantify the rates of nitrification and uptake in both the coral host and the *Symbiodinium* over gradual and longer NH_4^+ exposure. Both the host and *Symbiodinium* have the ability to fix NH_4^+; however, *Symbiodinium* have been found to uptake NH_4^+ much faster than the coral host especially under a pulse NH_4^+ exposure method (Grover et al. 2002, Pernice et al. 2012). We used a pulse method to add NH_4^+ during the course of our study and may have influenced uptake and nitrification rates by using this pulse method.

Under eutrophic stress, denitrification has been hypothesized to act as a pathway to decrease excess N in water surrounding coral (Siboni et al. 2008, Rädecker et al. 2015). In our study denitrification may have caused the amount of NO_3^- in the tank water to decrease to a similar level as found in the control tanks. This may have led to tanks exposed to the highest level of NH_4^+ to have the highest denitrification rates as anoxic conditions occur within varying

parts of the coral (Siboni et al. 2008). If these denitrification rates are correlated to the level of NH_4^+ treatment, these levels may have led to a lower concentration of NO_3^- which may have possibly influenced nitrification rates.

Overall, we provide rates for N cycling processes associated with two species of *Acropora* under varying NH_4^+ treatments. Future studies to quantify N cycling processes could consider denitrification to give further insight into what is occurring within the N cycle under varying levels of NH_4^+. We provide support for the hypothesis that remineralization, uptake, and nitrification appear to not be influenced by NH_4^+ levels in wild and aquacultured *Acropora* in addition to sites exposed to levels of probable differing anthropogenic activity in wild *Acropora*.

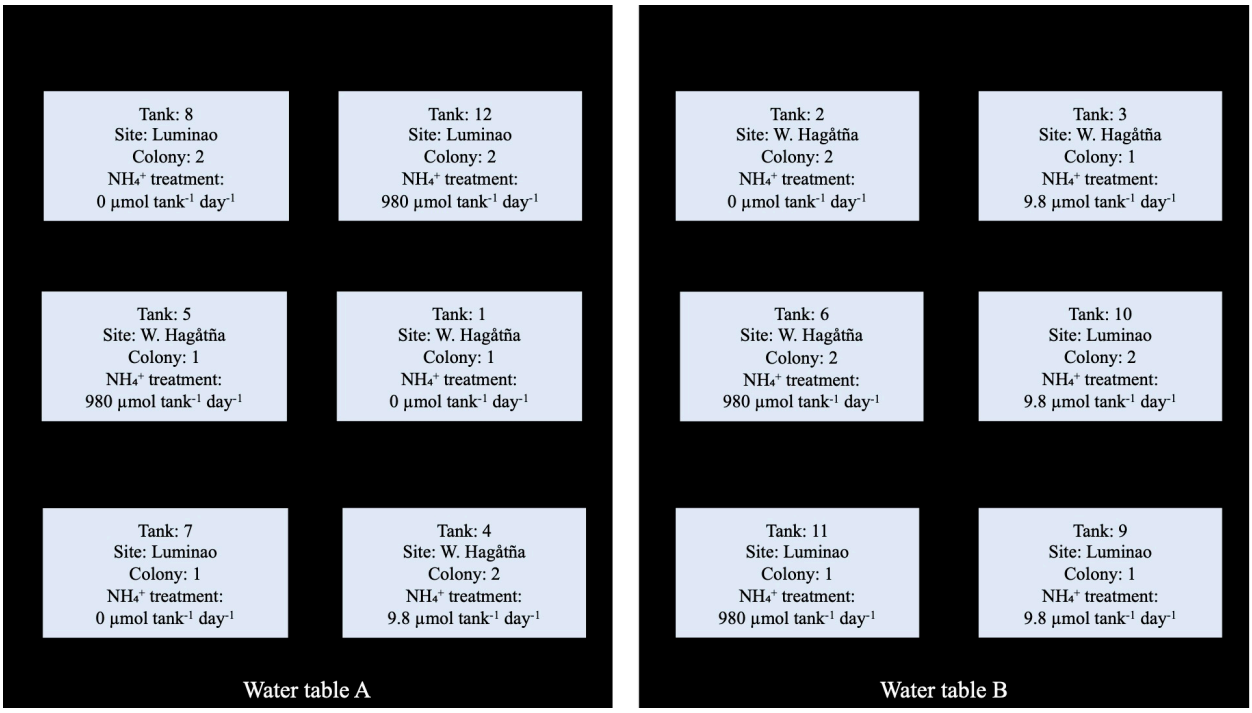

Figure 4.1 *A. pulchra* experimental tank set up in Guam. Each tank contained six *A. pulchra* fragments.

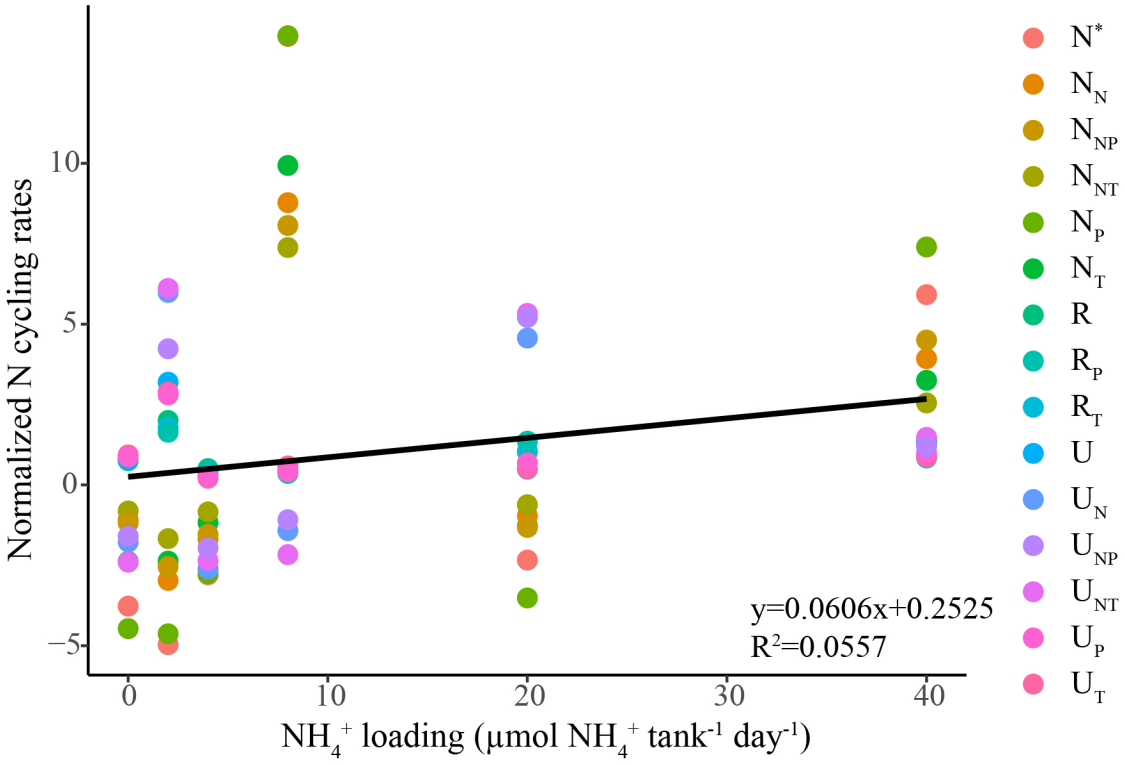

Figure 4.2 Normalized N cycling rates across six NH_4^+ treatment tanks containing aquacultured *Acropora.* We found rates significantly increased as the NH_4^+ treatment level (p=0.025). These results indicate that the level of NH_4^+ treatment corresponded to the level of N cycling throughout the tanks.

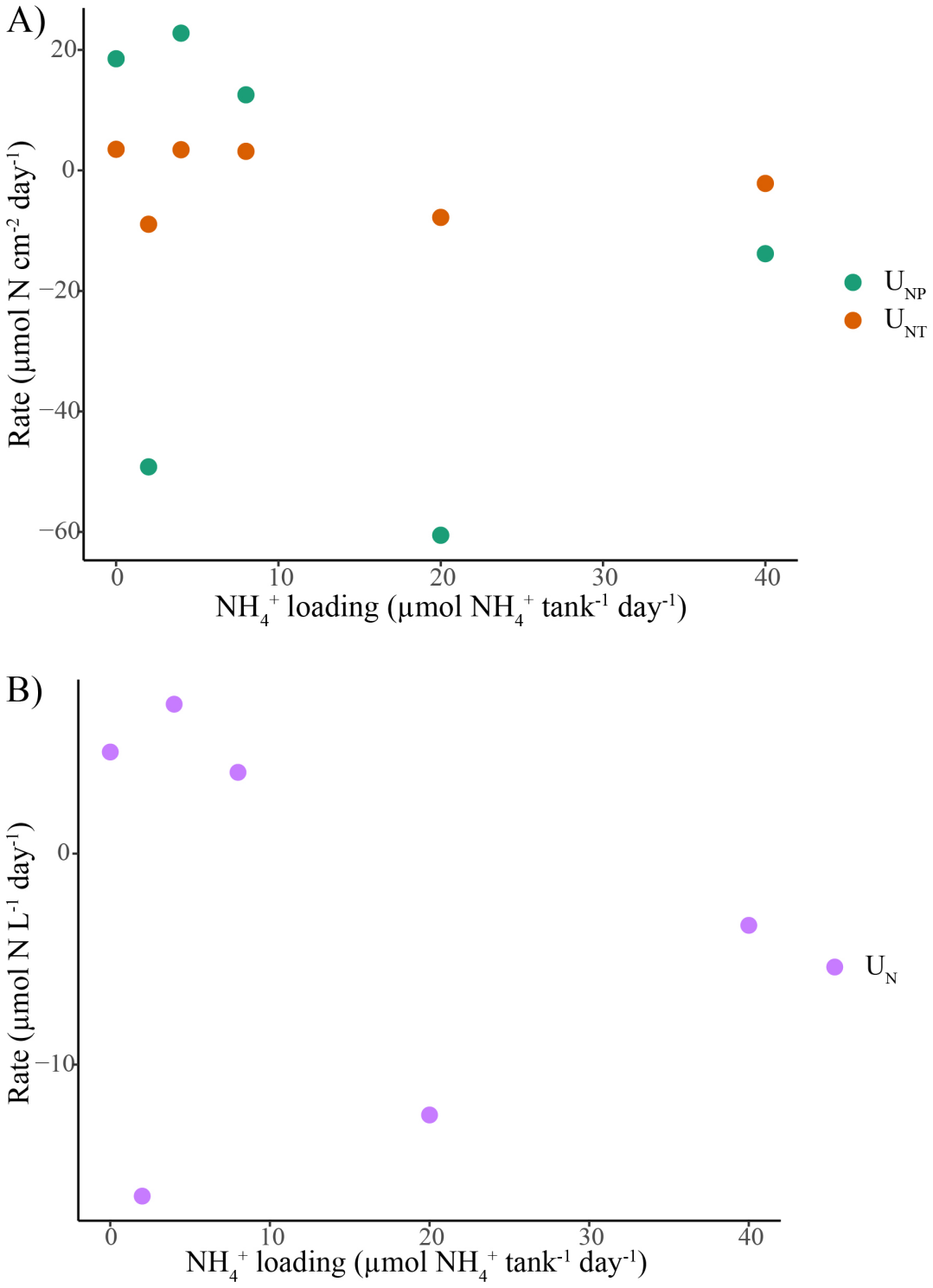

Figure 4.3 A) Rates of net uptake calculated per projectional and total surface area of the aquacultured *Acropora.* B) Rate of net uptake calculated for each tank containing aquacultured *Acropora*. See Table 4.1 for symbol identification.

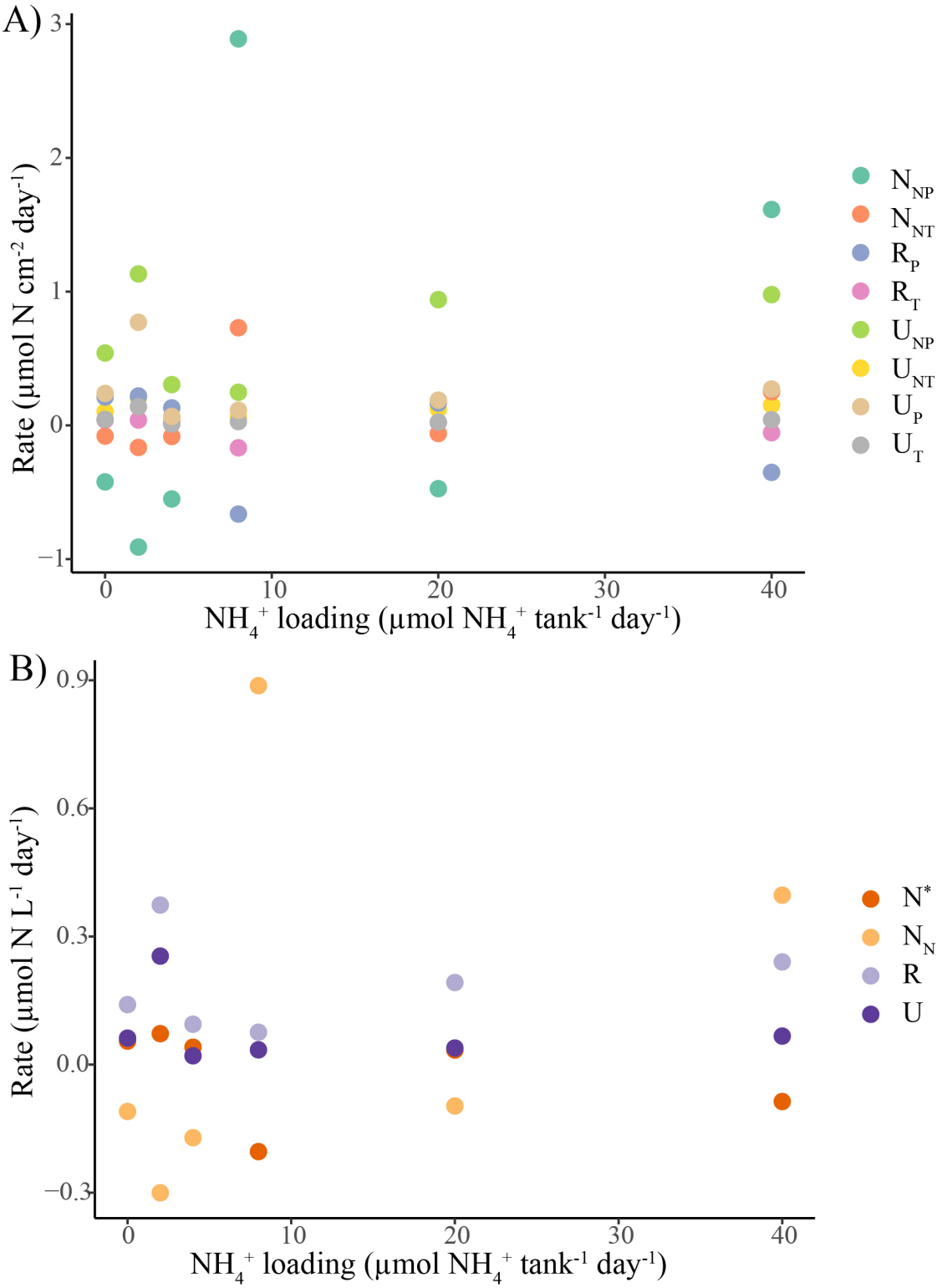

Figure 4.4 A) Rates of remineralization, nitrification, and uptake calculated per projectional and total surface area of the aquacultured *Acropora* inside each tank. B) Remineralization (R), nitrification (N^*), uptake (U), and net nitrification (N_N) rates scaled per each tank containing aquacultured *Acropora* (N_N). See Table 4.1 for additional symbol identification.

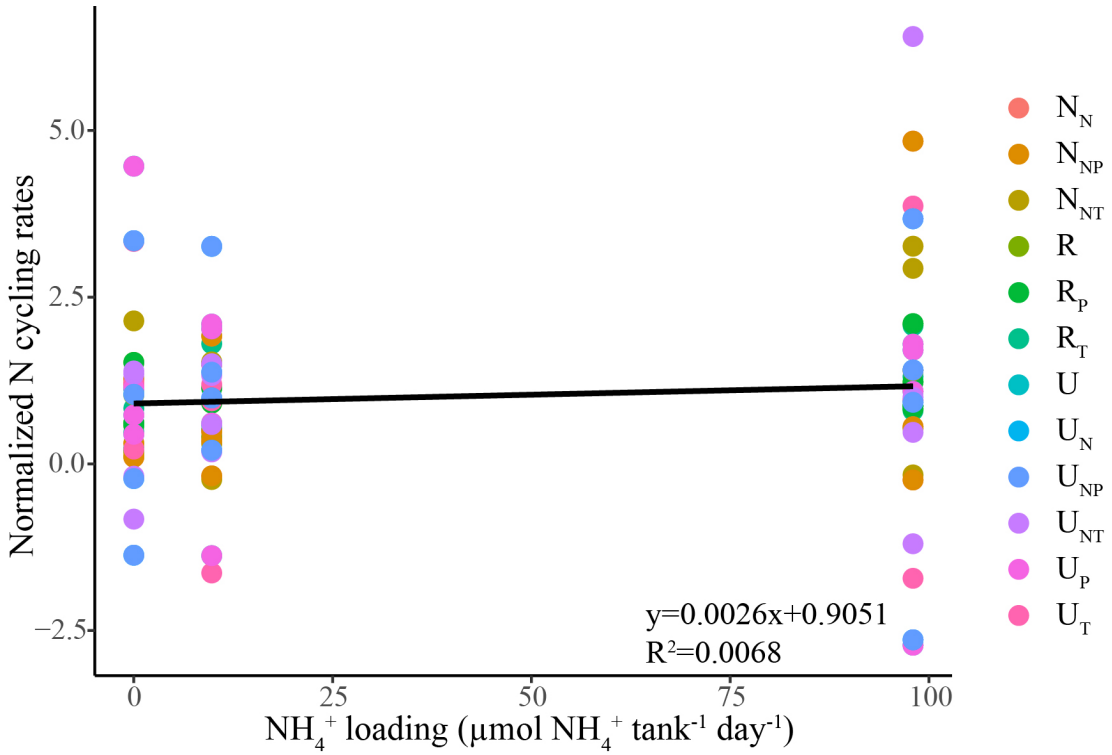

Figure 4.5 Normalized N cycling rates across NH_4^+ treatment tanks containing wild *Acropora pulchra* collected from two sites with differing anthropogenic activity. Rates were not impacted by the NH_4^+ treatment level (p=0.325). There appeared to be a slight positive correlation between the level of NH_4^+ treatment and the level of N cycling throughout the tanks, but it was not significant.

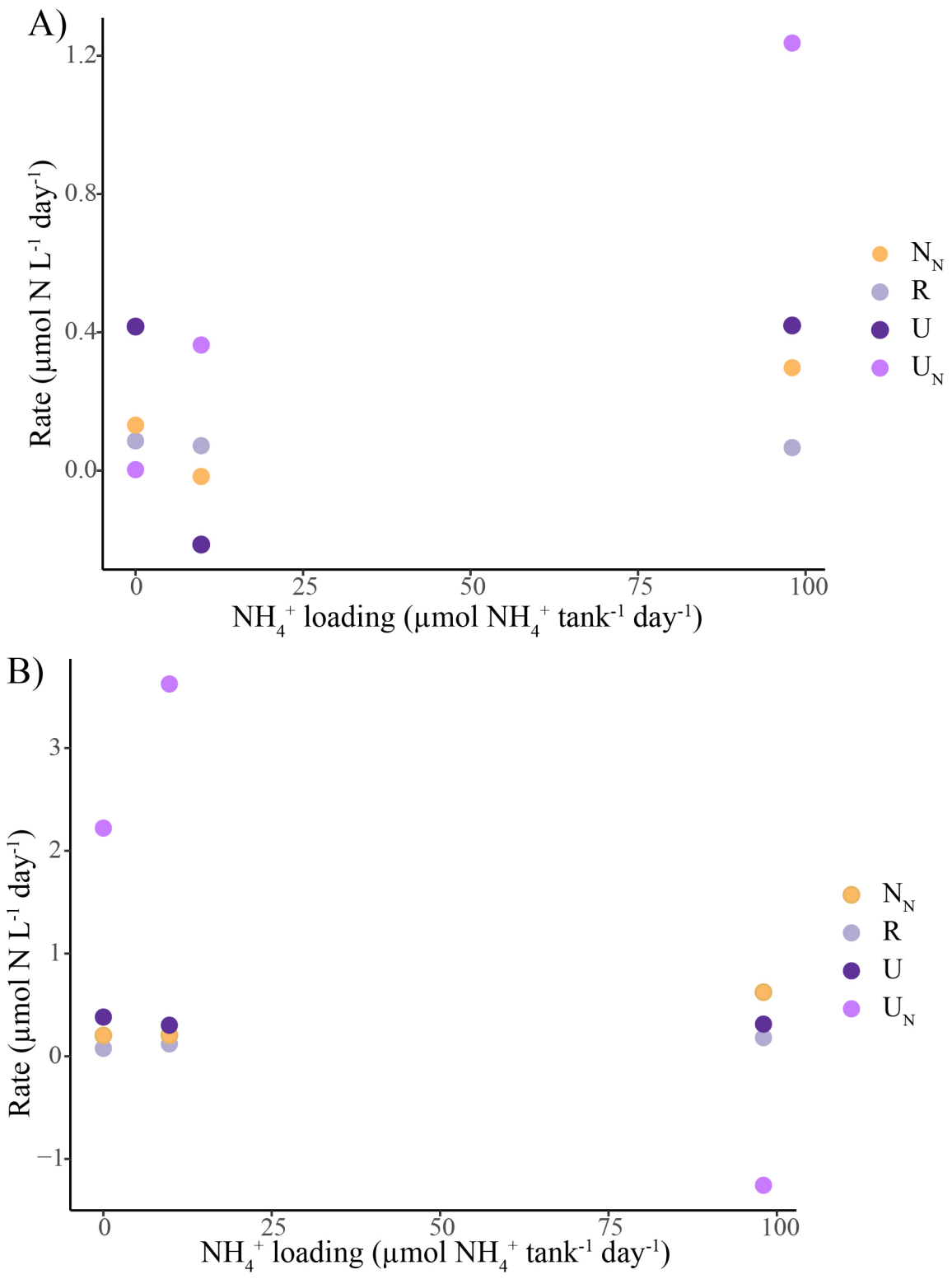

Figure 4.6 Average remineralization (R), nitrification (N_N), uptake (U), and net uptake (U_N) rates scaled per each tank containing wild *A. pulchra.* A) N cycling rates associated with coral collected from W. Hagåtña and B) N cycling rates associated with coral collected from Luminao.

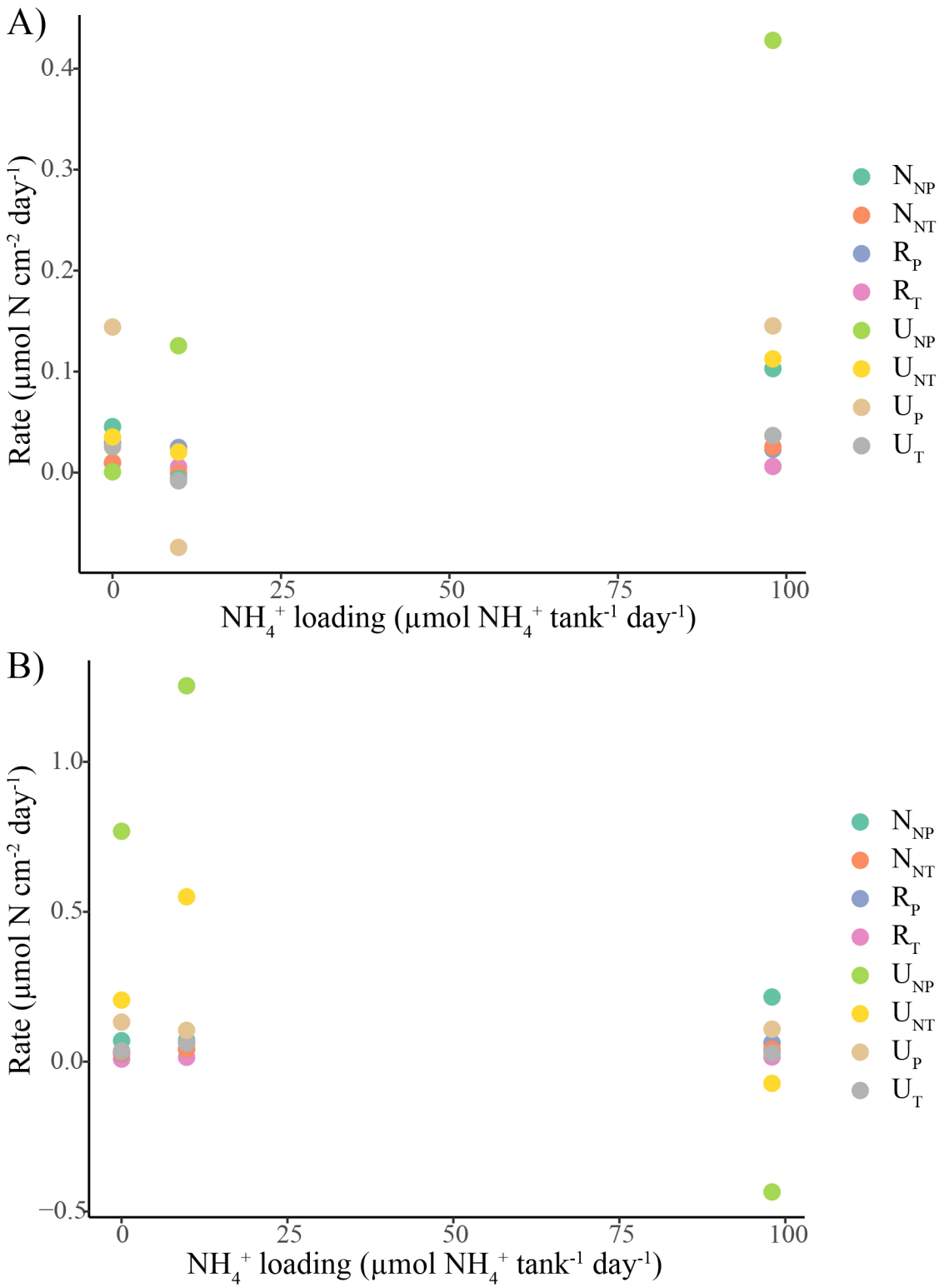

Figure 4.7 Average rates of remineralization, nitrification, and uptake calculated per projectional and total surface area of the wild *A. pulchra* inside each tank. A) Rates for coral collected from W. Hagåtña. B) Rates for coral collected from Luminao. See Table 4.1 for symbol identification.

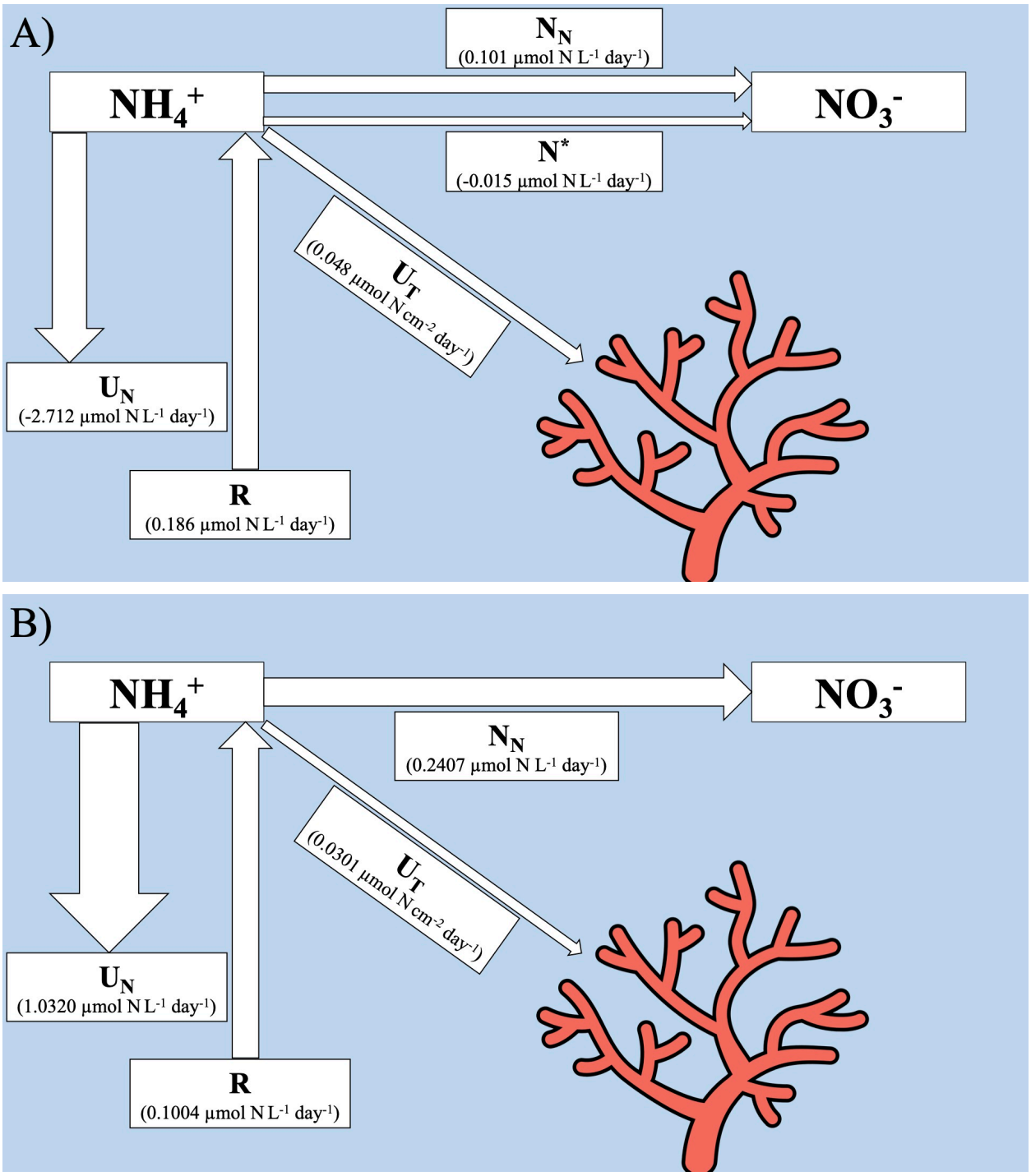

Figure 4.8 Visual diagram of average N cycling rates of A) aquacultured *Acropora* and B) wild *A. pulchra.* Rates were averaged among all tanks regardless of NH_4^+ treatment received or the collection origin. The width of arrows represent an approximate proportion to the flux rates.

Table 4.1 Variables and units for N cycling rates presented in this study.

Symbol	Variable	Units
R	Remineralization	μmol N L^{-1} day^{-1}
R_T	Remineralization (total surface area)	μmol N cm^{-2} day^{-1}
R_P	Remineralization (projectional surface area)	μmol N cm^{-2} day^{-1}
N^*	Nitrification	μmol N L^{-1} day^{-1}
N_T	Nitrification (total surface area)	μmol N cm^{-2} day^{-1}
N_P	Nitrification (projectional surface area)	μmol N cm^{-2} day^{-1}
N_N	Net nitrification	μmol N L^{-1} day^{-1}
N_{NT}	Net nitrification (total surface area)	μmol N cm^{-2} day^{-1}
N_{NP}	Net nitrification (projectional surface area)	μmol N cm^{-2} day^{-1}
U	Uptake	μmol N L^{-1} day^{-1}
U_T	Uptake (total surface area)	μmol N cm^{-2} day^{-1}
U_P	Uptake (projectional surface area)	μmol N cm^{-2} day^{-1}
UN	Net uptake	μmol N L^{-1} day^{-1}
U_{NT}	Net uptake (total surface area)	μmol N cm^{-2} day^{-1}
U_{NP}	Net uptake (projectional surface area)	μmol N cm^{-2} day^{-1}

Table 4.2 N cycling rates for aquacultured *Acropora* under varying NH_4^+ treatment levels. All rates were not statistically significant between NH_4^+ treatment levels ($p > 0.423$). See Table 1 for which variable and unit is associated with each symbol.

Tank #	Treatment (μmol NH_4^+ $tank^{-1}$ day^{-1})	R	R_T	R_P	N^*	N_T	N_P	N_N	N_{NT}	N_{NP}	U	U_T	U_P	U_N	U_{NT}	U_{NP}
1	0	0.141	0.103	0.541	0.055	0.040	0.212	-0.110	-0.080	-0.422	0.062	0.045	0.239	4.816	3.511	18.535
2	2	0.374	0.206	1.132	0.073	0.040	0.220	-0.300	-0.165	-0.910	0.254	0.140	0.771	-16.234	-8.930	-49.201
3	4	0.095	0.046	0.305	0.041	0.020	0.131	-0.171	-0.083	-0.550	0.021	0.010	0.067	7.082	3.437	22.784
4	8	0.076	0.063	0.248	-0.204	-0.167	-0.663	0.887	0.730	2.890	0.035	0.029	0.113	3.853	3.169	12.547
5	20	0.192	0.121	0.940	0.034	0.021	0.167	-0.097	-0.061	-0.473	0.039	0.025	0.189	-12.389	-7.796	-60.548
6	40	0.241	0.153	0.978	-0.086	-0.055	-0.351	0.397	0.252	1.614	0.067	0.043	0.272	-3.399	-2.159	13.820
Mean		0.186	0.115	0.691	-0.015	-0.017	-0.047	0.101	0.099	0.358	0.080	0.048	0.275	-2.712	-1.461	-11.617
Standard deviation		0.120	0.063	0.390	0.115	0.089	0.380	0.479	0.372	1.567	0.097	0.052	0.285	10.864	6.441	40.256

Table 4.3 N cycling rates of wild *A. pulchra* under three NH_4^+ treatments. Mean and standard deviation are included for rates. See Table 1 for which variable and unit is associated with each symbol.

Tank #	Site	Colony	Treatment (µmol NH_4^+ $tank^{-1}$ day^{-1})	R	R_T	R_P	N_N	N_{NT}	N_{NP}	U	U_T	U_P	U_N	U_{NT}	U_{NP}
1	W. Hagåtña	1	0	0.0576	0.0048	0.0199	0.3065	0.0255	0.1061	1.250	0.1003	0.4169	-1.4143	-0.1176	-0.4894
2	W. Hagåtña	2	0	0.1142	0.0151	0.0394	-0.0435	-0.0058	-0.0151	-0.3717	-0.0492	-0.1286	1.4198	0.1881	0.4910
3	W. Hagåtña	1	9.8	0.1234	0.0087	0.0427	-0.0581	-0.0041	-0.0201	-0.7341	-0.0516	-0.2541	0.9538	0.0607	0.330
4	W. Hagåtña	2	9.8	0.0206	0.0024	0.0070	0.0242	0.0027	0.0084	0.3063	0.0355	0.1059	-0.2273	-0.0262	-0.0787
5	W. Hagåtña	1	98	0.0506	0.0041	0.0175	0.4615	0.0379	0.1596	0.5463	0.0447	0.1891	1.0205	0.0837	0.3531
6	W. Hagåtña	2	98	0.0825	0.0081	0.0286	0.1339	0.0130	0.0463	0.2931	0.0286	0.1015	1.4530	0.1416	0.5028
7	Luminao	1	0	0.0621	0.0113	0.0214	0.2889	0.0531	0.0999	0.1975	0.0363	0.0684	1.0759	0.1977	0.3723
8	Luminao	2	0	0.0951	0.0058	0.0317	0.1162	0.0074	0.0401	0.5655	0.0358	0.1958	3.3663	0.2132	1.1649
9	Luminao	1	9.8	0.0874	0.0209	0.0302	0.3372	0.0809	0.1167	0.4845	0.1162	0.1677	3.7934	0.9096	1.3126
10	Luminao	2	9.8	0.1527	0.0084	0.0528	0.0742	0.0041	0.0257	0.1200	0.0067	0.0415	3.4551	0.1906	1.1955
11	Luminao	1	98	0.1512	0.0182	0.0523	0.0823	0.0099	0.0285	0.1651	0.0285	0.0571	0.2107	0.0254	0.0729
12	Luminao	2	98	0.2109	0.0132	0.0730	1.1650	0.0727	0.4030	0.4608	0.0288	0.1594	-2.7230	-0.1701	-0.9422
Mean				0.1004	0.0101	0.0347	0.2407	0.0248	0.0833	0.2699	0.0301	0.0934	1.0320	0.1419	0.3571
Standard deviation				0.0531	0.0058	0.0184	0.3325	0.0298	0.1150	0.4844	0.0488	0.1676	1.9453	0.2726	0.6731

Chapter 5 - Conclusions

Scleractinian coral are crucial coral reef ecosystem engineers. Coral reefs provide a habitat for a large portion of marine life in addition to providing key ecosystem services to humans such as coastal protection. Unfortunately, scleractinian coral and the reefs they support are under threat due to anthropogenic activities. Knowledge surrounding scleractinian coral is continuously expanding; however, much is yet to be investigated and understood. Specifically, eutrophication has largely been understudied in reef ecosystems compared to other stressors such as ocean warming and acidification especially in the genus *Acropora*, which is one of the most widespread and vulnerable scleractinian coral in the world. I was interested in determining how two different species of *Acropora* would respond to varying levels of ammonium (NH_4^+) through measuring metabolism presented in Chapter 3 and quantifying processes within the nitrogen (N) cycle which are presented in Chapter 4. Both metabolism and N cycling calculations depended upon surface area measurements. Methods to determine surface area measurements are ubiquitous throughout scientific literature; however, I determined which surface area method was best for our study which is presented in Chapter 2.

Foil wrapping is slightly more explanatory, no requirement to sacrifice coral tissue or skeleton

Foil wrapping and wax dipping are easily replicable and cost-effective methods to determine coral surface area. Our foil wrapping technique was slightly more explanatory than the single wax dipping method used; however, there were no statistical differences between surface area values produced between methods. Ultimately, I used the foil wrapping method to determine surface area values for our *Acropora* fragments. Although there was no statistical

difference in produced values, the foil method does not require a sacrifice of the coral tissue or skeleton.

***Acropora* primary production increased with NH_4^+ level, respiration uninfluenced**

I designed and constructed a chamber to specifically conduct metabolism measurements on small scleractinian coral fragments. Using this chamber, I measured primary production and respiration of aquacultured *Acropora* and respiration of wild *Acropora pulchra* after exposure to varying levels of NH_4^+. I found that primary production was positively correlated to the level of NH_4^+ aquacultured *Acropora* was exposed to, and respiration was not correlated to the level of NH_4^+ in either the aquacultured *Acropora* or *A. pulchra.* I hypothesize that *Acropora* primary production increases as NH_4^+ increases due to the possibility that the photosynthetic *Symbiodinium* uncontrollably multiplies within the coral tissue and retains the photosynthates. The retention of the photosynthates increases *Symbiodinium* respiration and decreases the coral host respiration resulting in an unchanged rate. I additionally found that respiration only differed between the sites in which the *A. pulchra* were collected from. I hypothesize that a factor other than NH_4^+ influences respiration, and further research needs to be conducted to determine this influence.

Nitrification, remineralization, and uptake uninfluenced by NH_4^+

I was able to detect nitrification, remineralization, and uptake rates in tanks including both aquacultured and wild *Acropora*. Nitrification, remineralization, and water uptake rates were not able to be separated and include the coral individual and the tank water. The rates at which these processes were occurring within aquacultured and wild *Acropora* did not differ between NH_4^+ levels. I suggest that future research focus on denitrification in addition to nitrification, remineralization, and uptake as denitrification may act as a pathway to decrease

excess N in water surrounding coral. All rates presented in this study serve as an important resource for understanding how scleractinian coral respond to increased N in an otherwise N limited environment as quantification of N cycling processes in the scientific literature are limited. I additionally found that the methods used to determine surface area of the coral may result in differing rates. Through this finding I stress that the method to determine coral surface area be selected carefully.

Coral surrounding island of Guam under numerous stressors, continued research needed

Guam, a U.S. territory in the western Pacific, is the largest and southernmost island of the Mariana Islands located 13º28'N, 144º46'E with an area of 549 km^2 supporting a population of 159,000 people (United States Census Bureau 2020). The island is characterized by 244 km of shoreline which includes fringing reefs, patch reefs, submerged reefs, offshore banks, and barrier reefs. Over 5,000 marine species can be found on Guam's coral reefs including an estimated 400 species of scleractinian coral (Randall 2003, Porter et al. 2005). These reefs have a total economic value of $183.8 million (adjusted for inflation) through the fishing industry and tourism (van Beukering et al. 2007). Guam reefs provide extraordinary benefits to both humans and the greater natural world; however, coral surrounding the island of Guam are under numerous stressors. Over a four-year period, from 2013-2017, ⅓ of coral cover on shallow seaward slopes was lost island-wide due to increased bleaching and extreme weather events (Raymundo 2019). Frequent *Acanthaster planci* outbreaks, depleted fish populations, and sedimentation have also led to the decline of scleractinian coral surrounding Guam and the change of the dominant coral cover of *Acropora* to Pocilloporidae within the last 100 years (Burdick 2008, Cybulski 2016). Although I did not find any significant impacts of NH_4^+ loading on *A. pulchra* N cycling or metabolism, previous research has found that increases in sewage-

derived N have been found to significantly correlate with disease severity within *Porites* surrounding the island of Guam indicating that N loading is an additional stressor facing Guam reefs (Redding et al. 2013). I stress that future studies need to continue to research how N and other stressors can impact scleractinian coral and whole reef ecosystems surrounding Guam. Through my research, I build upon the current knowledge of how N loading may influence coral surrounding Guam and provide a framework for future studies to build upon.

Overall, this work was focused on the response of *Acropora* subjected to varying levels of NH_4^+. While it does not appear that NH_4^+ exposure impacts respiration, remineralization, nitrification, or uptake, there was a significant correlation between primary production and NH_4^+ treatment level indicating that NH_4^+ does impact coral. Further work should incorporate molecular analyses as the coral host, *Symbiodinium*, or other associated microbes might be undergoing responses to NH_4^+ that are not reflected in N cycling or metabolic activities. This work presents some of the first N cycling and metabolism rates of two *Acropora* species subjected to varying levels of NH_4^+. I am exceedingly grateful to have had the opportunity to positively contribute to the knowledge base of how scleractinian coral respond to eutrophication as anthropogenic stressors in marine environments continue to amplify in our changing world. I am looking forward to seeing how future N-based work on scleractinian coral can contribute to the overall conservation of such a significant organism.

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