Increased glucose availability increases infection susceptibility in symbiotic Aiptasia.

Authors: Claire Cook, Carly Goldenberg, Samantha Masih, Sarah Davies

Abstract:
Host–pathogen interactions are intrinsically shaped by nutrient availability, including glucose, which fuels both host metabolism and immune activity. In the anemone *Exaiptasia pallida* (*Aiptasia*), symbiotic individuals exhibit elevated glucose levels, which creates a biologically relevant contrast in internal nutrient states. However, it is unknown whether symbiosis-driven differences in glucose availability alter susceptibility to bacterial infection in basal metazoans with open circulatory systems. Here, we show that elevated glucose, whether produced by algal symbionts or provided through diet, accelerates infection progression and increases mortality in *Aiptasia* challenged with *Pseudomonas aeruginosa*. Using aposymbiotic and symbiotic anemones fed either glucose-supplemented or control diets, we found that all infected individuals died within eleven days, with symbiotic and glucose-fed groups dying earliest. Symbiotic anemones experienced mortality approximately five days before aposymbiotic anemones, and glucose-fed anemones consistently died faster than control-fed counterparts. Infected individuals displayed severe stress phenotypes prior to death, whereas all non-infected controls survived with only mild and transient stress. These results indicate that high glucose availability intensifies infection severity in *Aiptasia* and shortens survival during bacterial challenge. Our findings reveal a metabolic cost of symbiosis under pathogenic stress, highlighting that nutrient fluxes can influence immune outcomes even in organisms lacking specialized circulatory systems. This work suggests that glucose-mediated exacerbation of infection may represent an evolutionarily conserved phenomenon across animal lineages. By establishing *Aiptasia* as a useful model for studying nutrient–pathogen interactions, these results open opportunities to explore how metabolic state influences immunity in both marine symbioses and broader host–pathogen biology.

Introduction:

A complex interplay of host physiology, immune functions, and resource availability shapes host-pathogen interactions. Among these factors, nutrient levels, particularly glucose, can strongly influence the progression and severity of infections (Ingels *et al.,* 2017). Glucose ($C_6H_{12}O_6$) is the most abundant monosaccharide in cells and is a central metabolic fuel for host cells. Glucose supports basic cellular functions and plays a significant role in powering immune responses (Morano *et al.*, 2020). However, while glucose is clearly beneficial to organisms, some pathogens can exploit this nutrient (Holt *et al.*, 2024). Since glucose is a readily accessible energy source, elevated glucose levels can create conditions that promote rapid microbial growth (Darweesh *et al.,* 2025). In humans, for example, individuals with diabetes often experience worsened infection outcomes due to chronically high blood glucose. These perpetually elevated blood glucose levels can impair immune defenses while simultaneously providing excess

nutrients for invading pathogens. This dual role of glucose underscores its importance as a mediator of disease dynamics across biological systems, especially when levels are too high.

Perhaps this model organism can be used to help with diabetes medication development due to potential similarities to human responses to elevated glucose. *Exaipitasia pallidia* (colloquially referred to as *Aiptasia*) is a tropical and sub-tropical anemone that is often found in the shallow waters of the Atlantic Ocean (Pale Anemone, n.d.). These anemones can establish a symbiotic relationship with algae in the Family Symbiodiniaceae. One function of the symbionts associated with the symbiotic Aiptaisa is the symbionts' role in glucose production through photosynthesis, with a study finding that the loss of symbionts leads to a reduction in glucose. Through this symbiosis, the anemone receives carbon sugars in the form of glucose as a byproduct of photosynthesis (Molina *et al.,* 2017). However, this anemone is also able to exist in both symbiotic ('brown', with algal symbionts) and aposymbiotic ('white', without algal symbionts) states (Baumgarten *et al*., 2015), which has led to these anemones emerging as a valuable model system for understanding symbiotic relationships. Aposymbiotic anemones are still able to survive without this algae-derived source of glucose; however, they rely on heterotrophic feeding methods, such as filter feeding (Cui *et al*., 2023). This difference manifests as distinct baseline glucose levels, with symbiotic *Aiptasia* generally possessing higher levels of internal glucose (Molina *et al.,* 2017). These differences in baseline glucose levels between aposymbiotic and symbiotic specimens may lead to glucose acting as a hotspot for invading pathogens in symbiotic anemones. *Aiptasia*'s reputation as a pest, resilience without a symbiont, and biological relation to stony coral species make it an optimal specimen to test metabolic processes in an open circulatory system.

Symbiotic and aposymbiotic *Aiptasia* differ in a lot of traits due to the difference due to the presence or absence of symbionts. Studies have shown that growth rates and bacterial density, and bacterial associations differ between aposymbiotic and symbiotic anemones, *Aiptasia,* with symbiotic individuals having faster growth rates and higher bacterial density. (Costa *et al*., 2021). Different nutritional supplies between these two symbiotic states of *Aiptasia* also account for faster pedal lacerate growth and development in symbiotic anemones (Presnel *et al*., 2022). However, there have been no studies examining whether the differences in internal nutrient levels of symbiotic vs aposymbiotic *Aiptasia* have an impact on host-pathogen interactions. Thus, it is unknown whether symbiotic *Aiptasia* with naturally higher internal glucose levels are more susceptible to bacterial infections. While we know that mammals with elevated glucose levels from diabetes are more susceptible to infection (Holt *et al.*, 2024), there is very little information on the role of elevated glucose for bacterial infection in more basal metazoans that lack a circulatory system.

Our study investigates whether glucose availability shapes infection severity in Aiptasia across symbiotic and aposymbiotic states. This system provides a natural contrast in internal nutrient levels, allowing us to ask whether hosts with elevated glucose experience worse outcomes when exposed to bacterial pathogens. We hypothesize that symbiotic *Aiptasia* with elevated internal glucose levels will experience a more severe response to pathogen challenge,

which may indicate a cost to symbiosis in these cnidarians. Results of this study can provide valuable knowledge on whether simple organisms without a circulatory system act in the same way as more complex organisms when experiencing heightened levels of glucose, and if increased infection rates are a universal biological phenomenon. Due to the role of symbiosis on infection rates, this study provides further information on the influence of symbionts on infection. This expands the limited knowledge we have on microbial processes and immune responses in relation to symbionts.

Methods:

2.1 Experimental Design

In total, we had forty-eight anemones, twenty-four were aposymbiotic, and twenty-four were symbiotic (Table 1). Half of each symbiotic state (24) were fed a high-glucose diet, while the others were fed the control, a brine shrimp *(Artemia salina)* diet. After seven days of glucose enrichment, half of the anemones in each feeding treatment were challenged with the *Pseudomonas aeruginosa* pathogen (6 anemones/symbiotic state/glucose treatment) to test whether excess glucose and symbiotic state influence infection severity and thus, survival in *Aiptasia*.

Table 1: Experimental groups of *Exaiptasia pallida* (strain H2) categorized by symbiotic state (symbiotic vs. aposymbiotic), infection state (control vs. infected), and diet (glucose-fed vs. control-fed). Group IDs reflect the combination of treatments applied to each experimental condition.

Symbiotic State	Group ID	Strain	Infection State	Diet
Symbiotic	CG	H2	Control	Glucose-Fed
	CC	H2	Control	Control-Fed
	TC	H2	Infected	Control-Fed
	TG	H2	Infected	Glucose-Fed
Aposymbiotic	CG	H2	Control	Glucose-Fed
	CC	H2	Control	Control-Fed
	TC	H2	Infected	Control-Fed
	TG	H2	Infected	Glucose-Fed

2.2 Pre-Infection Acclimation

Leading up to the infection of anemones, we conducted an acclimation period followed by a glucose-feeding period. After multiple trials, twenty-four symbiotic and twenty-four

aposymbiotic *Exaiptasia pallida* anemones were selected to be used in this experiment. The forty-eight anemones were placed in eight trays made up of six 60x15mm well plates. Anemones were placed in artificial seawater at a temperature of 18°C and maintained in an incubator with a 12-hour day and 12-hour night light and dark light cycle. For six days, anemones were fed freshly hatched brine shrimp (*Artemia salina*) every other day with water changes occurring thirty minutes after feeding. Prior to the glucose-feeding period, photographs of the anemones were taken. All photos taken during the experiment followed the same method using an Olympus Tough G-6 water-resistant camera on the microscope setting. Each anemone was positioned at the 10-centimeter mark on a ruler for scale, directly under a lamp at full light, and the camera lens was placed directly over the well. A full water change to artificial seawater was then conducted, and each well was wiped down with a Q-tip. The glucose feeding period lasted for seven days which half of the anemones were fed glucose. The specimens were maintained in the same temperature and light cycle conditions and were fed 20µL of 32% glucose brine shrimp made up of a solution of ½ homogenized brine shrimp and ½ 64% glucose. They were fed by using a pipette to slowly deposit the solution directly into the mouth. Similar to the acclimation period, water was changed thirty minutes after feeding.

2.3 *Anemone Susceptibility Trials*

The day prior to infection, we created *Pseudomonas aeruginosa* cultures to be used for the treatment group for susceptibility trials. The frozen *P. aeruginosa* cultures were preserved in ethanol and inoculated in 50mL of Luria broth for twenty-four hours. 15µL of these preserved pathogen cultures was transferred into three Erlenmeyer flasks, which were placed in a shaking incubator overnight at 37°C to allow the pathogen to replicate.

On the day of infection (day 0), we conducted post-glucose-feeding imaging, water changes, pre-infection imaging, PAM measurements, and pathogen infection. Post-glucose-feeding photographs of all individual anemones were taken using the same protocol described above for the pre-feeding photos. All wells then received a water change with 33ppt artificial seawater to remove any leftover glucose and food from the water before infection. Next, we conducted Pulse-Amplitude Modulated (PAM) fluorometry, which is a non-invasive technique used to assess the photosynthetic efficiency of photosystem II (PSII; F_v/F'_m). Prior to PAM measurements, anemones were dark-acclimated for one hour to ensure that all PSII reaction centers were fully open. For each individual, three F_v/F'_m readings were recorded and averaged. The PAM measurements quantify the maximum quantum yield of PSII, where F_0 represents the minimum fluorescence of all PSII centers open, F'_m represents the maximum fluorescence after a saturated pulse (when all PSII centers are closed), and F_v, variable fluorescence, equals $F'_m - F_0$. The resulting F_v/F'_m ratio reflects the photosynthetic efficiency of symbiotic algae, and healthy, symbiotic individuals typically show values around 0.6-0.7, whereas values near 0 indicate severely reduced or absent symbionts–consistent with expectations for aposymbiotic anemones. Pre-infection images were taken following the same

procedure as post-feeding photographs to be used as a day 0 reference for how the anemones' physiology responds to infection.

In preparation for infection, three 50mL cultures of *Pseudomonas aeruginosa* were centrifuged at 3300 xg for five minutes, the supernatant was then discarded, and the pellets were resuspended in seawater; this wash step was repeated twice. Final pellets were resuspended in 1mL volumes of seawater, and optical density (OD) readings were taken in triplicate for each sample on a Biotek Synergy H1M hybrid multimode microplate reader. The three preparations were then combined and diluted to 30mL, yielding an OD 600 of 3.0, and the final working culture measured to be an OD 600 of 9.4327. Each of the anemones that were being infected (*Section 2.1*) received 500µL of this pathogen suspension diluted into 9.5mL of artificial seawater (final OD ≈ 0.4716). Pathogen was gently mixed into each well by pipetting up and down about eight times to ensure homogenization.

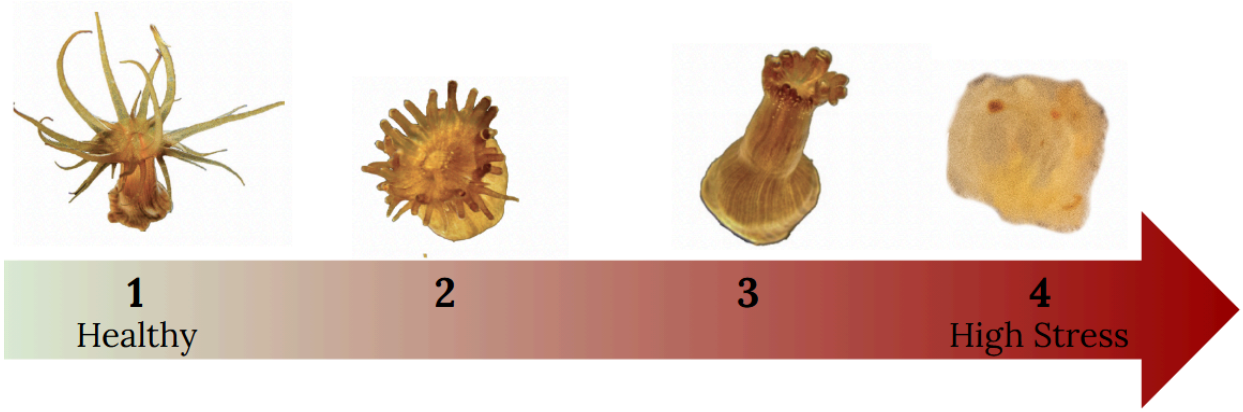

Figure 1: Phenotypic assessment of anemone stress from level 1 healthy to level 4 high stress. Created by Chloe Costa (2025).

2.4 Phenotypic Assessments Post-Infection

Using the same photography methods as those from pre-infection and Day 0, post-infection photos were taken daily in the morning to monitor the tentacle length of all 48 specimens. Using an open source image processing program, ImageJ, each photo was calibrated using the ruler in the background to set approximately 120px:1unit. Then, five of the longest tentacles were measured and averaged. Photos were also used to monitor the mortality of all specimens. Approximately seven hours after the photos were taken, phenotype was recorded using a numerical scale of 1-4 (Figure 1). 1 indicates that the anemone appeared healthy, represented by the full extension of tentacles and a color consistent with the specimen's symbiotic status. 2 signifies that the anemone is experiencing mild stress, manifested as the slight retraction of tentacles and the fading of the brown color in symbiotic anemones. 3 conveys moderate stress, the polyp becomes highly retracted, with a notable reduction in tentacle movement. Finally, 4 indicates high stress, which exhibits a strong discoloration in symbiotic

specimens and fully contracted tentacles, forming a rigid ball shape. Mortality was recorded using a binomial metric, where 1 designates that the anemone is dead, while 0 designates that the anemone is alive.

2.5 Statistical Analysis

All statistical analyses and visualizations were conducted in RStudio 2025.09 using the *tidyverse*, *ggplot2*, *survival*, *dplyr*, and *tidyr* packages. PAM values were summarized by treatment group and visualized using boxplots to compare average PAM measurements among groups. Phenotype scores were analyzed by calculating mean values and standard errors for each day, treatment group, and symbiotic state, and results were visualized using line plots with error bars, faceted by symbiotic condition. Differences in phenotype across treatment groups, symbiotic states, and time were assessed using factorial analyses of variance (ANOVA), with day, treatment group, and symbiotic state included as fixed effects; post hoc comparisons were used when appropriate to evaluate pairwise differences. Mortality data were analyzed as binomial outcomes, summarized as proportions dead per day with associated binomial standard errors, and compared among groups and symbiotic states using generalized linear models with a binomial error distribution and chi-squared tests. Average tentacle length was analyzed by calculating mean tentacle length ± standard error for each day, treatment group, and symbiotic state, and differences among groups were assessed using Tukey's HSD. For all analyses, assumptions of normality and homogeneity of variance were evaluated where applicable, and statistical significance was assessed at $\alpha = 0.05$.

Results:

3.1 Photosynthetic Efficiency ($F_v/F\square$; PAM fluorescence)

Average PAM fluorescence ($F_v/F\square$) did not differ significantly among treatment groups ($F_{3,20} = 0.09$, $p > 0.05$). While residuals showed slight deviation from normality (Shapiro-Wilk, $p = 0.026$), variances were the same across groups (Bartlett's test, $p = 0.83$), so an ANOVA was performed. A one-way ANOVA revealed no effect of treatment on $F_v/F\square$ values ($p > 0.005$). These data show consistent photosynthetic performance across all symbiotic anemones prior to experimental treatments.

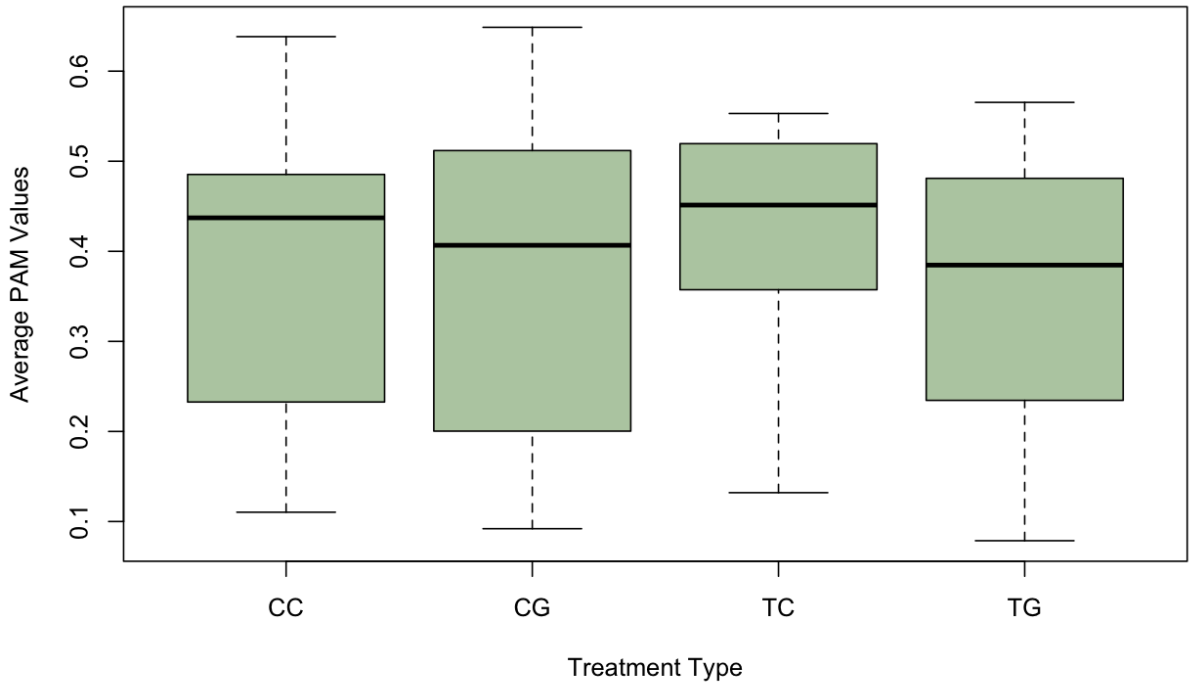

Figure 2: No differences in photosynthetic efficiencies across anemones at the start of experimentation. Mean photosynthetic efficiency of photosystem II (F_v/F□) measured via Pulse Amplitude Modulation (PAM) by treatment group prior to infection. The black line represents the mean, and the dashed lines represent the extent of the outlying data points.

3.2 Phenotype Scores

A repeated measure ANOVA revealed that phenotype differed significantly across treatment groups and changed over time between both aposymbiotic and symbiotic anemones (aposymbiotic: $F_{3,20} = 133.6$, $p < 0.001$, symbiotic: $F_{3,20} = 259.8$, $p < 0.001$). Overall stress is observed the most in the treatment symbiotic, glucose-fed anemones. Glucose feeding did not alter the phenotype of the control anemones, regardless of symbiotic state. Phenotype scores remained between 1-2 in all non-infected control groups, regardless of glucose feeding or symbiotic state. In contrast, anemones exposed to the pathogen exhibited a gradient from mild stress (phenotype 2) to severe stress (phenotype 4), as time went on (aposymbiotic: $F_{3,20} = 4.37$, $p = 0.016$, symbiotic: $F_{3,20} = 9.96$, $p < 0.001$). This transition occurred most rapidly amongst glucose-fed symbiotic anemones, while aposymbiotic treatment groups showed a more gradual gradient in stress phenotype.

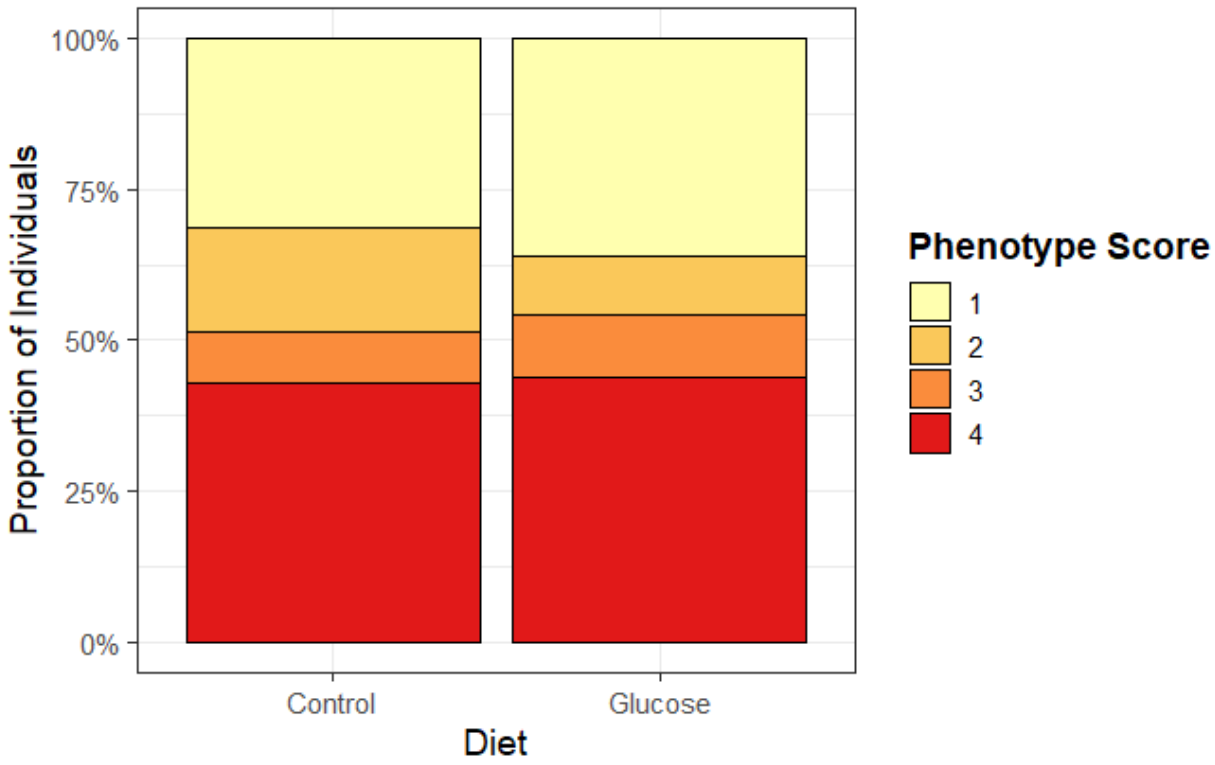

Figure 3: Stacked bar plot showing phenotype scores of aposymbiotic and symbiotic *Aiptasia* under 4 treatment levels across all experimental days. The phenotype is scored from 1-4, 1 being healthy and 4 being high stress (refer to Fig.1), across a period of 11 days. Results reveal that anemones present as phenotype 4 when exposed to a pathogen.

3.3 Mortality Analysis

A binomial generalized linear model revealed significant effects of day and treatment on mortality ($\chi^2_3 = 310.7$, $p < 0.001$), as well as a significant day-by-treatment interaction ($p = 0.00069$), which signifies that mortality rates increased over time, yet at different rates depending on treatment. Symbiotic state had no significant effect on mortality ($\chi^2_1 = 0.21$, $p = 0.65$), and did not interact with day or treatment group. Mortality was absent in the non-infected control groups throughout the entire experiment. Infected anemones began to show mortality within the first 5 days post-infection, with glucose-fed groups exhibiting a more rapid increase in mortality ($\chi^2_1 = 16.1$, $p < 0.001$). Symbiotic state did not significantly correlate with mortality, yet symbiotic anemones that were glucose-fed reached complete mortality faster than aposymbiotic treatment groups ($\chi^2_1 = 4.07$, $p = 0.044$). This indicates faster infection progression with elevated glucose levels.

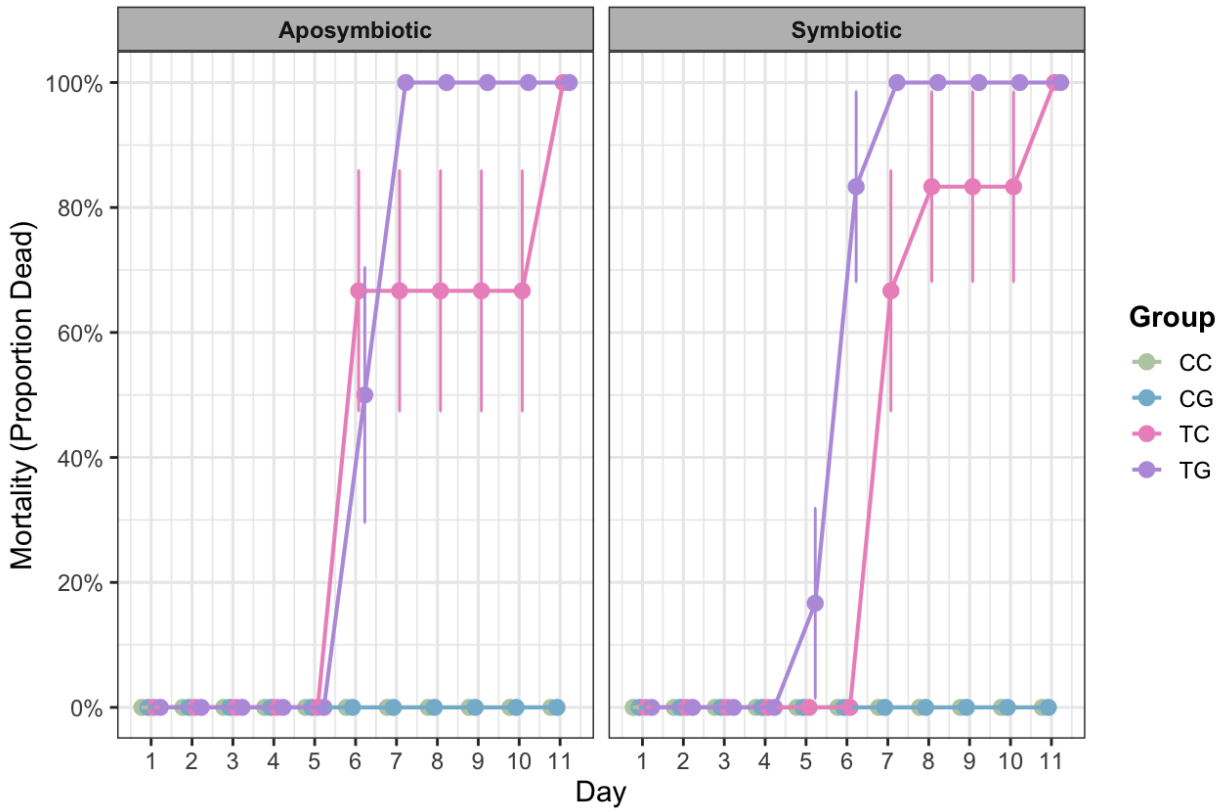

Figure 4: Proportion of *Aiptasia* mortality across treatment groups over time. Mortality was recorded as a binary response, alive = 0 and dead = 1. This was then summarized as the proportion of individuals who died per group across the 11 days of the experiment. No significant mortality occurred in control groups; however, pathogen-exposed anemones increased mortality over time, with glucose-fed anemones reaching complete mortality faster.

3.4 Average Tentacle Length

Average tentacle length differed significantly across treatment groups ($p < 0.001$) and changed over time ($p < 0.001$) between symbiotic states. On day 11, tentacle lengths were significantly shorter in anemones exposed to the pathogen regardless of treatment when comparing TC vs CC, and TG vs CC. Tentacle lengths were lower in pathogen-exposed treatment groups that were fed glucose (0.50mm ± 0.21), relative to the control (0.38mm ± 0.21), and glucose-fed groups exhibited quicker tentacle reductions over time; however, these trends were not significantly different ($p = 0.90$). Average tentacle length was reduced in all anemones treated with the pathogen, which reflects the immediate manifestation of tentacle retraction following infection. However, the control groups maintained stable tentacle lengths throughout the experiment. Glucose feeding did not significantly affect tentacle length; however, glucose-fed anemones showed more rapid and more pronounced tentacle retraction over time. All in all, up to day 11, tentacle length could either not be monitored due to dead infected anemones or was greatly reduced in infected groups that were still alive, compared to the control

group, which remains consistent with stress phenotypes observed. On day 11, all infected anemones were dead, and tentacle length could not be monitored.

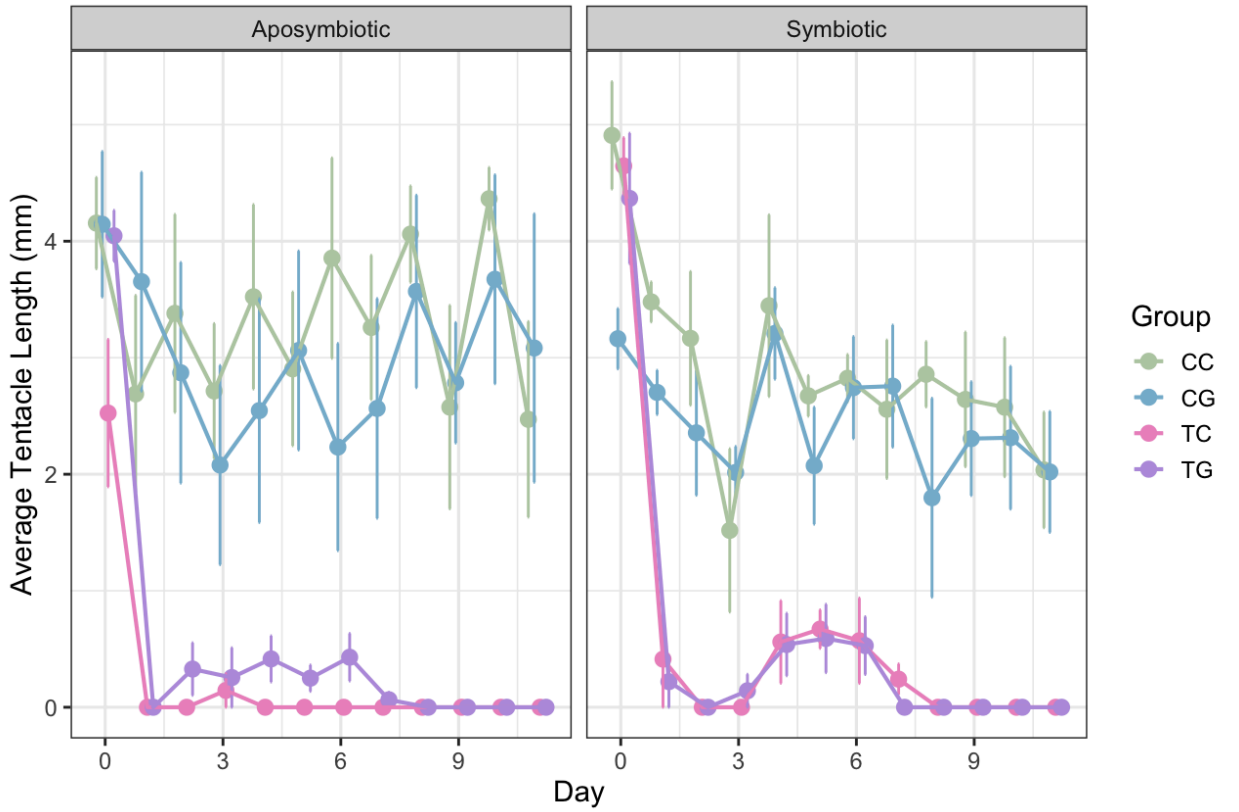

Figure 5: Changes in average tentacle length of aposymbiotic and symbiotic *Aiptasia* across treatments over time. Mean tentacle length (mm) was calculated by averaging the length of the five longest tentacles per individual. Values represent group means over time. Pathogen-exposed groups (TC and TG) displayed progressive tentacle retraction, while control groups (CC and CG) remained stable.

Discussion:

4.1 Interpretation of Results

This study analyzed how experimental treatment and symbiotic state influenced phenotype, tentacle growth, photosynthetic efficiency, and survival in *Aiptasia* over 11 days. The results of this study indicate that pathogen-exposed *Aiptasia* exhibit more phenotypic and morphological stress responses and mortality. The results also suggest that glucose could be a factor in increasing the rate of these effects, which is true in prior work on more complex systems that evaluate how glucose elevates the risk and rate of infection (Holt *et al.,* 2024 & Darweesh *et al.,* 2025). Both control groups, glucose-fed or non-glucose-fed, did not differ significantly throughout the experience and exhibited stable results in photosynthetic efficiency (Fig. 2) and across each test variable (Figs. 3-5), indicating that excess glucose alone does not have a large effect on *Aiptasia* in either symbiotic state.

` Anemones in the treatment groups died faster than those not infected with the pathogen, as expected. The results also suggest that diet influences anemone survival, as anemones that were fed glucose died faster as well. In terms of response between symbiotic states, a significant difference was found, with glucose-fed symbiotic anemones experiencing faster mortality than non-glucose-fed symbiotic anemones (Fig 4). As symbiotic *Aiptasia* produce their own glucose through symbiotic processes (Molina *et al.,* 2017), these results support that there is a cost to receiving an excess amount of glucose in symbiotic anemones. Furthermore, aposymbiotic anemones do not experience as many of these effects, as evidenced by the less pronounced difference between the treatment control and the treatment glucose. These results provide insight into host-symbiont interactions and how exposure to glucose can negatively impact these interactions in symbiotic specimens by hindering the survival of these anemones when infected with a pathogen.

The phenotype results suggest that anemones exposed to pathogens will eventually exhibit phenotype level 4, meaning they will present as balled up and severely stressed, which is consistent with our expected results. These effects were found to present the fastest in the control-fed pathogen-exposed aposymbiotic group (Fig. 3). This suggests that glucose may have been an initial buffer against the pathogen, as the glucose-fed aposymbiotic treatment group experienced a more gradual progression into the unhealthy phenotypes. The shift into level 4 for symbiotic anemones took slightly longer, which indicates that the presence of symbionts may also initially buffer the effects of the pathogen. However, the symbiotic anemones that were fed glucose and exposed to treatment exhibited a faster shift in phenotypic level than the symbiotic treatment control group that was not fed glucose. This suggests that the extra glucose in glucose-fed symbiotic anemones is more susceptible to the effects of the pathogen.

The tentacle analysis suggests similar stress response patterns to pathogen exposure and glucose. There was a significant difference in tentacle length between groups. Anemones exposed to the pathogen showed an immediate retraction of tentacles. This stress response after exposure to bacterial infection has been observed in the Aiptasia species *Exaiptasia diaphana* (Billaud & Czerucka, 2025). For symbiotic TG anemones, tentacle length showed a slight increase between days 2 to 6, and for aposymbiotic anemones in both the TC and TG groups, a slight increase was found between days 3 to 6 (Fig. 5). Although our results did not show that glucose alone had a significant impact on the outcome of tentacle length, the more rapid retraction of tentacles that were observed in glucose-fed anemones exhibits the stress responses that would be expected. Eventually, all pathogen-treated anemones experienced full tentacle retraction as consistent with our phenotype results.

This study reveals how treatment of a pathogen directly impacts anemone mortality, yet future research could aim to further analyze the physiological processes, such as symbiont density, to better understand the fluctuating phenotype presentations. The duration of our experiment may have limited the examination of the surviving anemones. The study also provides significant insight into how glucose specifically influences host-symbiont interactions when hosts are exposed to pathogens. Our results indicate trends in symbiotic anemones

experiencing more rapid mortality when fed extra glucose, and while not significant, trends in tentacle length and phenotype response also suggested that the presence of glucose negatively influenced these data.

4.2 Comparison of Other Studies

Our findings align with and extend previous research demonstrating that elevated glucose availability can exacerbate infection severity across diverse biological systems. In mammals, chronic hyperglycemia is well known to impair immune defenses while simultaneously enhancing pathogen proliferation, resulting in worsened infection outcomes in individuals with diabetes (Ingels *et al*., 2017; Holt *et al.,* 2024). Although *Aiptasia* lacks a closed circulatory system and specialized immune cells, the accelerated mortality observed in glucose-fed symbiotic anemones suggests that glucose-mediated enhancement of infection susceptibility may be a deeply conserved phenomenon across animal lineages. This supports the idea that basic metabolic conditions alone, independent of circulatory complexity, can strongly influence host-pathogen dynamics.

Within cnidarian systems, previous studies have shown that symbiotic Aiptasia exhibit higher baseline glucose levels due to photosynthate transfer from algae in the family Symbiodiniaceae (Molina *et al.,* 2017), as well as increased bacterial density and altered microbial community composition relative to aposymbiotic individuals (Costa *et al.,* 2021). Our results build upon this work by directly linking nutrient availability to infection outcomes, showing that symbiotic state and exogenous glucose supplementation both accelerate pathogen-induced mortality. While Costa *et al.,* (2021) demonstrated differences in microbial abundance between symbiotic states, our study is among the first to show that these metabolic and microbial differences translate into heightened vulnerability during an active bacterial challenge.

Interestingly, photosynthetic efficiency ($F_v/F\square$) did not differ among treatment groups prior to infection, indicating that differences in infection progression were not driven by initial variation in symbiont health or density. This suggests that glucose availability itself, rather than symbiont performance, plays a dominant role in shaping infection severity. Similar findings have been observed in other host–microbe systems, where excess nutrients disproportionately benefit pathogens relative to host immune responses (Darweesh *et al.*, 2025). Together, these comparisons position *Aiptasia* as a powerful comparative model for investigating how nutrient-rich internal environments can favor pathogen success, even in basal metazoans.

4.3 Implications for Future Studies

This study highlights several important directions for future research on nutrient-mediated host–pathogen interactions. First, direct quantification of internal glucose concentrations and symbiont densities throughout infection would allow for a more mechanistic understanding of how glucose availability shifts during pathogen exposure. Measuring host metabolic markers, immune-related gene expression, and bacterial load could help disentangle

whether glucose primarily enhances pathogen growth, suppresses host defenses, or both. Extending experimental durations may also reveal whether any individuals are capable of recovery following infection and whether glucose influences long-term resilience rather than only acute mortality.

Additionally, future studies could explore whether different types of nutrients (e.g., lipids or amino acids) similarly alter infection outcomes, or whether glucose uniquely exacerbates disease severity. Given that nutrient enrichment is common in coastal environments due to runoff and eutrophication, understanding how external nutrient availability interacts with symbiosis and disease could have important ecological implications for cnidarians and coral reef systems. Investigating whether glucose-driven susceptibility extends to other pathogens, including *Vibrio* species that are ecologically relevant to corals, would further strengthen the relevance of this model.

More broadly, our results suggest that *Aiptasia* can serve as a simplified experimental system for studying metabolic regulation of immunity. Because *Aiptasia* naturally transitions between symbiotic and aposymbiotic states, it offers a rare opportunity to manipulate internal nutrient availability without genetic or pharmacological intervention. This makes it a valuable comparative platform for examining whether principles observed in human metabolic diseases, such as diabetes-associated infection risk, reflect universal biological trade-offs between energy availability and immune defense. By leveraging this model, future work can bridge marine biology, immunology, and metabolic research to uncover evolutionarily conserved links between nutrition and disease susceptibility.

4.4 Experimental Design Flaws

While this study demonstrates clear patterns linking glucose availability to increased infection severity in *Aiptasia*, several aspects of the experimental design limit the mechanistic resolution of our conclusions. Most notably, internal glucose concentrations were not directly quantified in either symbiotic or aposymbiotic anemones throughout the experiment. As a result, glucose availability was inferred based on symbiotic state and dietary treatment rather than measured directly. Although prior studies have established that symbiotic *Aiptasia* possess higher internal glucose levels (Molina *et al.*, 2017), direct measurements would strengthen causal links between glucose concentration and infection outcomes.

Similarly, symbiont density was assessed indirectly using photosynthetic efficiency ($F_v/F\square$), which reflects algal performance rather than absolute symbiont abundance. While PAM data indicated comparable photosynthetic health across groups at the start of the experiment, it does not rule out subtle differences in symbiont density that could influence internal nutrient flux. Direct quantification of symbiont cells would allow for clearer separation of glucose-driven effects from symbiont-related physiological variation.

Another limitation is the absence of direct measurements of pathogen load and host immune activity. Mortality, phenotype scores, and tentacle retraction provide strong indicators of disease severity, but they do not distinguish whether elevated glucose primarily enhanced bacterial proliferation, impaired host defenses, or both. Quantifying bacterial abundance over

time, along with host immune markers such as antimicrobial gene expression or oxidative stress responses, would allow for more precise interpretation of the underlying mechanisms driving accelerated mortality.

Sample size also constrained statistical power, particularly for detecting interactions involving the symbiotic state. Although clear trends were observed - especially faster mortality in glucose-fed symbiotic anemones - some effects did not reach statistical significance, which may reflect limited replication rather than biological absence of effect. Increasing replicate numbers would improve the ability to resolve these interactions and reduce variability associated with individual physiological differences.

Finally, the experiment was conducted over an acute time scale and at a single pathogen dose. Because all infected individuals ultimately died, it was not possible to assess whether glucose influences recovery, tolerance, or long-term resilience following infection. Varying pathogen dose and extending experimental duration could reveal threshold effects or survivorship differences that were not captured here.

Despite these limitations, the experimental design successfully isolated glucose availability and symbiotic state as key variables shaping infection outcomes. The observed patterns provide a strong foundation for future work that incorporates direct metabolic, microbial, and immunological measurements to more fully resolve the mechanisms underlying glucose-mediated susceptibility in basal metazoans.

References:

Baumgarten, S., Simakov, O., Esherick, L. Y., Liew, Y. J., Lehnert, E. M., Michell, C. T., & Aranda, M. (2015). The genome of Aiptasia, a sea anemone model for coral symbiosis. *Proceedings of the National Academy of Sciences, 112*(38), 11893-11898. https://doi.org/10.1073/pnas.1513318112

Beredo, S. J., Bosacoma, E. K., Martorana, S. I., Sanchez, E. A., Davies, S. W., & Da-Anoy, J. K. (2025). The microbiome strikes back: How Aiptasia pallida combats environmental stress. *Boston University Marine Program and Department of Biology.* https://sites.bu.edu/davieslab/files/2024/11/Aiptasia_Manuscript_2024.pdf

Billaud, M., & Czerucka, D. (2025). Exploring different methods of Exaiptasia diaphana infection to follow Vibrio parahaemolyticus dissemination in the whole animal. BMC Microbiology, 25(1), 83. https://doi.org/10.1186/s12866-025-03744-8

Costa, R. *et al*. (2021). Surface topography, bacterial carrying capacity, and the prospect of microbiome manipulation in the sea anemone coral model *Aiptasia*. *Frontiers in Microbiology, 12*, 637834. https://doi.org/10.3389/fmicb.2021.637834

Cui, G. et al. (2023). Molecular insights into the Darwin paradox of coral reefs from the sea anemone Aiptasia. *Sci. Adv.* 9, eadf7108. https://www.science.org/doi/10.1126/sciadv.adf7108

Darweesh, M., Mohammadi, S., Rahmati, M., Al-Hamadani, M., & Al-Harrasi, A. (2025). Metabolic reprogramming in viral infections: the interplay of glucose metabolism and immune responses. *Frontiers in immunology*, *16*, 1578202. https://doi.org/10.3389/fimmu.2025.1578202

Holt, R.I.G., Cockram, C.S., Ma, R.C.W., Luk, A. O. Y. (2024). Diabetes and infection: review of the epidemiology, mechanisms and principles of treatment. *Diabetologia,* 67, 1168–1180. https://doi.org/10.1007/s00125-024-06102-x.

Davy, S., Cook, C. (2001). The relationship between nutritional status and carbon flux in the zooxanthellate sea anemone *Aiptasia pallida*. *Marine Biology* 139, 999–1005. https://doi.org/10.1007/s002270100640

Lehnert, E.M., Burriesci, M.S. & Pringle, J.R. (2012). Developing the anemone *Aiptasia* as a tractable model for cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic *A. pallida*. *BMC Genomics 13*, 271. https://doi.org/10.1186/1471-2164-13-271

Molina, V. H., Castillo-Medina, R. E., & Thomé, P. E. (2017). Experimentally induced bleaching in the sea anemone *Exaiptasia* supports glucose as a main metabolite associated with its symbiosis. *Journal of Marine Sciences*, 3130723. https://doi.org/10.1155/2017/3130723.

Ingels, C., Vanhorebeek, I., Van den Berghe, G. (2017). Glucose homeostasis, nutrition, and infections during critical illness. *Clinical Microbiology and Infection*, 24, 1. https://doi.org/10.1016/j.cmi.2016.12.033.

Pale anemone (*Aiptasia padilla). iNaturalist.* https://www.inaturalist.org/taxa/132539-Aiptasia-pallida

Poole, A. Z., Kitchen, S. A., & Weis, V. M. (2016). The role of complement in cnidarian–dinoflagellate symbiosis and immune challenge in the sea anemone *Aiptasia pallida*. *Frontiers in Microbiology, 7*, 519. https://doi.org/10.3389/fmicb.2016.00519.

Valadez-Ingersoll, M., Aguirre Carrión, P. J., Bodnar, C. A., Desai, N. A., Gilmore, T. D., & Davies, S. W. (2024). Starvation differentially affects gene expression, immunity, and pathogen susceptibility across symbiotic states in a model cnidarian. *Proceedings of the Royal Society B: Biological Sciences, 291*, 2017, 20231685. https://doi.org/10.1098/rspb.2023.1685.