# Effects of Nicotinamide Riboside and NAD+ on Photosynthetic Efficiency of *Aiptasia pallida* under Heat Stress


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Global coral reefs are among the most delicate and important ecosystems in the oceans. Due to the rapidly changing climatic conditions, especially the rise in shallow ocean water temperature, this ecosystem is severely threatened by coral bleaching. Finding solutions to prevent the global demise of corals is of the utmost urgency.

## 1. Introduction

The core issue:

The specific problem addressed in this research is the lack of data on whether nicotinamide riboside, a NAD+ precursor known to activate protective sirtuin proteins in other organisms, can stabilize photosynthetic efficiency in coral model organisms under thermal stress. While NR has demonstrated protective effects against cellular aging and stress in mammalian studies, its potential application in marine invertebrates, particularly in preserving coral-algae symbiosis, remains unexplored. This knowledge gap limits the development of innovative conservation strategies that could enhance coral resilience through targeted cellular protection mechanisms. Current conservation efforts, although helpful, lack in addressing the scale and pace of coral reef decline. Traditional approaches focus primarily on reducing local stressors, planting healthy coral, and establishing marine protected areas (MPAs), but these strategies do not directly address the cellular and molecular mechanisms connected to thermal stress responses in corals.

### *Why Aiptasia pallida?*

Aiptasia pallida is a species of sea anemone. They are originally brown or orange in their normal condition, but can be white when bleached. Aiptasia size ranges from 2.5 cm to 5 cm when growing into an adult. In addition, they hunt for prey using their 100 tentacles, which contain a long, thin column and narrow rings around it. Normally, these species live in mangrove roots, dead coral, and rocks. Anemones, including Aiptasia, have life spans that vary depending on their regeneration mechanisms and the ocean's conditions. They are widely used as model organisms in coral research.

## 2. Research Scope

This research project examined the protective potential of nicotinamide riboside (NR) on stabilizing the photosynthetic efficiency of Aiptasia pallida under normal temperature conditions and under thermal stress.

## 3. Materials & Methodology

This experiment was conducted through three experimental phases. The first experimental phase focused on NR effects under normal conditions of maturing and mature specimens. The second phase focused on NR effects under thermal stress in adults. The third phase of the experiment focuses on RNA sequencing of the treated Aiptasia individuals to detect Sirtuin-1 activation, with results pending.

- **Two Experimental + Two Control Tanks**
- **Two Weeks**
- **7x TRU NIAGEN Application**
- **33.0 ± 0.5°C vs. 26.5 ± 0.5°C**
- **PAM Fluorometry [Y(II) of photosystem II]**

*Materials:*

4x 10-gallon tank, 40x perforated petri dishes, 100 wild mature *Aiptasia pallida* individuals, sterile scalpel, 70% ethyl alcohol, 60 glass slides, Laboratory film (Amcor Parafilm PM992, 5.08 cm), Marineland Bio-Wheel Penguin 150 Power Filters, API Marine Reef Master Test Kit, Milwaukee MR100ATC automatic temperature compensation refractometer, UV aquarium lights, liquid coral feed (Seachem, ZooPlankton™, 12.5-500 µm, NR tablet (TRU™, Niagen 100 mg), Pulse Amplitude Modulation (PAM) fluorometer, Life Extension®, NAD+ Cell RegeneratorTM, 100 mg), RNAlater™ RNA Stabilization Solution (Sigma Life Science, Product R0901-500ML, Lot# MKCT6402).

## 4. Results

PHASE 1:

**Non-adults**

Fo/Fm in non-adult Aiptasia (**t =** 0.6835, df = 25.12, p = 0.5006, mean difference = 0.0336). Cohen's d=0.2291, α = 0.05 power= 0.101, power=10.1%, power= 80% (W = 166, p = 0.6799, location shift = 0.009, rank biserial correlation = -0.085). (median exp. = 0.692, median contr. = 0.6775), p-value = 0.6799, Cohen's d= 0.1706. α=0.05 p=0.078, p=7.8%, p= 80%.

**Adults**

Fo/Fm in adult aiptasia (t = -1.1178, df = 4.01, p = 0.3261, mean difference = -0.0398). Cohen's d equaling (-0.8064). α = 0.05 was p= 0.138, =13.8%, p=80%. (t = 1.5378, df = 4.54, p = 0.1905, mean difference = 0.0993). Cohen's d= 1.1719. α = 0.05 was p= 0.238 =23.8%, p=80%.

PHASE 2:

**Adults**

We found that NR significantly preserves Y(II) in heat-stressed coral organisms compared to controls.

**Two-way ANOVA**

(p = 0.1247 ), (p = 0.00423 ) Tukey's HSD showed that NR-supplemented corals =33°C Y(II) is greater than control corals at 33°C.

**Chi-Square Tests**

Treatment/bleaching X-squared = 10.334, p = 0.001306, temperature/bleaching X-squared = 19.158, p = 1.203e-05

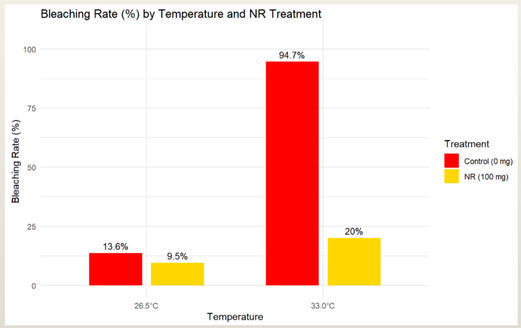
**Logistical Regression**

(p = 0.01266 | OR = 0.0208) interaction temperature/NR supplementation.

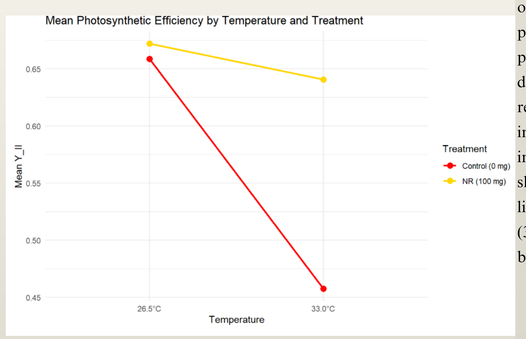
## 5. Data Analysis

STATISTICAL TESTS:

**Assumption Testing:** Shapiro-Wilk test for normality; Levene's test for homogeneity of variance.
**Two-way ANOVA**: Tested the main effects and interaction on Y(II) (excluding zeros).
**Kruskal-Wallis Test**: Non-parametric sensitivity check.
**Post-hoc Tukey's HSD**: Pairwise comparisons when significant effects were found.
**Chi-Square Tests**: Compared bleaching rates by treatment and temperature.
**Logistic Regression**: Tested the interaction effect on bleaching probability.




***This bar chart shows the percentage bleaching rate as a function of temperature and treatment. It is evident that severe bleaching has occurred in the control group under heat-stressed conditions without NR treatment, compared with the heat-stressed and NR-treated groups.***




***The interaction plot shows how the untreated group declined more steeply than the treated group as the temperature increased. It shows the significant benefits of NR in retaining photosynthetic efficiency under heat-stressed conditions.***

## 6. Conclusion

This research investigated the protective potential of 100 mg NR supplementation in stabilizing photosynthetic efficiency in coral under normal and extreme conditions as a possible intervention to coral bleaching due to climate change. The project addressed a critical knowledge gap regarding coral supplementation research and NAD+ metabolism research, extending knowledge of mammalian cellular protection mechanisms to cnidarian organisms. It also contributed new knowledge that may be used to save depleting coral reefs, improve future conservation strategies, and guide future coral research projects. Through phase one of the experiment, we discovered that while NR does not affect photosynthetic efficiency under optimal conditions, it is a biologically safe pharmaceutical to use on coral model organisms. In phase two, the Aiptasia data showed that corals at 33.0°C had significantly lower photosynthetic efficiency than corals at 26.5°C. We also found that while NR supplementation did not alter Y(II) under ambient temperature, the significant interaction effect (p = 0.00423 ) revealed that it protects photosynthetic efficiency under thermal stress. There is a significant interaction between temperature and NR treatment. Both the (p = 0.00423 ) of the ANOVA interaction and logistic regression interaction (p = 0.01266 ) were significant. These results show that the protective effect of NR treatment is temperature-dependent, meaning it provides little benefit at (26.5°C), but provides significant protection to corals under thermal stress (33.0°C). Additionally, NR treatment substantially reduces the temperature increase in bleaching odds.


Reference: National Oceanic and Atmospheric Administration [NOAA]. (2025c, July 21). Restoring Coral Reefs. NOAA Fisheries. https://www.fisheries.noaa.gov/national/habitat conservation/restoring-coral-reefs
Sharma, C., Donu, D., & Cen, Y. (2022). Emerging role of nicotinamide riboside in health and diseases. Nutrients, 14(19), 3889. https://doi.org/10.3390/nu14193889
Vaughn, C. (n.d.). Aiptasia pallida (pale anemone). Animal Diversity Web. https://animaldiversity.org/accounts/Aiptasia_pallida/
Vreones, M., Mustapic, M., Moaddel, R., Pucha, K. A., Lovett, J., Seals, D. R., Kapogiannis, D., & Martens, C. R. (2023). Oral nicotinamide riboside raises NAD+ and lowers biomarkers of neurodegenerative pathology in plasma extracellular vesicles enriched for neuronal origin. Aging Cell, 22(1), 1–7. https://doi-org.mylibrary.wilmu.edu/10.1111/acel.13754