# Nutrient transfer experiments with host coral and symbionts under varying environmental conditions conducted March 2014 and March 2015

**Website**: https://www.bco-dmo.org/dataset/907003
**Data Type**: Other Field Results, experimental
**Version**: 1
**Version Date**: 2023-08-29

**Project**
» Collaborative Research: Stability, flexibility, and functionality of thermally tolerant coral symbioses (Thermally tolerant coral)


| Contributors | Affiliation | Role |
|---|---|---|
| Kemp, Dustin | University of Alabama at Birmingham (UA/Birmingham) | Principal Investigator |
| Gerlach, Dana Stuart | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |


**Abstract**
Symbiotic mutualisms are essential to ecosystems and numerous species across the tree of life. For reef-building corals, the benefits of their association with endosymbiotic dinoflagellates differ within and across taxa, and nutrient exchange between these partners is influenced by environmental conditions. Furthermore, it is widely assumed that corals associated with symbionts in the genus Durusdinium tolerate high thermal stress at the expense of lower nutrient exchange to support coral growth. We traced both inorganic carbon ($H^{13}CO_3^-$) and nitrate ($^{15}NO_3^-$) uptake by divergent symbiont species and quantified nutrient transfer to the host coral under normal temperatures as well as in colonies exposed to high thermal stress. Colonies representative of diverse coral taxa associated with Durusdinium trenchii or Cladocopium spp. exhibited similar nutrient exchange under ambient conditions. In contrast, heat-exposed colonies with D. trenchii experienced less physiological stress than conspecifics with Cladocopium spp. while high carbon assimilation and host transfer was maintained. This discovery is different from the prevailing notion that these mutualisms inevitably suffer trade-offs in physiological performance. These findings emphasize that certain host-symbiont combinations adapted to high temperature equatorial environments; and why their increase in prevalence is likely important to the future productivity and stability of coral reef ecosystems.

## Table of Contents


- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
  - BCO-DMO Curation Notes
- Data Files
- Related Publications
- Parameters
- Instruments
- Project Information
- Funding


## Coverage

**Spatial Extent**: **N**:7.3245 **E**:134.4939 **S**:7.248833 **W**:134.235817
**Temporal Extent**: 2014-03-01 - 2015-03-01

### Methods & Sampling

Corals from Rebotel Reef on the western barrier reef of Palau were collected for offshore samples, while nearshore corals were collected in Nikko Bay approximately 28 km away. The corals *Acropora*

*muricata* and *Coelastrea aspera* were sampled in March of 2014 from both locations and used in the initial thermal experiments. Two additional coral species, *Pachyseris rugosa*, and *Cyphastrea chalcidicum*, were sampled from the same locations and treated the same way in March of 2015. A total of 8 colonies of each species were collected using a hammer and chisel at a depth of 5–10 meters (offshore) or 1–5 meters (nearshore) to ensure similar irradiance conditions, and each colony was sampled a minimum distance of 10 meters from surrounding colonies to better ensure sampling of unique coral genets. Despite the thermal experiments being conducted in multiple years (2014 and 2015), the thermal histories and light levels indicate similar conditions during this time period and allowed physiological comparisons across host species and population origin (see Hoadley et al. 2021). Colonies were transported to the Palau International Coral Research Center (PICRC) and fragmented into replicate pieces (clone ramets) and placed into 1200 L flow-through aquariums supplied with natural seawater and held at 27.5°C. Corals were allowed to heal for a minimum of 2 days and were then placed on individual 5 cm square PVC tiles with marine epoxy (splash zone compound A-788) and returned to the holding aquariums for 12 – 16 days to recover before the start of the experiment.

For each treatment, two replicate fragments from each coral colony were placed in separate treatment bins. In the heated treatment, the temperature was gradually increased from 27.5°C to 32°C over 4 days, and then maintained at 32°C for an additional 10 days, totaling 14 days of heating. The control treatment was kept at a constant temperature of 27.5°C throughout the 14-day experiment. All the experimental coral fragments were kept outdoors, and covered by non-UV filtering clear plastic film (Sun Selector, Ginegar Plastic Products) to protect them from periodic rainfall. Additionally, a 60% shade cloth was used to provide a peak midday light intensity of 800 µmol quanta $m^{-2}s^{-1}$, measured with a PAR sensor (LiCor LI-192), similar to the maximum light levels of natal colony habitats at collection depth.

At the beginning of the experiment (day 0), one fragment from each coral colony (if available; n=4-8) was removed, and $^{13}C$ and $^{15}N$ isotope measurements of unlabeled colonies were made for enrichment comparison. On day 14 (4 days of temperature ramping and 10 days at 32°C), coral fragments were removed from treatments and processed the same as day 0.

A pulse amplitude modulation fluorometer (Diving PAM, Waltz, Germany) was used to measure the maximum quantum yield of photosystem II (PSII, Fv/Fm) one hour after sunset in three separate locations using a 0.6 second saturation pulse (saturation intensity > 1000 mmol quanta m-2s-1). Three intracolony Fv/Fm measurements were averaged together to calculate the mean Fv/Fm for each fragment.

Coral tissue was removed using an airbrush (100 psi) and filtered (0.22 µm) seawater. The resulting slurry, containing coral tissue and symbiotic dinoflagellates, was homogenized for approximately 10 seconds using a Tissue Tearor (BioSpec Products, Bartlesville, OK, USA). Aliquots (1 ml) were taken from the homogenized slurry and preserved with 1% glutaraldehyde for symbiotic algal enumeration. Algal densities were quantified using an EVOS digital fluorescent microscope from 4–6 replicate haemocytometer counts (AO Spencer Bright Line Improved Neubauer haemocytometer) and normalized to coral surface area using the aluminum foil method (Marsh 1970) for *C. aspera, C. chalcidicum*, and *P. rugosa*, and the hot wax method (Stimson and Kinzie 1991) for the branching coral *A. muricata*. The influence of thermal treatments (32°C) on areal symbiotic dinoflagellate densities were compared to clone fragments at the control temperature (28°C).

On day 14, control and treatment fragments were placed into glass beakers containing 400 ml of freshly filtered seawater (0.45 µm) that was enriched with 0.633 mM of $NaH^{13}CO_3$ (99 atom % 13C, Cambridge Isotope Lab Inc., Andover, MA, USA), and 1.5 µM of $Na^{15}NO_3^-$ (98 atom % $^{15}N$, Cambridge Isotope Lab Inc., Andover, MA, USA). The beakers were fitted with false bottoms and continually stirred with magnetic stir bars. All beakers were held constant at the experimental temperatures for 5 h (28°C or 32°C) and illuminated by LED lights (Cree Cool White XP-G R5) set to a light intensity of 500 µmol quanta $m^{-2}s^{-1}$. Preliminary measurements determined this irradiance level was sufficient to maximize photosynthesis (Pmax) and the $H^{13}CO_3$ and $^{15}NO_3^-$ concentrations were sufficient to be used for elemental tracing across the biological compartments. After isotopic labeling, the fragments were removed, rinsed in filtered seawater, and immediately frozen at -60°C. The impact of symbiotic dinoflagellates on the uptake and assimilation of $^{13}C$ and $^{15}N$ across biological compartments (symbiotic dinoflagellates, coral tissue, and coral skeleton) was assessed by comparing colonies containing *D. trenchii* with colonies containing *Cladocopium* spp. at a temperature of 28°C and the influence of thermal treatments (32°C) on $^{13}C$ and $^{15}N$ uptake and assimilation was compared.

Coral tissue was removed with an airbrush as previously described, followed by the addition of 0.02% (w/v) sodium dodecyl sulfate (SDS) and homogenization for 10 s with a Tissue-Tearor (Biospec Products, Inc). Symbiotic dinoflagellates and coral tissue were separated by 2–3 centrifugation washes (550 *g* for 5 min) with

10 second homogenization between each wash (Lesser and Shick 1989). Algal fractions were microscopically verified to ensure the efficiency of the separation technique and to confirm the homogeneity and removal of the bulk animal material (Tremblay et al. 2012). Clean algal cells were pelleted via centrifugation (5,000 *g* for 5 min) and frozen at -20°C until analyzed. Accumulated supernatants (animal portion) were microscopically verified to not contain symbiotic dinoflagellates or skeletal material and were filtered onto pre-combusted (450° C for 5h) glass 0.7 mm filters (Whatman GF/F) until clogged and frozen at -20°C.

Due to the relatively high concentration of $^{13}C$ assimilation by the symbiotic dinoflagellates during incubations, coral skeletons were placed in 100% bleach for 24 hours to remove any remnant organic material from host-algal tissue, rinsed in freshwater for 24 hours, and dried under low heat. Approximately 20 mg of $CaCO_3$ was sampled from the corallite and coenosarc regions of the coral skeleton using a Dremel tool with a diamond bit. Skeletal samples were stored at -20°C until analyzed. Elemental $^{13}C$ and $^{15}N$ analyses were performed on a Carlo Erba CHN Elemental Analyzer (Model NA1500) coupled to Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer via a Thermo Finnigan Conflo III Interface at the University of Georgia, Center for Applied Isotope Studies.

#### Data Processing Description

Enriched isotopic data are reported as atom % of the heavy isotope (AP13C & AP15N).

#### BCO-DMO Curation Notes

- Imported data from source file "Tracer_metadata.csv" into BCO-DMO system
- Added columns for latitude and longitude based on reef site locations
- Converted temperature column to numerical (removed letter C)
- Modified parameter (column) names to conform with BCO-DMO naming conventions
- Checked taxonomic names in the dataset with the World Register of Marine Species (WoRMS) taxa match tool. All names matched accepted names exactly as of 2023-08-29

[ table of contents | back to top ]

## Data Files

| File | |
|---|---|
| **907003_v1_nutrient_transfer_tracers.csv** | (Comma Separated Values (.csv), 29.55 KB) MD5:7227361ad5d286d780b9e8cd634d0fab |
| Primary data file for dataset 907003 | |

[ table of contents | back to top ]

## Related Publications

Hoadley, K. D., Pettay, Daniel. T., Lewis, A., Wham, D., Grasso, C., Smith, R., Kemp, D. W., LaJeunesse, T., & Warner, M. E. (2021). Different functional traits among closely related algal symbionts dictate stress endurance for vital Indo-Pacific reef-building corals. Global Change Biology, 27(20), 5295–5309. Portico. https://doi.org/10.1111/gcb.15799
*Methods*

Kemp, D.W., Hoadley, K.D., Lewis, A.M., Wham, F., Smith R.T., Warner, M.E., LaJeunesse T.C. Thermotolerant coral-algal mutualisms maintain high rates of nutrient transfer while exposed to heat stress. Proceedings of the Royal Society, B. (Accepted).
*Results*

Lesser, M. P., & Shick, J. M. (1989). Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of Aiptasia pallida: primary production, photoinhibition, and enzymic defenses against oxygen

toxicity. Marine Biology, 102(2), 243–255. https://doi.org/10.1007/bf00428286
https://doi.org/10.1007/BF00428286
*Methods*

Marsh, J. A. (1970). Primary Productivity of Reef-Building Calcareous Red Algae. Ecology, 51(2), 255–263. doi:10.2307/1933661
*Methods*

Stimson, J., & Kinzie, R. A. (1991). The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions. Journal of Experimental Marine Biology and Ecology, 153(1), 63–74. doi:10.1016/s0022-0981(05)80006-1
https://doi.org/10.1016/S0022-0981(05)80006-1
*Methods*

Tremblay, P., Grover, R., Maguer, J. F., Legendre, L., & Ferrier-Pagès, C. (2012). Autotrophic carbon budget in coral tissue: a new 13C-based model of photosynthate translocation. Journal of Experimental Biology, 215(8), 1384–1393. https://doi.org/10.1242/jeb.065201
*Methods*

[ table of contents | back to top ]

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Latitude | Latitude of the sampled reef | decimal degrees |
| Longitude | Longitude of the sampled reef | decimal degrees |
| Site | Site location (NR=Nearshore Reef, OR=Offshore Reef) | unitless |
| Species | Genus and species of collected coral colony sample | unitless |
| Symbiont | Genus and species of symbiotic dinoflagellate | unitless |
| Temperature | Temperature (control = 28°C, treatment = 32°C) | degrees Celsius |
| Replicate | Colony (ramet) identification | unitless |
| Fraction | Biological compartment measured (symbiont, host, or skeleton) | unitless |
| AP13C | Atom percent of 13C | percent |
| AP15N | Atom percent of 15N | percent |
| Fv_Fm | Maximum photosynthetic yield of photosystem II | unitless |
| Densities | Areal densities of symbiotic dinoflagellates | cells per squared centimeter (cells cm-2) |

[ table of contents | back to top ]

## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | airbrush |
| **Generic Instrument Name** | Airbrush |
| **Dataset-specific Description** | Coral tissue was removed using an airbrush (100 psi) and filtered (0.22 µm) seawater. |
| **Generic Instrument Description** | Device for spraying liquid by means of compressed air. |

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|---|---|
| **Dataset-specific Instrument Name** | Flow through aquarium |
| **Generic Instrument Name** | Aquarium |
| **Dataset-specific Description** | Colonies were fragmented into replicate pieces (clone ramets) and placed into 1200 L flow-through aquariums supplied with natural seawater and held at 27.5°C |
| **Generic Instrument Description** | Aquarium - a vivarium consisting of at least one transparent side in which water-dwelling plants or animals are kept |

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|---|---|
| **Dataset-specific Instrument Name** | Carlo Erba CHN Elemental Analyzer (Model NA1500) |
| **Generic Instrument Name** | Carlo-Erba NA-1500 Elemental Analyzer |
| **Dataset-specific Description** | Elemental 13C and 15N analyses were performed on a Carlo Erba CHN Elemental Analyzer (Model NA1500) coupled to Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer via a Thermo Finnigan Conflo III Interface at the University of Georgia, Center for Applied Isotope Studies. |
| **Generic Instrument Description** | A laboratory instrument that simultaneously determines total nitrogen and total carbon from a wide range of organic and inorganic sediment samples. The sample is completely and instantaneously oxidised by flash combustion, which converts all organic and inorganic substances into combustion products. The resulting combustion gases pass through a reduction furnace and are swept into the chromatographic column by the carrier gas which is helium. The gases are separated in the column and detected by the thermal conductivity detector which gives an output signal proportional to the concentration of the individual components of the mixture. The instrument was originally manufactured by Carlo-Erba, which has since been replaced by Thermo Scientific (part of Thermo Fisher Scientific). This model is no longer in production. |

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| **Dataset-specific Instrument Name** | IEC clinical centrifuge |
| **Generic Instrument Name** | Centrifuge |
| **Dataset-specific Description** | Symbiotic dinoflagellates and coral tissue were separated by 2–3 centrifugation washes (550 g for 5 min) using IEC clinical centrifuge (Damon/IEC Division, Needham Heights., MA) |
| **Generic Instrument Description** | A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids. |

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| **Dataset-specific Instrument Name** | Thermo Finnigan Conflo III Interface |
| **Generic Instrument Name** | Continuous Flow Interface for Mass Spectrometers |
| **Dataset-specific Description** | Elemental 13C and 15N analyses were performed on a Carlo Erba CHN Elemental Analyzer (Model NA1500) coupled to Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer via a Thermo Finnigan Conflo III Interface |
| **Generic Instrument Description** | A Continuous Flow Interface connects solid and liquid sample preparation devices to instruments that measure isotopic composition. It allows the introduction of the sample and also reference and carrier gases. Examples: Finnigan MATConFlo II, ThermoScientific ConFlo IV, and Picarro Caddy. Note: This is NOT an analyzer |

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|---|---|
| **Dataset-specific Instrument Name** | EVOS digital fluorescent microscope |
| **Generic Instrument Name** | Fluorescence Microscope |
| **Dataset-specific Description** | Algal densities were quantified using an EVOS digital fluorescent microscope. |
| **Generic Instrument Description** | Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments. |

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|---|---|
| **Dataset-specific Instrument Name** | Walz Diving-PAM pulse amplitude modulation fluorometer |
| **Generic Instrument Name** | Fluorometer |
| **Dataset-specific Description** | A pulse amplitude modulation fluorometer (Diving PAM, Waltz, Germany) was used to measure the maximum quantum yield of photosystem II (PSII, Fv/Fm) one hour after sunset in three separate locations using a 0.6 second saturation pulse |
| **Generic Instrument Description** | A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ. |

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| **Dataset-specific Instrument Name** | AO Spencer Bright Line Improved Neubauer haemocytometer |
| **Generic Instrument Name** | Hemocytometer |
| **Dataset-specific Description** | Algal densities were quantified from replicate haemocytometer counts (AO Spencer Bright Line Improved Neubauer haemocytometer) |
| **Generic Instrument Description** | A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html. |

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| **Dataset-specific Instrument Name** | Tissue Tearor (BioSpec Products) |
| **Generic Instrument Name** | Homogenizer |
| **Dataset-specific Description** | The resulting slurry, containing coral tissue and symbiotic dinoflagellates, was homogenized for approximately 10 seconds using a Tissue Tearor (BioSpec Products, Bartlesville, OK, USA) |
| **Generic Instrument Description** | A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. |

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| **Dataset-specific Instrument Name** | Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer |
| **Generic Instrument Name** | Isotope-ratio Mass Spectrometer |
| **Dataset-specific Description** | Elemental 13C and 15N analyses were performed on a Carlo Erba CHN Elemental Analyzer (Model NA1500) coupled to Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer via a Thermo Finnigan Conflo III Interface at the University of Georgia, Center for Applied Isotope Studies. |
| **Generic Instrument Description** | The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer). |

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| **Dataset-specific Instrument Name** | LED lights (Cree Cool White XP-G R5) |
| **Generic Instrument Name** | LED light |
| **Dataset-specific Description** | All beakers were illuminated by LED lights (Cree Cool White XP-G R5) set to a light intensity of 500 µmol quanta m-2s-1. |
| **Generic Instrument Description** | A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons. |

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| **Dataset-specific Instrument Name** | PAR sensor (LiCor LI-192) |
| **Generic Instrument Name** | LI-COR LI-192 PAR Sensor |
| **Dataset-specific Description** | A 60% shade cloth was used to provide a peak midday light intensity of 800 µmol quanta m-2s-1, measured with a PAR sensor (LiCor LI-192), similar to the maximum light levels of natal colony habitats at collection depth. |
| **Generic Instrument Description** | The LI-192 Underwater Quantum Sensor (UWQ) measures underwater or atmospheric Photon Flux Density (PPFD) (Photosynthetically Available Radiation from 360 degrees) using a Silicon Photodiode and glass filters encased in a waterproof housing. The LI-192 is cosine corrected and features corrosion resistant, rugged construction for use in freshwater or saltwater and pressures up to 800 psi (5500 kPa, 560 meters depth). Typical output is in um s-1 m-2. The LI-192 uses computer-tailored filter glass to achieve the desired quantum response. Calibration is traceable to NIST. The LI-192 serial numbers begin with UWQ-XXXXX. LI-COR has been producing Underwater Quantum Sensors since 1973. These LI-192 sensors are typically listed as LI-192SA to designate the 2-pin connector on the base of the housing and require an Underwater Cable (LI-COR part number 2222UWB) to connect to the pins on the Sensor and connect to a data recording device. The LI-192 differs from the LI-193 primarily in sensitivity and angular response. 193: Sensitivity: Typically 7 uA per 1000 umol s-1 m-2 in water. Azimuth: < ± 3% error over 360° at 90° from normal axis. Angular Response: < ± 4% error up to ± 90° from normal axis. 192: Sensitivity: Typically 4 uA per 1000 umol s-1 m-2 in water. Azimuth: < ± 1% error over 360° at 45° elevation. Cosine Correction: Optimized for underwater and atmospheric use. (www.licor.com) |

[ table of contents | back to top ]

## Project Information

**Collaborative Research: Stability, flexibility, and functionality of thermally tolerant coral symbioses (Thermally tolerant coral)**

**Coverage**: Coral Reefs of Palau, Micronesia

NSF abstract:

All reef-building corals require large numbers of internal symbiotic microalgae (called Symbiodinium) for their survival and growth. These mutualisms have shown considerable sensitivity to changes in the environment in recent decades, especially due to global increases in ocean temperatures. When exposed to severe thermal stress, corals loose their symbionts and often die. However, recent experiments show that some symbionts

may be more stress-tolerant. Corals with these heat-resistant symbionts continue to receive high amounts of algal derived nutrients and grow under elevated temperatures. If the global trend in seawater warming continues to increase, these heat-resistant symbioses may become more ecologically prevalent on reef systems around the world and could play a critical role in maintaining healthy and productive coral communities. This project will examine the ecological and physiological attributes of stress-tolerant symbioses from the Indo Pacific where coral communities are the largest, most diverse, and productive in the world. The researchers will conduct a series of experiments to (1) evaluate host and symbiont attributes that contribute to thermal tolerance and (2) characterize the relative flexibility and functionality of various corals and symbionts exposed to typical ambient and stressful temperatures. Broader impacts of the project include the training of several Ph.D. students, undergraduates, and high school students in the disciplines of physiology and ecology. The researchers will partner with Global Ocean Exploration, Inc. to communicate this research to the general public through short documentary videos, editorials, and podcasts. An interactive K-5 program, "Invertebrates on the Road," will introduce elementary students in Pennsylvania to marine invertebrate diversity. Research results will also be disseminated to the public at the University of Delaware via educational seminars, as well as through hands-on research displays and demonstrations presented at the annual open house "Coast Day" festival in each year of the project.

This project will examine several attributes important to the functional ecology of coral-dinoflagellate symbioses. Specifically, the research team seeks to understand the interplay between coral and symbiont physiologies under different environmental conditions and determine the relative influence of biotic factors crucial to the performance of stress tolerant symbioses. Results from recent experiments on Indo-west Pacific corals found that Clade D (S. trenchii) symbionts are stress-tolerant. These symbionts are able to maintain function and provide nutrients to their hosts under high temperatures that typically elicit the breakdown of symbioses involving many other species of symbiont. A number of questions arise about how enhanced thermal tolerance symbioses may be aided by a combination of factors; for example: Are symbionts physiologically hardier in corals that are routinely feeding? Do host genotypes that are adapted to high temperatures affect the physiology of their symbionts in ways that make the partnership more stress-tolerant? A series of experiments over three years will examine the functionality of different coral-symbiont pairings exposed to ambient and high temperatures. Reciprocal transplants between inshore (stress-tolerant) and offshore (stress-susceptible) reef sites will be used to produce specific host-symbiont parings. Controlled experiments will test the relative importance of coral trophic status (nutrient content) while holding symbiont type constant and how changes in both coral trophic status and symbiont species identity of the resident affect thermal tolerance. Tank experiments on shore will track rates of photosynthesis as well as carbon translocation and assimilation from symbiont to host tissues and skeletons. Long-term growth rates via skeletal density, linear extension, and biomass gain will also be measured. This project will help elucidate how biochemical, physiological and ecological differences among host-symbiont pairings may respond to rising ocean temperatures and enhance the future viability of coral reefs.

[ table of contents | back to top ]

## Funding


| Funding Source | Award |
|---|---|
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1719684 |
| NSF Division of Integrative Organismal Systems (NSF IOS) | IOS-1719675 |


[ table of contents | back to top ]