Comparative analysis of tissue biomass and energy reserves of six coral species from nearshore and offshore reefs in Palau, Micronesia during March 2017

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Data Type: experimental, Other Field Results
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Project
» Collaborative Research: Stability, flexibility, and functionality of thermally tolerant coral symbioses (Thermally tolerant coral)

Contributors	Affiliation	Role
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Abstract
The nearshore reef habitats in Palau, Micronesia, serve as a representation of the challenges that coral populations may face in a future with warmer and more acidic oceans. Interestingly, corals inhabiting these nearshore habitats demonstrate a greater ability to withstand and recover from episodes of thermal stress compared to their offshore counterparts. In order to investigate the underlying physiological mechanisms behind this tolerance, we conducted a comparative analysis of six coral species found in both offshore and nearshore environments. Specifically, we examined parameters such as tissue biomass (ash-free dry weight cm−2), energy reserves (including protein, total lipid, and carbohydrate content), as well as several crucial lipid classes.

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Coverage

Spatial Extent: **N**:7.3245 **E**:134.4939 **S**:7.248833 **W**:134.235817
Temporal Extent: 2017-03-12 - 2017-03-25

Dataset Description

Six coral species from two reef environments were sampled in March 2017 for comparative analysis in order to investigate the underlying physiological mechanisms for thermal tolerance. Offshore samples were collected at Rebotel Reef on the western barrier reef of Palau (7.248833° N, 134.235817° E) while nearshore corals were collected at Ngermid Bay (aka Nikko Bay) approximately 28 km away (7.3245° N, 134.4939° E).

Methods & Sampling

Summarized from: *Keister, E.F., Gantt, S.E., Reich, H.G., Turnham, K.E., Bateman, T.G., LaJeunesse, T.C., Warner, M.E., Kemp, D.W. Similarities in biomass and energy reserves among coral colonies from contrasting reef environments. Scientific Reports (2023).DOI:10.1038/s41598-023-28289-6*

Field sampling

For each species, coral colonies were sampled (n= 3 to 14 individuals) at depths of 5 to 10 meters for offshore colonies and 2 to 5 meters for nearshore colonies. The sampled colonies were spaced at least 10 meters apart. All colonies selected for sampling were of similar sizes, representative of typical sizes for each species, and fragments were taken from the top and center of the colonies. Prior to sample collection, the coral colonies displayed no visible signs of stress, and there were no reported thermal anomalies or bleaching events.

The sampled coral colonies were carefully transported back to the Palau International Coral Research Center (PICRC) in coolers filled with seawater. At PICRC, the coral samples were individually placed into Whirlpaks® and immediately frozen at a temperature of -40°C. The frozen samples were then transported to the University of Alabama at Birmingham (UAB) in the United States, where they were stored at -80°C until further processing.

Laboratory processing

While still frozen, the coral fragments were cut into approximately 4 cm^2 pieces using a Torque Master Tile Saw (QEP) equipped with a diamond blade. All excess skeleton, boring sponges, and epibionts were carefully removed from the coral fragments. To determine the surface area of each fragment, 3D scanning was performed using a Capture Mini 3D scanner along with Geomagic® Controlx64™ software from 3DSystems. Subsequently, the coral fragments were lyophilized for 36 hours using a Labconco Freeze Dry System and weighed to determine their total dry mass.

The lyophilized coral fragments were individually pulverized into a fine, homogenized powder using a SPEX Sample Prep ball mill. This powder consisted of the complete coral holobiont, including the animal host, endosymbiotic dinoflagellate communities, and microbiome. The powdered samples were then partitioned for further analysis, including the examination of tissue biomass and energy reserves such as total lipids, soluble protein, and carbohydrates.

Tissue biomass

To assess tissue biomass, the dry powdered coral fragments were weighed, ranging from approximately 0.5 to 3.3 grams. These fragments were then subjected to combustion in a muffle furnace for a duration of 12 hours at a temperature of 500°C. This process allowed for the determination of the total organic content. The ash-free dry weight (AFDW) was calculated by subtracting the weight of the ash after combustion from the initial dry weight of the fragments. The ratio of AFDW to the total dry weight was utilized to calculate the overall AFDW, representing the total coral tissue biomass per surface area of the entire fragment.

Energy reserves

For the measurement of energy reserves, the sample sizes varied depending on the availability of samples. For some species, the offshore sampling yielded anywhere from 3 to 14 individuals, while the nearshore sampling had 6 to 8 individuals. Energy reserves were assessed alongside the tissue biomass as part of the study.

Lipid analyses were conducted to determine the total lipids and quantify different lipid classes present in the coral samples. Approximately 0.6 grams of lyophilized coral fragment powder were used for the lipid extraction process. Triplicate, independent lipid extractions were performed for each sample using a modified Folch method. In brief, a solvent system consisting of chloroform, methanol, and 0.88% NaCl in specific ratios (8:4:3) was used to extract the total lipids. The solvent volume was maintained at a 20:1 ratio relative to the dry sample weight. The lower chloroform phase was collected in pre-weighed glass tubes and dried under a continuous stream of nitrogen gas. The gravimetric determination of total lipid concentration was performed, and the values were converted to Joules (J) per unit of ash-free dry weight (AFDW) per gram. To achieve a concentration of 10 mg/mL, 100% chloroform was added to the dried lipid extract.

For the quantification of lipid classes, 1 µL of the lipid extracts was spotted in duplicate on individual silica Chromarods®. Thin-layer chromatography (TLC) was then conducted using a two-step solvent system. The first step involved a mixture of chloroform, methanol, and water (50:20:2 by volume) and allowed for the elution of phosphatidylethanolamine (PE), phosphatidylserine and phosphatidylinositol (PS-PI), phosphatidylcholine (PC), and lysophosphatidylcholine (LPC). The second step involved a mixture of hexane, ethyl ether, and formic acid (60:15:1.5 by volume) and allowed for the elution of wax ester (WAX), triacylglycerol (TAG), sterol (ST), and diacylglycerols (DAG). The Chromarods were dried at 100°C for 10 minutes before being analyzed using an Iatroscan MK 6S thin-layer flame ionization detector (TLC-FID) for

identification and quantification of lipid classes. Calibration of the Iatroscan MK 6S was performed using compound classes in the concentration range of 0.1-10.0 mg/mL, including l-alpha-phosphatidyl-l-serine for PS-PI, l-alpha-phosphatidylethanolamine for PE, l-alpha-phosphatidylcholine for PC, l-alpha-lysophosphatidylcholine for LPC, palmityl palmitate for WAX, tripalmitin for TAG, cholesterol for ST, and dipalmitin for DAG.

All phospholipid lipid classes (PE, PS-PI, PC, LPC) were grouped together and analyzed as a single unit. The values for all lipid classes were presented in units of milligrams per unit of ash-free dry weight (AFDW) per gram.

For the analysis of carbohydrates and proteins, specific protocols were followed. To quantify soluble proteins, approximately 0.5 grams of lyophilized and crushed coral samples were used. Following the procedure described by McLachlan et al., the samples were placed in 15 mL tubes, and 1 mL of a diluted 1x solution of radioimmunoprecipitation (RIPA, Sigma-Aldrich) was added. The samples underwent three freeze-thaw cycles to lyse the cells and solubilize the proteins. After centrifugation for 20 minutes at 4122g at 4°C, 1 mL of the supernatant containing the solubilized protein was transferred to a 2 mL tube. A modified Bradford assay, using bovine serum albumin (BSA) as a standard, was then employed to quantify the soluble protein levels. All samples were analyzed in triplicate using a microplate reader (EPOCH 2, Agilent), measuring absorbance at 465 nm and 595 nm. The values for soluble proteins were converted to Joules per unit of ash-free dry weight (AFDW) per gram.

For the quantification of carbohydrates, around 0.3 grams of lyophilized and crushed coral samples were placed in a 2 mL tube with 1 mL of Milli-Q water. The samples were sonicated at 35% intensity for 2 minutes using a Fisher Scientific model CL-18 sonicator. After centrifugation for 10 minutes at 1000g, 1 mL of the supernatant containing total carbohydrates was collected. A modified DuBois method, described in Masuko et al., was employed to quantify carbohydrates, with glucose used to create a standard curve. All samples were run in triplicate on a microplate reader (EPOCH 2, Agilent), measuring absorbance at 485 nm and 750 nm for the determination of total carbohydrates. The carbohydrate values were then converted to Joules per unit of ash-free dry weight (AFDW) per gram.

Data Processing Description

Protein, lipid, and carbohydrate data were converted to Joules per unit of ash-free dry weight per gram following Gnaiger & Bitterlich 1984.

BCO-DMO Processing Description

- Imported data from source file "Keister et al. 2023 Tissue data.csv" into BCO-DMO system
- Added columns for latitude and longitude based on reef site locations
- Added column for surface area based on PI-provided information
- 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. Corrected spelling of one so all species names matched accepted taxon names exactly as of 2023-09-06

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Data Files

File	
907507_v1_kemp_keister_tissue_data.csv	(Comma Separated Values (.csv), 12.98 KB) MD5:2ddc7d25de77e26dc2108a8abf12a6c2
Primary data file for dataset ID 907507 version 1	

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Related Publications

Conlan, J. A., Humphrey, C. A., Severati, A., & Francis, D. S. (2017). Influence of different feeding regimes on the survival, growth, and biochemical composition of Acropora coral recruits. PLOS ONE, 12(11), e0188568. https://doi.org/10.1371/journal.pone.0188568
Methods

Conlan, J. A., Jones, P. L., Turchini, G. M., Hall, M. R., & Francis, D. S. (2014). Changes in the nutritional composition of captive early-mid stage Panulirus ornatus phyllosoma over ecdysis and larval development. Aquaculture, 434, 159–170. https://doi.org/10.1016/j.aquaculture.2014.07.030
Methods

Delmas, R. P., Parrish, C. C., & Ackman, R. G. (1984). Determination of lipid class concentrations in seawater by thin-layer chromatography with flame ionization detection. Analytical Chemistry, 56(8), 1272–1277. https://doi.org/10.1021/ac00272a018
Methods

Enochs, I. C., Manzello, D. P., Carlton, R., Schopmeyer, S., van Hooidonk, R., & Lirman, D. (2014). Effects of light and elevated pCO2 on the growth and photochemical efficiency of Acropora cervicornis. Coral Reefs. https://doi.org/10.1007/s00338-014-1132-7
Methods

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A SIMPLE METHOD FOR THE ISOLATION AND PURIFICATION OF TOTAL LIPIDES FROM ANIMAL TISSUES. Journal of Biological Chemistry, 226(1), 497–509. https://doi.org/10.1016/s0021-9258(18)64849-5
Methods

Gnaiger, E., & Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia, 62(3), 289–298. doi:10.1007/bf00384259 https://doi.org/10.1007/BF00384259
Methods

Keister, E. F., Gantt, S. E., Reich, H. G., Turnham, K. E., Bateman, T. G., LaJeunesse, T. C., Warner, M. E., & Kemp, D. W. (2023). Similarities in biomass and energy reserves among coral colonies from contrasting reef environments. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-28289-6
Results
,
Methods

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Analytical Biochemistry, 339(1), 69–72. doi:10.1016/j.ab.2004.12.001
Methods

Mclachlan, R., Price, J., Dobson, K., Weisleder, N., & Grottoli, A. (2020). Microplate Assay for Quantification of Soluble Protein in Ground Coral Samples v1 (protocols.io.bdc8i2zw). Protocols.io. doi:10.17504/protocols.io.bdc8i2zw
Methods

Nichols, P. D., Mooney, B. D., & Elliott, N. G. (2001). Unusually high levels of non-saponifiable lipids in the fishes escolar and rudderfish. Journal of Chromatography A, 936(1–2), 183–191. https://doi.org/10.1016/s0021-9673(01)00894-9 https://doi.org/10.1016/S0021-9673(01)00894-9
Methods

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Parameters

Parameter	Description	Units
Latitude	Latitude of reef site	decimal degrees
Longitude	Longitude of reef site	decimal degrees
Sample_ID	Identification given for individual samples and technical replicates	unitless
Species	Genus and species of collected sample	unitless
Site	Location of sampled colony (Rebotel Reef is offshore; Ngermid Bay is nearshore)	unitless
Collection_Date	Date of sample collection	unitless
AFDW	Ash Free Dry Weight	grams
Total_lipids	Total lipids in joules	joules
Surface_Area	Surface area of coral fragment from 3D scan	squared centimeters (cm^2)
Protein	Total protein	joules
Carbohydrate	Total carbohydrate	joules
WAX	Wax esters	milligrams Ash Free Dry Weight per gram (mg AFDW/g)
TAG	Triacylglycerol	milligrams Ash Free Dry Weight per gram (mg AFDW/g)
ST	Sterol	milligrams Ash Free Dry Weight per gram (mg AFDW/g)
Phospholipids	Phospholipid	milligrams Ash Free Dry Weight per gram (mg AFDW/g)

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Instruments

Dataset-specific Instrument Name	Capture Mini 3D scanner
Generic Instrument Name	3D scanner
Dataset-specific Description	To determine the surface area of each fragment, 3D scanning was performed using a Capture Mini 3D scanner along with Geomagic® Controlx64™ software from 3DSystems.
Generic Instrument Description	A 3D scan captures digital information about the shape of an object with equipment that uses a laser or light to measure the distance between the scanner and the object.

Dataset-specific Instrument Name	IEC clinical centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	After centrifugation for 10 minutes at 1000g, 1 mL of the supernatant containing total carbohydrates was collected.
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.

Dataset-specific Instrument Name	Iatroscan MK 6S thin-layer flame ionization detector (TLC-FID)
Generic Instrument Name	Flame Ionization Detector
Dataset-specific Description	Lipid extracts on Silica Chromarods® were dried at 100°C for 10 minutes before being analyzed using an Iatroscan MK 6S thin-layer flame ionization detector (TLC-FID) for identification and quantification of lipid classes.
Generic Instrument Description	A flame ionization detector (FID) is a scientific instrument that measures the concentration of organic species in a gas stream. It is frequently used as a detector in gas chromatography. Standalone FIDs can also be used in applications such as landfill gas monitoring, fugitive emissions monitoring and internal combustion engine emissions measurement in stationary or portable instruments.

Dataset-specific Instrument Name	SPEX Sample Prep ball mill
Generic Instrument Name	Homogenizer
Dataset-specific Description	The lyophilized coral fragments were individually pulverized into a fine, homogenized powder using a SPEX Sample Prep ball mill.
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.

Dataset-specific Instrument Name	Labconco Freeze Dry System
Generic Instrument Name	Lyophilizer
Dataset-specific Description	The coral fragments were lyophilized for 36 hours using a Labconco Freeze Dry System and weighed to determine their total dry mass.
Generic Instrument Description	A lyophilizer, also known as freeze dryer or liofilizador, is a device that is used to freeze-dry material.

Dataset-specific Instrument Name	Torque Master Tile Saw (QEP) with a diamond blade
Generic Instrument Name	Manual Biota Sampler
Dataset-specific Description	While still frozen, the coral fragments were cut into approximately 4 cm^2 pieces using a Torque Master Tile Saw (QEP) equipped with a diamond blade.
Generic Instrument Description	"Manual Biota Sampler" indicates that a sample was collected in situ by a person, possibly using a hand-held collection device such as a jar, a net, or their hands. This term could also refer to a simple tool like a hammer, saw, or other hand-held tool.

Dataset-specific Instrument Name	microplate reader (EPOCH 2, Agilent)
Generic Instrument Name	plate reader
Dataset-specific Description	All samples were analyzed in triplicate using a microplate reader (EPOCH 2, Agilent), measuring absorbance at 465 nm and 595 nm.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Sonicator model CL-18, Fisher Scientific
Generic Instrument Name	ultrasonic cell disrupter (sonicator)
Dataset-specific Description	The samples were sonicated at 35% intensity for 2 minutes using a Fisher Scientific model CL-18 sonicator.
Generic Instrument Description	Instrument that applies sound energy to agitate particles in a sample.

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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.

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Funding

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

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