

Led by:

Prof. Christian R. Voolstra, Ms. Hannah Manns, Mr. Luigi Colin Organized by:

Dr. Davide Seveso, Dr. Enrico Montalbetti, Dr. Yohan Didier Louis

For information:

workshop.marhe@unimib.it davide.seveso@unimib.it marhe.unimib.it



INTRODUCTION

Researchers have recently identified a number of coral populations, reef regions, and coral genotypes with enhanced bleaching resilience. These are critical resources/targets for conservation and restoration in a changing climate. However, until recently, there was no standardized procedure for determining bleaching susceptibility and assessing populations, individuals, and regions for these resilient characteristics.

Commonly employed approaches range from: observational surveys of naturally occurring bleaching severity and mortality, to multiple months of controlled thermal exposure or years of field transplantation, to rapid, single/multi-day acute heat shocks. Field surveys are one of the most informative measures of bleaching susceptibility, however, natural bleaching events are difficult to predict and measure, large-scale bleaching surveys are costly, and monitoring recovery and mortality is a lengthy process requiring months to years. Longer-term lab exposures (weeks to months) to elevated temperature are designed to approximate natural thermal stress events and have a proven track record in the literature over many decades; yet they require extensive/expensive aquarium systems capable of sustaining

corals for weeks on end. These systems are not practical in many remote locations where coral reefs exist, and the approach takes weeks to months for an assessment of just a single set of individuals from a single population. By comparison, experiments utilizing short-term (1 day), acute thermal exposures in remote field settings show a promising ability to reveal fine-scale differences in thermal tolerance across small spatial scales.

We have recently developed a portable experimental system termed CBASS (Coral Bleaching Automated Stress System) that allows determination of 'ED50' (effective dose) thermal thresholds within 11 to 18 hours. The ED50 thermal threshold is a standardized metric signifying the temperature at which the initial thermal performance is decreased by 50%. Typically, algal photosynthetic efficiency or coral whitening is used as a physiological measure, which have both been shown to be indicative of coral holobiont biology under heat stress [1–3]. There is a suite of CBASS studies available now that show its applicability to reproducibly determine thermal thresholds for a large number of colonies [4, 5] as well as its utility as a standardized platform to assess factors affecting bleaching susceptibility or identify mechanisms underlying thermal resilience. Thus, CBASS assays are ideally suited to assay a large number of colonies for their thermal tolerance. Importantly, building instructions, run protocols, and analytical routines are freely and openly accessible [2, 6]. At large, short-term acute thermal assays as made prominent by the CBASS platform are becoming increasingly popular, experiencing wide adoption globally to identify reefs and corals with increased thermal tolerance, due to their mobility, rapidity, and standardization. Implementation of standardization is critical to establish effective tools for high-throughput screening, inter-experiment comparability, and monitoring of coral thermal tolerance within restoration programs [7–9].

This workshop will be run by leading experts in CBASS assays and diagnostics to present state-of-the-art methodological and analytical procedures. Besides learning how to run CBASS assays, we discuss integration of short-term diagnostic approaches to inform research and conservation efforts. Thus, the workshop should be of interest to students, researchers,

resource managers, restoration specialists, practitioners, and funders.

The course will consist of a series of lectures and field activities held by researchers with experience in running and analyzing short-term acute thermal assays (CBASS). This workshop will result in determining thermal tolerances of Magoodhoo Island corals.

After completion of the workshop, you will have gained the following skills:

- Build your own CBASS assay
- Design CBASS experiments
- Analyze CBASS thermal stress data using R
- Learn about climate variables that affect coral thermal resilience and query climate databases for these variables to contextualize your coral thermal stress data
- Conduct standardized underwater coral transect surveys using artificial intelligence-assisted analysis to contextual your coral thermal stress data
- Improve your knowledge on coral identification
- Provide standardized reporting of thermal stress experiment
- Upload and query CBASS data using the coralfuture.org database.









ORGANIZATION OF THE WORKSHOP

This workshop is an intensive 9-day handson, in-water/out-of-water practical program and will take place from 9 to 17 December 2024 on Magoodhoo Island, Faafu Atoll, Maldives. The logistical support base will be the Marine Research and High Education Center (MaRHE Center) of the University of Milano-Bicocca, Italy (www.marhe.unimib.it). Magoodhoo and the MaRHE Center are about 3 hours by speed boat from the airport of Malé, the Maldivian capital.

The program includes a series of classroom lessons, training sessions, CBASS practical activities, and field activities including SCUBA diving and snorkeling in the reefs surrounding the island. Students will be divided into groups in order to carry out the practical and field activities and each group will be accompanied and assisted both underwater and during the practical sessions by the organizers of the workshop and the MaRHE Center staff.

The workshop will be held in English language and will be led by **Prof. Christian R Voolstra**, a renowned coral reef researcher

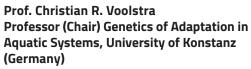
who established and co-developed CBASS thermal assays [1,2], the SymPortal framework [10,11], the reefgenomics.org database [12], the analytical framework Coracle [13], and most recently the coralfuture.org database (https://coralfuture. org) and the CBASSED50 R package [6] for CBASS thermal tolerance data. In addition to learning how to run CBASS assays, the workshop touches on experimental design considerations, best practices, logistics, and analysis of CBASS data, including contextualization with environmental metadata, how to query relevant databases (e.g., NOAA's Coral Reef Watch), and meaningful additional data to generate/collect (e.g., Symbiodiniaceae and microbiome assemblage).

The workshop is coordinated by researchers from the University of Milano-Bicocca and MaRHE Center. These professionals have extensive experience in tropical marine research and possess comprehensive expertise in the Maldivian coral ecosystem from various scientific perspectives.









Christian has pioneered studies on coral metaorganism function, particularly in deciphering the mechanistic underpinnings of coral-microalgal symbioses and the role of bacteria in coral acclimation and adaptation. His team produced the first hologenome of the coral Stylophora pistillata, its associated microalgal symbiont Symbiodinium microadriaticum, and its pervasive bacterial associate Endozoicomonas. which spawned further research on this ubiquitous genus. Christian's team developed and built SymPortal, an analytical framework for genetically resolving algal symbionts of reef corals

using next-generation sequencing (NGS) data of the ITS2 rDNA; the online platform symportal.org offers fully automated analysis providing a global description of coral symbiont diversity. Christian's and collaborators long-standing interest in diagnosing coral thermal tolerance spurred the recent development of a standardized heat stress assay, the Coral Bleaching Automated Stress System (CBASS), which is now the de facto approach for screen and identifying naturally heat resistant coral populations for conservation and restoration. Christian was awarded the 2022 mid-career award by the International Coral Reef Society (ICRS) and is the society's current president. His research is regularly featured in digital and print news and outlets, with some appearances on TV and in documentaries. Google Scholar / Webpage



Ms. Hannah Manns Lab and Field Technician at Voolstra Lab, University of Konstanz (Germany)

As a marine enthusiast I got my masters degree in marine environmental sciences at the University of Oldenburg where I also earned the certification as a German and European Scientific Diver. Since April 2022 I am now a Lab and Field Technician in the Voolstra Lab at the University of Konstanz performing and supervising CBASS experiments and working on various projects related to coral heat stress resistance and resilience as well as coral calcification.



Mr. Luigi Colin PhD student at Voolstra Lab, University of Konstanz (Germany)

Luigi's PhD project focuses on the study of coral holobiont diversity using metagenomics and metabarcoding techniques, particularly analyzing the coral's bacterial signature in conjunction with environmental and climate data. Luigi holds an International Master of Science in Marine Biological Resources from the University of Ghent, Belgium. Prior to his current appointment, Luigi worked as a Postgraduate Research Assistant at the Zoological Society of London, where he studied the genetic connectivity of corals in the Chagos Archipelago. His expertise includes molecular techniques such as DNA/RNA extraction and sequencing, as well as bioinformatics analysis of next-generation sequencing data. He is proficient in programming languages such as R, Python, Nextflow, and Django. He has contributed to publications on coral genetics, mesophotic fish communities, and aquarium coral collections.



Dr. Davide Seveso Assistant Professor at Dept. of Earth and **Environmental Science, University of** Milano-Bicocca (Italy) & Vice-director of MaRHE Center (Maldives)

Davide is a marine biologist interested in the ecology and biodiversity of coral reef systems and their related organisms. His main research activities focus on the impact of different environmental stressors on corals and other reef inhabitants, and in particular on the analysis of the coral stress response from a cellular perspective and the molecular mechanisms involved during coral bleaching events, by using different cellular biomarkers and multi-

omics approaches. Davide's research aims to acquire information from the molecular processes underlying the coral holobiont thermotolerance to develop heat tolerant corals and bleaching mitigation strategies. Most of his research activities were performed on the coral reefs of the Maldives, where he started to work more than 14 years ago. In addition, he has field experience in other tropical marine environments of the Indo-Pacific, the Red Sea and the Caribbean. Davide is a PADI diving Instructor with an Advanced author of about 80 peer-reviewed papers in international scientific journals and has contributed to 3 book chapters. Google Scholar / ResearchGate

European Scientific Diver license. He is the



Dr. Yohan D. Louis Researcher at Dept. of Earth and **Environmental Science, University of** Milano-Bicocca (Italy) & MaRHE Center (Maldives)

Yohan holds a Ph.D. in Marine Molecular Biology and Ecology at University of Mauritius. His research focuses on the biodiversity and responses of marine benthos to climate change and anthropogenic threats in tropical ecosystems, with a particular emphasis on coral reefs. Through field and aquarium experiments, he investigates bleaching tolerance in hard corals. In particular, he uses a multi-biomarker and multi-omics approach, combining techniques such as PAM fluorometry, DNA barcoding, and gene expression analysis via next-generation sequencing to understand the mechanisms of coral tolerance to thermal stress. Yohan aims to improve the efficiency of coral reef restoration techniques through his research. With a decade of combined fieldwork experience in Mauritius and the Maldives, he has substantial expertise in conducting research in tropical marine environments.



Dr. Enrico Montalbetti Researcher at Dept. of Earth and **Environmental Science, University of** Milano-Bicocca (Italy) & MaRHE Center (Maldives)

Enrico earned his Ph.D. from Bicocca University in Marine and Environmental Sciences, focusing his research on the mitigation strategies adopted by tropical corals at the cellular and molecular level to cope with environmental stress. Enrico's actual research draws upon the use of different omics approaches to address big questions in coral reefs and Mediterranean ecology. He is also a passionate diving instructor, certified scientific diver, and underwater enthusiast with 12 years of diving experience in tropical, temperate, and cold waters. Google Scholar / ResearchGate

PARTICIPATION REQUIREMENTS

The workshop is aimed at students (enrolled in master's degree courses or in a PhD course), researchers and professionals with degrees preferably in the scientific field (Biology, Environmental Sciences, Natural Sciences).

The workshop can accommodate a maximum of **16 participants**. In the event that this limit is surpassed, selection criteria will be applied based on the applicants' experience and inter-ests. Therefore, as part of the enrollment process, we kindly request that you also submit your Curriculum Vitae and, optionally, a brief motivation letter. These documents will be considered for evaluation and potential selection purposes.

DIVING REQUIREMENTS

This workshop necessitates a scuba diving certificate for the enrollment. Each participant must hold a valid diving license of any level, with a minimum number of 20 logged dives, or with a minimum of 15

dives of which 5 performed in the 6 months preceding the workshop.

All divers must have a **valid diving insurance** (**DAN or similar**) in order to perform diving activities.

Each diver has to fill in upon his/her arrival at MaRHE Center a medical declaration for suitability for diving (see Appendix 1 as example). In case of positive answer to any questions, an additional medical certificate will be required to attest the suitability to undertake diving activities. This must be obtained before travelling to the Maldives. To carry out some practical activities it is also advisable (not mandatory) to be in possession of an underwater camera.

CREDITS

At the end of the workshop, participants will receive a **certificate of completion** to recognize the participant's skills and achievements in the field of coral thermal stress testing and resilience diagnostics.

Students of the International Master's
Degree in Marine Sciences (University of
Milano-Bicocca) can expect recognition
of training credits for attending the
workshop. All the activities carried out
during the workshop, both theoretical
(lectures plus elaboration and presentation
of results), and practical (field activities and
data collection) will reach a number of hours
corresponding to 4 ECTS (practical training).

Students enrolled in other degree courses and/or PhD course and/or at other Universities or Institutions are requested to inquire at the respective administrative secretariats for the possible recognition of credits ECTS.





PROGRAM

DAY 1 - (December 9th, Monday):

- Meeting at Male' International Airport and transfer by speedboat to the MaRHE Center on Magoodhoo Island (about 2.5-3 h trip depending on sea conditions)
- Room allocation and lunch
- Introduction to the Workshop structure and the MaRHE Center and tour of the island
- Registration for diving activities
- Briefing on the next day's activities

DAY 2 - (December 10th, Tuesday):

- Dive 1- check dive
- Lecture Introduction to the Maldives and their coral reef ecosystem
- Lecture Review of coral biology and ecology and importance of coral conservation/restoration; history of CBASS; broad overview over workshop program
- Lecture climate variables and why they matter; extraction of climate variables from NOAA databases
- Training session Determine climate variables from sampling site
- Lecture Running a CBASS experiment;
 CBASS thermal thresholds ED50 modeling
- Recap of day, briefing on next day's activities, assign groups

DAY 3 - (December 11th, Wednesday):

- Dive 2 CBASS coral sampling (2 species)
- Training session Run CBASS system
- Lecture Identification of coral genera; standardized reef survey using AI assisted annotation
- Training session Diving PAM measurements and bleaching score photos
- Recap of session and briefing on next day's activities

DAY 4 - (December 12th, Thursday):

- Training session Diving PAM measurements and bleaching score photos
- Training session CBASS cleanup
- Dive 3 Reef transect survey
- Training/analysis session Reef transect survey data processing
- Recap of session and briefing on next day's activities

DAY 5 - (December 13th, Friday):

- Dive 4 CBASS coral sampling (2 species)
- Training session Run CBASS system
- Lecture 5 Identification of coral genera; standardized reef survey using AI assisted annotation
- Training session Diving PAM measurements and bleaching score photos
- Recap of session and briefing on next day's activities

DAY 6 - (December 14th, Saturday):

- Training session Diving PAM measurements and bleaching score photos
- Training session CBASS cleanup
- Dive 5 Reef transect survey
- Training/analysis session Reef transect survey data processing
- Recap of session and briefing on next day's activities

DAY 7 - (December 15th, Sunday):

- Analysis session CBASS thermal thresholds ED50 modeling; prepare and upload data to coralfuture.org; bleaching score determination
- Lecture Symbiodiniaceae and microbiome: biology, data collection, data interpretation

DAY 8 - (December 16th, Monday):

- Dive 6 Fun dive
- Analysis session conclusions and interpretation of data
- Recap of workshop
- Free time

DAY 9 - (December 17th, Tuesday):

 Preparation and departure for Male International Airport

IMPORTANT NOTES

- The program is indicative, as it is subject to modifications due to logistical needs or weather and sea conditions. In case of extreme marine conditions, diving activities could be replaced by snorkeling activities.
- The dives will be carried out both from the shore and from the boat and will be performed in different reefs located in the southern part of the Faafu Atoll at a maximum of 1 hour of navigation from the island of Magoodhoo.
- Each dive is preceded by a briefing during which the dive profile and organization as well as the scientific activities are presented and summarized to the participants.
- It is mandatory to bring a personal laptop for data analysis and presentation.
 Specific instructions on software to be installed on personal laptops to ease data analysis and exploration will be provided in detail in due time.









PRACTICAL INFORMATION

DOCUMENTS

To enter the Maldives it is necessary to have a passport with at least 6 months of validity from your departure from the Maldives and at least 2 empty pages to allow the application of the local visa. If you don't have a passport, we invite you to request it promptly since the waiting time may be long. If it is necessary to speed up the release times, Italian students only can request, after registering for the workshop, a letter from MaRHE Center to be handed to the police headquarters. Please let us know if you need this.

CLOTHING AND DIVE EQUIPMENT

The air temperature is around 28-29°C. The weather is always pleasant, even in the rainy months. Please note that most of the day will be occupied with field activities and lectures; it is, therefore, advisable to wear comfortable sportive and light clothes.

It is required to avoid too skimpy clothing, as the island of Magoodhoo is not a tourist destination and local costumes are those of an Islamic country.

For **diving** it is necessary to have your own equipment and in particular:

- Mask and fins (either with or without booties);
- Wetsuit (3mm) or lycra wetsuit/shirt (water is warm, avoid 5mm or thicker wetsuits);

- Diving computer (<u>it is mandatory to have your own</u>);
- BCD and regulators;
- Diving flashlight and underwater camera (optional but strongly recommended).

It is possible to rent, for a fee and based on availability, diving equipment (BCD, fins and regulators) at the diving center of the Centre. The dive computer is not available. Please inform us in advance in case you need to rent diving equipment or anything.

ACCOMMODATION & MEALS

During the workshop the accommodation is organized at the Center, in multiple rooms (with 4 beds) with services and meals included (breakfast, lunch and dinner). Sheets and towels are provided. Each room has a private bathroom, air conditioning and a fan. The rooms are cleaned and tidied up every day. There is no accommodation in single rooms, nor in mixed rooms for boys and girls. The food is prepared according to local customs, so it will mostly consist of fish, chicken, rice, pasta and vegetables prepared in different ways. Meals are served as a buffet. Special dietary requirements and allergies are to be mentioned in the application form.





HEALTH & SAFETY

No vaccination is required for travelers coming to Maldives from Europe. However, each participant should take care of this aspect in order to enter the country. In any case, it is good practice to check the coverage of the anti-tetanus vaccination, checking the expiry date. If it has expired, it can be renewed in time. Any field activity presents risks and dangers, and there are some basic rules to be observed in order to minimize them. In the water it is necessary to respect the buddy system: working groups will be settled, and, within the group, working couples will be established.

MEDICAL STATEMENT

Please carefully read the medical statement which you will need to fill only once on the island of Magoodhoo. Should you reply YES to any of the questions, you are required to present a medical certificate following a Physical Examination by your physician. This must be obtained before traveling to the Maldives.

INSURANCE

The insurance included with the Business Visa does not cover COVID-related costs and has a cover limit of 100.000 Maldivian Rufyiaa (MVR), approximately 6.500 USD\$,

for health issues. It is, therefore, up to you to decide whether to buy private health insurance in addition to the mandatory diving insurance.

CURRENCY

The Maldivian currency is the Maldivian Rufiyaa (MVR). Euros and dollars are accepted in any bank and exchange offices. 1 Euro = about 16,5 Maldivian Rufyiaa (MVR); 1 US \$ = about 15 MVR. It is possible to exchange money at the airport. We recommend bringing small denominations (€10 or €20), as these banknotes are also accepted also in shops on Magoodhoo Island.

COMMUNICATION

In MaRHE Center there is a free **WI-FI service** that can be used for educational purposes during the day and for communicating at home only in the evening. However, it is possible to purchase at the airport or in Male city a **local SIM** with data traffic, if you want to be always connected. Internet top-ups can be purchased online or also in Magoodhoo shops. It is now available an **e-sim card** on the Maldivian Ooredoo website, however compatibility with mobile phones must be verified beforehand as not all the brands and models support e-sim cards.





FEES & REGISTRATION

FEES

The registration fees for the workshop "Coral Bleaching Automated Stress System (CBASS) in Maldives" are as follows:

- <u>Student fee</u>: €1.500 (only for Master's students and PhD students);
- Non-student fee: €1.700.

One scholarship is available for a Maldivian national with 50% discount.

The above amount **includes:**

- Transfer by speed boat from/to Male airport;
- Transfers by local boat for all the activities, dives and excursions included in the program;
- Food and lodging at the MaRHE Center of Magoodhoo: full board treatment and accommodation in multiple rooms (single rooms are not available);
- Lectures and activities provided for the workshop program.

The above amount does not include:

- Flight to/for Maldives (Male International Airport);
- Entry Business visa: it is mandatory and the cost is €200, to be paid with the workshop fee;
- Passport issuing or renewal costs, if any;
- Possible rental of diving equipment (weights and weight belts are included);
- Anything else not specified above.

REGISTRATION

To proceed with the registration, you are asked to fill in the Google form which can be found at the following link:

https://shorturl.at/6RhkT

After completing the online questionnaire through the Google form, please send an email to workshop.marhe@unimib.it specifying in the subject: "Name Surname - CBASS workshop registration" and attaching:

- A good quality color scan of your passport as .pdf file (first 2 pages containing personal data), naming the file with CBASS24 Surname Name PPT.
- Your CV.

Important! Wait for our confirmation before purchasing your flight.

The workshop will be activated once a minimum number of participants has been reached. Therefore, we will give you the green light to purchase flights once this threshold is met. At that point, we will explain the procedure for paying the workshop fee and Business Visa, after which you will receive an invoice. Any errors you make (wrong flight dates or other) cannot be refunded. The maximum number of participants is set to 16 people.

THE REGISTRATION DEADLINE IS SET FOR 20 SEPTEMBER 2024

FLIGHT TICKETS

You will be responsible for purchasing your own flight. The choice of the airline is yours, the important thing is to be at the airport in Male, Maldives, on the day and at the time established (we will share with you the necessary information once registered). Pay attention while purchasing the flight tickets as your mistakes (incorrect flight dates or other) cannot be reimbursed. Furthermore, it will be necessary to send us by mail (workshop.marhe@unimib.it) a copy of the air ticket to be able to proceed with the entry visa request, which we will take care of.

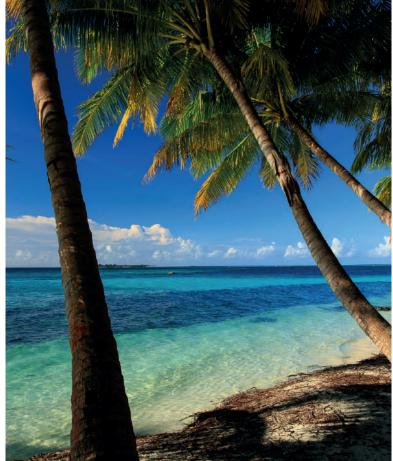
CANCELLATION POLICY

Following the payment of the fee, reimbursements for cancellations will be subject to 10% admin fee. In case of cancellation from the workshop after 15 October 2024, no refund will be possible.











References

Appendix 1

MEDICAL STATEMENT

Pneumothorax (collapsed lung)?

(Panic attack, fear of closed or open spaces)?

medication to prevent them?

Other chest disease or chest surgery?

Behavioral health, mental or psychological problems

Epilepsy, seizures, convulsions or take medications to

Blackouts or fainting (full/partial loss of consciousness)?

Recurring complicated migraine headaches or take

Frequent or severe suffering from motion sickness?

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 genomics data. Database. 2016;2016.
 doi:10.1093/database/baw152
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The purpose of this Medical Questionnaire is to find out if yor recreational diving. A positive response to a question does not means that there is a preexisting condition that may affect you physician prior to engaging in dive activities. Please answer the with a YES or NO. If you are not sure, answer YES. If any of the aphysician prior to participating in scuba diving. Your instructional Scuba Diver's Physical Examination	ot necessarily disqualify you from diving. A positive response our safety while diving and you must seek the advice of you he following questions on your past or present medical histor ese items apply to you, we must request that you consult with ructor will supply you with an RSTC Medical Statement and
Could you be pregnant, or are you attempting to become pregnant? Are you presently taking prescription medications? (with the exception of birth control or anti-malarial) Are you over 45 years of age and can answer YES to one or more of the following? Currently smoke a pipe, cigars or cigarettes Have a high cholesterol level Have a family history of heart attack or stroke Are currently receiving medical care High blood pressure Diabetes mellitus, even if controlled by diet alone Have you ever had or do you currently have Astma, or wheezing with breathing, or wheezing with exercise?	Any dive accidents or decompression sickness? Inability to perform moderate exercise? Head injury with loss of consciousness in the past 5 years? Recurrent back problems? Back or spinal surgery? Diabetes? Back, arm or leg problems following surgery, injury or fracture? High blood pressure or take medicine to control blood pressure? Heart disease and/or attack? Angina, heart surgery or blood vessel surgery?
exerciser Frequent or severe attacks of hayfever or allergy? Frequent colds, sinusitis or bronchitis? Any form of lung disease?	Sinus surgery? Ear disease or surgery, hearing loss or problems with balance? Recurrent ear problems?

Bleeding or other blood disorders?

Recreational drug use or treatment for, or alcoholism in

The information I have provided about my medical history is

responsibility for omissions regarding my failure to disclose

Signature:

accurate to the best of my knowledge. I agree to accept

Ulcers or ulcer surgery?

A colostomy or ileostomy?

any existing or past health condition.

Hernia?

the past 5 years?

Date:

Summary

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