THE ROLE OF REBREATHER DIVERS IN THE STUDY OF A MARINE DEEP-WATER CAVE ECOSYSTEM

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Abstract: This study delves into the diverse marine habitats of Jubilee Shoals, a submerged rocky reef off Pissouri Bay, Cyprus (eastern Mediterranean). The habitats include *Posidonia oceanica* meadows on the plateau, coralline communities on the vertical walls, and a cave system at its base. Utilizing closed-circuit rebreathers, trained cave divers quantitatively described the epibenthic communities and biodiversity within the cave system, ensuring both safety and high-quality data collection. Rebreathers allowed silent, bubble-less operation, efficient gas usage, extended dive times, reduced decompression times essential for detailed observation. The findings underscore the need for rebreathers in scientific exploration of deep-water environments, emphasizing their advantages. This approach facilitates undisturbed marine life observation and minimizes environmental impact, paving the way for future research and conservation in similar ecosystems.

Keywords: Scientific Diving, Marine Caves, Dark Environment, Epibenthic Communities

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Introduction

Submerged caves in the Mediterranean Sea serve as remarkable repositories of information, showcasing unique ecological characteristics. Marine caves have historically intrigued scientists, fostering thus early understanding of these ecosystems. Scientific research has revealed the rich biodiversity found within these caves [1,3].

Within these caves, different sections of the galleries (zones) host distinct species assemblages influenced by gradients of biotic and abiotic factors. Among these factors, the illumination gradient from the exterior to the interior creates unique ecological conditions that support diverse species [7, 4]. Species richness and coverage decrease along this axis [6, 3].

Based on light availability, four zones can be defined: a) cave entrance (CE), b) semi-dark environment (SD), c) dark environment (DE), and d) unspecified environment [8, 3]. Marine cave formations may exhibit a variety of geomorphological features and submersion levels, including fully or partially submerged blind caves, tunnels, and pits, each displaying different degrees of topographical complexity [5].

Rebreathers have a long and complex history in scientific diving, while originally used primarily for military and commercial purposes, rebreathers began to find applications in scientific research with innovations such as Walter Starck's electronically-controlled closed-circuit rebreather (CCR) for undersea biological studies [10]. With the advent of state-of-the-art electronically-controlled, closed-circuit, mixed-gas rebreathers (eCCRs), the capabilities of scientific divers have expanded dramatically, allowing for safer and more efficient exploration of deeper and previously inaccessible marine environments.

Modern eCCR technology addresses many of the limitations of early self-contained underwater breathing apparatus, providing enhanced safety and functionality for underwater researchers [10]. Traditional diving methods using air are safe for shallow short dives, but as depth increases, available bottom time decreases due to decompression obligations, gas consumption, and thermal considerations. Beyond 30 meters, helium-enriched gas mixtures such as trimix are employed to mitigate the narcotic effects of nitrogen and reduce the risk of oxygen toxicity, though these require managing bulky and heavy open-circuit systems with multiple gas cylinders. This rebreathing principle not only enhances mobility and reduces the physical burden on divers but also extends dive durations and depths, making eCCRs an invaluable tool for in-situ scientific research in challenging underwater environments.

We conducted an ecological survey of four marine habitats of the Jubilee Shoals (Cyprus, eastern Mediterranean) between 17÷45 m depth. The use of eCCR was particularly convenient for the evaluation of the epibenthic communities (e.g., photo-quadrats, sediment and plankton samples) and mapping at the deepest areas of the shoals, including inside a cave system. Motivated by the extremely time-efficient survey, we report here the eCCR protocol we followed and the partial results of the evaluation of the epibenthic community (e.g., percentage of cover) inside the cave. We concentrate on the analysis to determine the number of photo-quadrats needed for a proper representation of the species richness in the cave's zones and discuss the advantages that research divers can have using eCCR.

Materials and Methods

The Jubilee Shoals (Petra tis Avdhimou, original toponymy) consists of a submerged rocky reef with a plateau situated 2.4km off Avdhimou Bay (SW coast Cyprus; Fig. 1). The shoals support a complex ecosystem, including three priority habitats recognized by the EU Habitats Directive (Council Directive 92/43/EEC): *Posidonia oceanica* beds at the top, coralligenous on the shoal's walls, and sciaphilic communities within a cave system near the sandy bottom (Fig. 1). The cave system has three entrances between 40÷45 m depth, with an average cave depth of 38 m.

The surveys to evaluate the percent of benthic cover inside the cave were carried out from May to November 2023 by means of eCCR dives (Fig. 2). The cave system was divided into three zones—entrance, semi-dark, and dark—using a bionomic model developed by Pérès [9]. Photo-quadrats (13 cm x 19 cm) to measure benthic cover were taken on the cave walls and ceiling using an Olympus TG-6 underwater camera equipped with a WEEFINE smart focus 5000 light to document the hard substrate epibenthic communities. This compact underwater photographic setup enabled divers to easily navigate narrow passages in the cave to record the system's biodiversity.

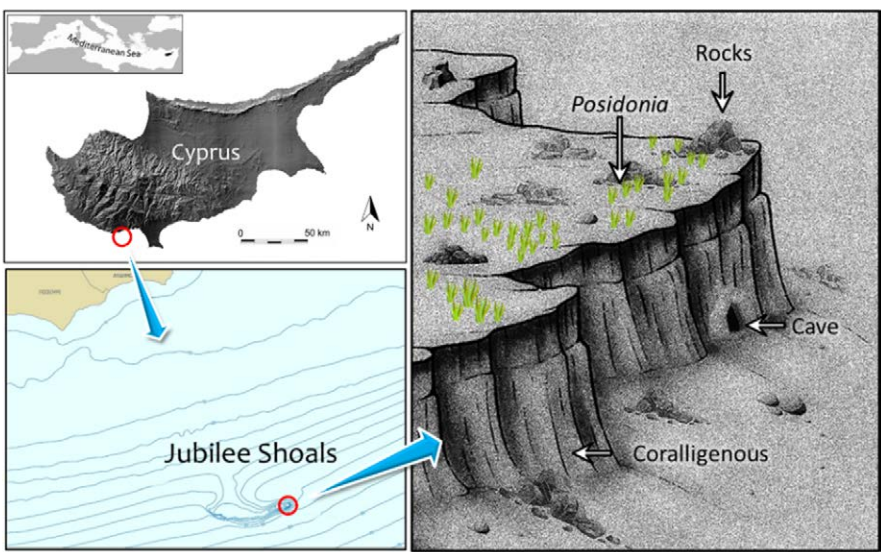

Figure 1 – Jubilee Shoals are located in the SW coast of Cyprus. The rocky outcrop (17÷45 m depth) supports important habitats, such as *Posidonia oceanica* meadows, rocky refs, coralligenous and sciaphilic communities inside and around the cave system.

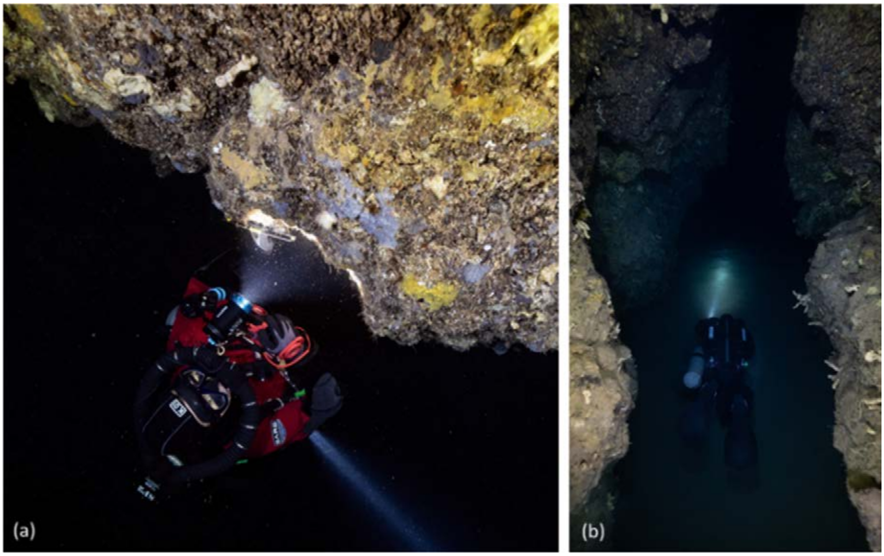

Figure 2 – (a) Epibenthic community on the cave's ceiling surveyed with photo-quadrats (b) Silent and bubble-free surveys of mobile species in the cave's galleries.

At each zone of the cave, 60 replicates (photo-quadrats) were taken from the cave walls (two flanks, 20 replicates each) and ceiling (20 replicates). The PhotoQuad v1.4 software [11] was used for the analysis (N=180 photo-quadrats, 100 points uniform spawn). Additionally, high-resolution macro photos and targeted samples were collected to identify organisms to the lowest possible taxonomic level; organisms whose taxonomy remains to be resolved were assigned to morphospecies. Percentage of cover was classified into seven main categories: Porifera, Cnidaria, Calcareous Algae, Bryozoa, Polychaeta, Others (e.g., Ascidia, Brachiopod, Mollusca, Foraminifera), and non-living Substrate. The R package 'vegan' was used to calculate the Rarefaction curves (version 4.4.1), using random re-sampling of the taxonomic richness of 20 quadrats/zone.

For the eCCR dive calculation and planning, a specialized multi-decompression (multi-deco) program was used. This program, when provided with basic information, such as depth and duration of the dive (among other factors), generates a comprehensive diving plan to ensure safe decompression procedures are followed.

Dive planning Following SSI guide standards

Fixed Parameters: 40 meters Depth, 90 minutes Bottom time, EAD: 30 meters Depth, Limiting ppO2: 1.10, Oxygen in non-narcotic, RMV Bottom: 15, RMV Deco: 17, Setpoint: 1.3, Decompression Model ZHL 16-C + GF, GF: 50/80, Surface interval = 1 day, Elevation = 0 m.

Gas Selection: Step 1: Calculate the oxygen content. Fraction of oxygen (FO2) = Limiting ppO2 / Depth = 1.10/5 bar = 0.22. Step 2: Calculate the nitrogen content: Fraction of nitrogen (FN2) = (Atmospheric partial presser of nitrox * END) / Depth = (0.79 * 4) / 5 = 0.632. Step 3: Calculate the helium content: Fraction of helium = 1- (FO2+ FN2) = 0.148. The best Trimix (TMX): 22/15.

Gas Fraction in the Breathing Loop: Step 1: Calculate the fractions of oxygen in the Breathing Loop: Fractions of oxygen = Setpoint / Depth = 0.26. Step 2 Calculate the fractions of nitrogen in the Breathing Loop: Fractions of nitrogen = [(Depth – Setpoint) * (Nitrogen + Helium)] / Depth = [(5 bar – 1.3) * (62 (%) / 62 (%) + 15 (%))] / 5. Fractions of nitrogen = (3.7 * 0.8) / 5 = 0.592. Step 3: Calculate the fractions of helium in the Breathing Loop. Fractions of helium = 1 – (Fractions of oxygen + Fractions of nitrogen) = 0.148

Equivalent Narcotic Depth (END) = {[FN2 * (Depth + 10)] / 0.79 – 10 = {[0.6 * (40 meters + 10)] / 0.79} – 10 = 27.9 meters

Based on these calculations, a diver can be confident that, when diving to 40 meters with a diluent trimix 22/15 and with a setpoint of 1.3, the actual gas on the loop is trimix 26/15. For logistical reasons, it was not possible to use trimix in this diving operation, so the dives were carried out without trimix. But it is important for this study to show you in detail how to calculate gases under ideal conditions.

Results

Benthic cover

The percentage of benthic cover for each of the seven categories is shown in Figure 3. In general, one category, Porifera, was on average higher than 20 % of benthic cover. Cnidaria (e.g., scleractinian corals), Calcareous Algae and Polychaeta showed noticeable range variations, between 1.7÷35.6 %, 0÷27.1 % and 1.3÷12.1 %, respectively. Bryozoa and Other were consistently low, between 0.2÷11.8 % and 2.1÷15.6 %, respectively. Not surprisingly, the Non-living Substrate was the category with the largest range, between 5.2÷53.5 % of benthic cover. Since the main purpose of this paper is to determine the adequate number of photo-quadrats needed for a good representation of the taxonomic richness from different zones of the Jubilee Shoals' cave, no further analysis was made (e.g., statistical comparison of benthic cover between zones).

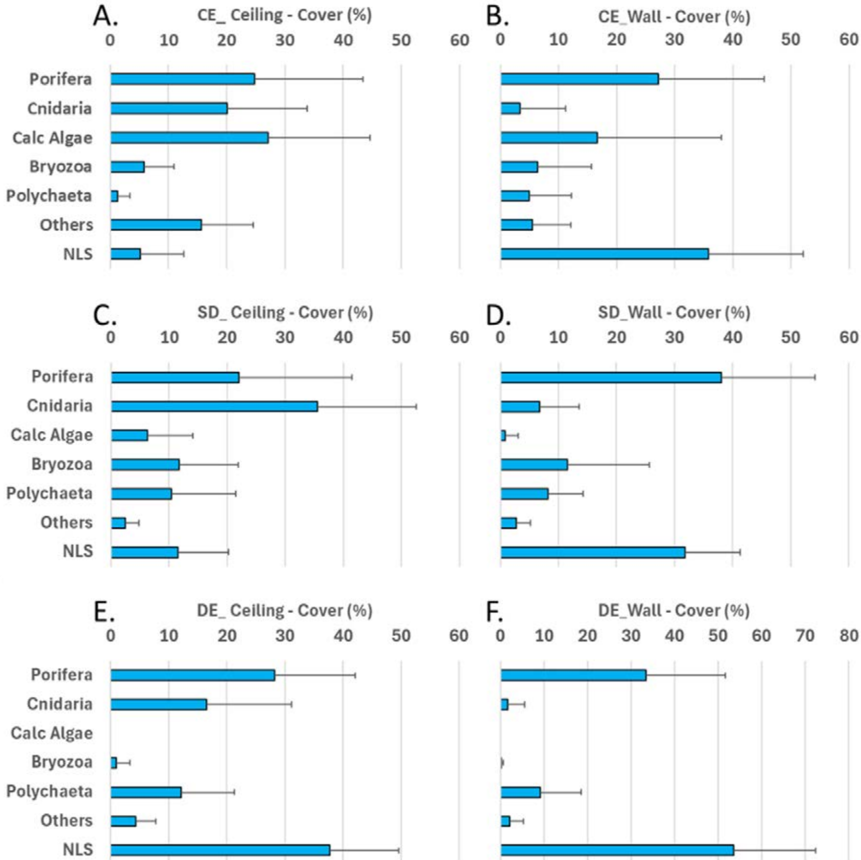

Figure 3 – Percent benthic cover (mean + 1SD; n=20 photo-quadrats) according to seven major categories in different sections of the cave. (A, B) Entrance zone: CE; (C, D) Semi-dark zone: SD; (E, F) Dark zone: DE. NLS stands for Non-living substrate.

The number of species as a function of the number of samples (photo-quadrats) taken, rarefaction curves, are shown in Figure 4. An adequate representation of the number of species, taxonomic richness, is achieved between 11-18 photo-quadrats in most of the cave's zones. The case of the Entrance zone Celling (C, Figure 4A) is different; the results from this zone suggest that more than 20 photo-quadrats are needed for an adequate representation. This is the zone where three categories (Porifera, Cnidaria and Calcareous Algae) are on average higher than 20 % of benthic cover (Figure 3A).

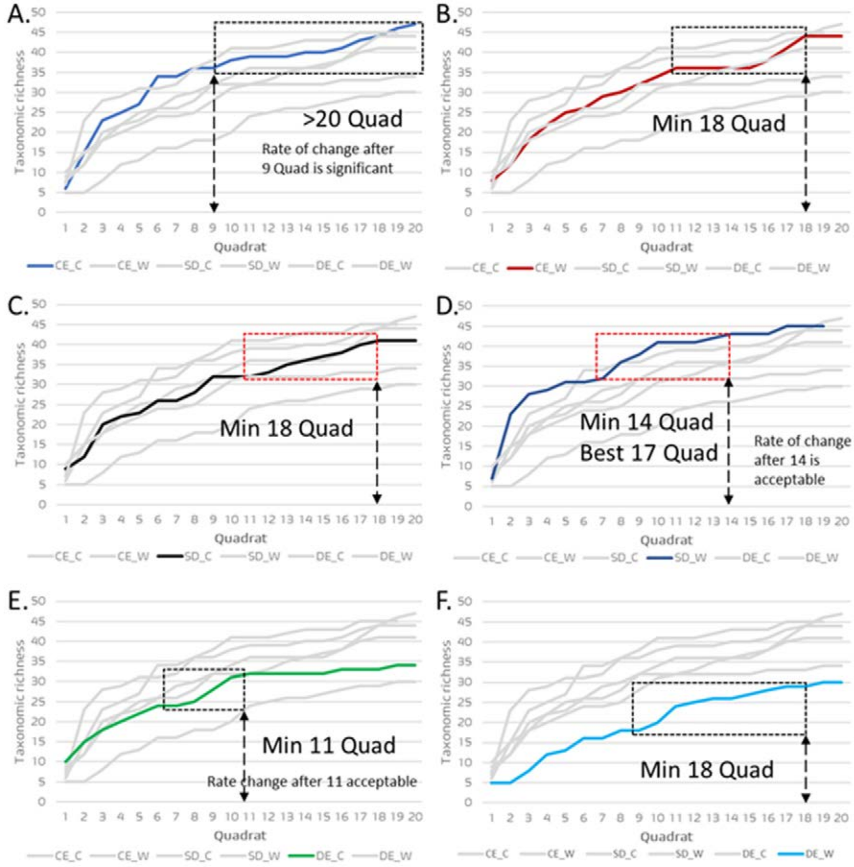

Figure 4 – Rarefaction curves to estimate the expected number of species (taxonomic richness) according to the number of photo-quadrats from different section of the cave: (A, B) Entrance zone: CE; (C, D) Semi-dark zone: SD; (E, F) Dark zone: DE. Rectangles indicate a high rate of change in species number. The suggested number of photo-quadrates necessary for a proper representation is shown with dashed lines and arrows.

Diving plan

By inputting all necessary data into the multi-decompression software, the dive profile was generated (Figure 5). This process also determined the required volume of gas for the dive, applicable to both open circuit and closed-circuit systems (Table 1). It is important to note that the Nitrox 50 and Oxygen used in the Closed Circuit Rebreather (CCR) are designated as bailout gases. This means they are reserved for emergency use and will not be utilized during the dive unless a problem with the CCR arises. Additionally, discussions with experienced closed circuit diving instructors led to the recommendation of different gas mixtures for this dive (Table 2).

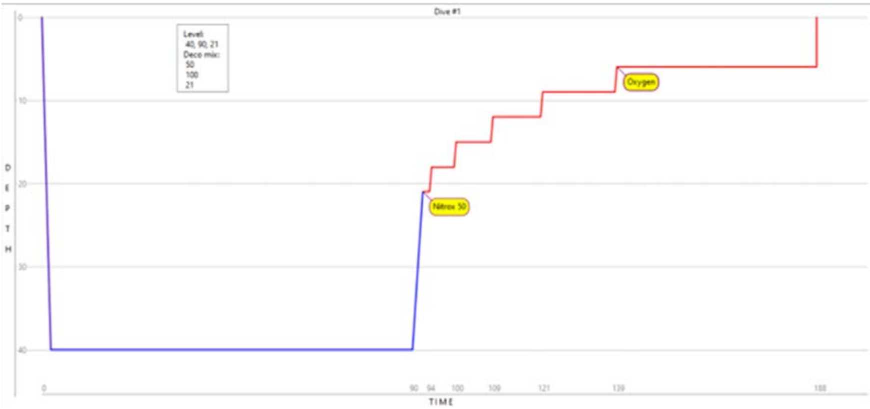

Figure 5 – The diving profile uses atmospheric air (21 %) as diluent.

Table 1 – Gases that will be needed performing the same dive with open circuit: OC and closed circuit: CCR.

	Air (litre)	Nitrox 50 (litre)	Oxygen (litre)
OC	6785.5	1786.1	1337.4
CCR	143.4	1428.4	1174.7

Table 2 – Data generated by multi-decompression software using different diluent.

	Dive time (min)	CNS Total	OTU's this dive	Gas density
OC	189	141.4 %	248	6.0 g/l
CCR	175	143.3 %	261	6.0 g/l
CCR TRIMIX (22/15)	167	139.8 %	251	5.3 g/l
CCR TRIMIX (20/20)	165	139.3 %	250	5.1 g/l
CCR TRIMIX (20/30)	167	142.3 %	252	4.6 g/l
CCR TRIMIX (21/35)	168	145.8 %	255	4.4 g/l

Discussion & Conclusion

The global marine environment has been modified by human activities and climate change with negative consequences, such as loss of biodiversity and disrupted ecological processes. In the Mediterranean, submerged marine caves are considered reservoirs of native biodiversity under the pressure of diverse agents of deterioration [2]. The cave system at the Jubilee Shoals in Cyprus, is an example of an important ecosystem with an almost unknown biodiversity. This study is part of a major effort to document the shoals' habitats biodiversity in order to take actions for their protection and management. However, the conditions to study the cave system are highly demanding because the depth and the restricted environment inside the cave. These factors pose a logistical challenge for the estimation of the benthic cover, particularly when the safety of the scientific divers is unquestionable. The minimum number of samples or photo-quadrats determined in this study, reflects the particular nature of the different zones inside the cave. Only in one case (entrance of the cave) a larger sample number that what was tested in this study is required (more than 20). Nevertheless, determining the sample number is far from being a trivial exercise; more often than not, studies are being conducted without pilot-testing in order to identify the appropriate sample number. It translates into efficiency and effort requirements and logistical aspects (bottom time). This important outcome of the study can be used when deciding the methodology to survey similar environments in other areas.

Up to a depth of 10 meters, open-circuit diving proves more efficient due to its simplicity and practicality. These shallow depths offer extensive bottom times and necessitate relatively modest gas volume requirements. However, challenges arise in buoyancy control and equipment management when employing closed-circuit systems in such environments.

At greater depths exceeding 15÷20 meters, closed-circuit rebreathers offer distinct advantages, notably in extended bottom times. By enabling precise gas mixture regulation tailored to specific depths, these systems minimize nitrogen absorption by the diver's body, thereby substantially reducing decompression obligations. Additionally, the ample gas volume available to the diver enhances both bottom time and operational efficiency. Notably, closed-circuit systems also reduce tank volume and refill frequency requirements, further enhancing operational efficiency.

Nonetheless, a noteworthy drawback of closed-circuit systems lies in their reliance on a 100 % oxygen supply. For dives deeper than 30 meters, helium-enriched gas is recommended, with minimal helium volumes required for depths between 30 and 50 meters compared to the significant advantages it offers, including mitigating nitrogen narcosis and ensuring clearer cognitive function during dives. Furthermore, open-circuit diving generally offers superior breathing quality.

When evaluating the merits and drawbacks of open-circuit versus closed-circuit systems, careful consideration must be given to the specific demands of both dives and equipment provisioning at the chosen location. Enhanced safety stands as a significant advantage of closed-circuit systems, particularly when utilizing helium-enriched gas, although atmospheric air can also suffice for such dives.

A prevalent concern in project planning involves designs crafted predominantly by scientists employing diving as a research tool, often at the expense of safety considerations. Instances where scientists exceed their diving limits to collect requisite data pose significant safety risks, indicative of a broader knowledge gap in diving theory among scientific practitioners. Such scenarios underscore the necessity for scientists to possess comprehensive theoretical and practical diving expertise to foster trust and ensure safety within diving teams.

Crucially, effective scientific diving missions demand the assignment of two responsible individuals—one overseeing the scientific aspect and the other managing diving operations. A harmonious collaboration between these two roles ensures informed decision-making and maximizes data collection efficacy while upholding stringent safety standards.

Based on the results in Table 2, we conclude that a diluent with less than 30% helium is most suitable for scientific dives at this depth. This choice reduces gas density, which in turn facilitates easier work of breathing, lowers the risk of CO2 buildup, and enhances safety during underwater operations.

Our study addressed the dual requisites of producing quality data of the epibenthic communities while ensuring divers' safety by determining the minimum quantity of samples (photo-quadrats) necessary for a proper representation of the taxonomic richness, and by using an eCCR diving protocol. If known, the minimum quantity of photo-quadrats reduces the sampling effort and the eCCR diving protocol maximizes the divers' bottom time. Both are important contributions of this study that can be applied to similar cave environments assisting in the generation of knowledge of these remarkable ecosystems.

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