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Cover: Marine invertebrates - made with acrylic paint. © P. Kritika.



## INTRODUCTION

Coral diseases are one of the major factors implicated in the decline of shallow water coral reef ecosystems worldwide (Sutherland et al. 2004; Hazraty-Kari et al. 2021). Among the diseases recognized to date, cyanobacterial Black Band Disease (BBD) is one of most well-studied diseases, affecting at least 24 scleractinian, one hydrozoan, and six gorgonian species in the Atlantic and Indo-Pacific oceans/seas (Antonious 1973; Bruckner 2015; Roff 2016). Within the Japanese archipelago, BBD has been reported from both mesophotic and shallow reefs affecting six coral genera: *Montipora*, *Acropora*, *Echinopora*, *Pachyseris*, *Goniastrea* and *Gardineroseris* (Wada et al. 2017; Kubomura et al. 2018; Das et al. 2022a) and among these, the encrusting form of genus *Montipora* can be considered one of the primary hosts in the region (Wada et al. 2018; Das et al. 2022a).

Coral diseases have been widely studied by field-based in situ observations, which obviously provide several benefits such as ease of observation and the need for few pieces of equipment. However, such observer-based data are also prone to bias and inconsistency, as well as often only being able to cover only limited areas. An alternate method of observation to cover large areas is remote sensing-based disease assessments (Kabiri et al. 2013; Maynard et al. 2015), although such methods have their own limitations such as interference via clouds and dust (Purkis 2018). Recently, consumer-grade drones have been increasingly used to monitor coral reef ecosystems (Casella et al. 2017; Kabiri et al. 2020), and this relatively low-cost equipment can clearly increase the ease of monitoring coral reefs (Murfitt et al. 2017). While the commercialization of cheap drones is recent, the concept existed earlier with suggestions of utilizing high-resolution cameras along with multiple spectrum/hyper-spectral sensors on hexacopters, and the usage of hydrogen balloons (Rützler 1978; Kabiri et al. 2014). Here, we utilized such drone technology to detect and identify cyanobacterial BBD infecting individual coral colonies on a nearshore intertidal habitat in Okinawa, Japan.

## MATERIALS AND METHODS

On a clear day on the 09<sup>th</sup> of September 2021, we flew a commercial drone (Mini2, DJI Technology Co. Ltd.) equipped with a 1/2.3" inch CMOS sensor camera (12 MP) over the shallow nearshore reefs of Sesoko Island (off central western Okinawajima Island), near Sesoko Station, University of the Ryukyus (26.6340°N,

127.8641°E) (Das & Yamashiro 2018; Das et al. 2022a). The reef in this area is comprised of various morphotypes and species of the genus *Montipora*, such as *Montipora* cf. *aequituberculata*, *Montipora* cf. *digitata*, and encrusting *Montipora* cf. *informis*, *Montipora* cf. *efflorescens*, and other *Montipora* spp. (Yamashiro et al. 2000; Baird et al. 2018; Das et al. 2022a). *Montipora* spp. in Okinawa have been recently reported to be easily infected by BBD (Das et al. 2022a). The time and the day of our survey were chosen based on low wind and tidal conditions. We identified BBD-infected encrusting *Montipora* colonies from drone imagery. Simultaneously, reef walking and snorkeling was conducted to locate and confirm BBD-infected colonies initially identified by the drone imagery.

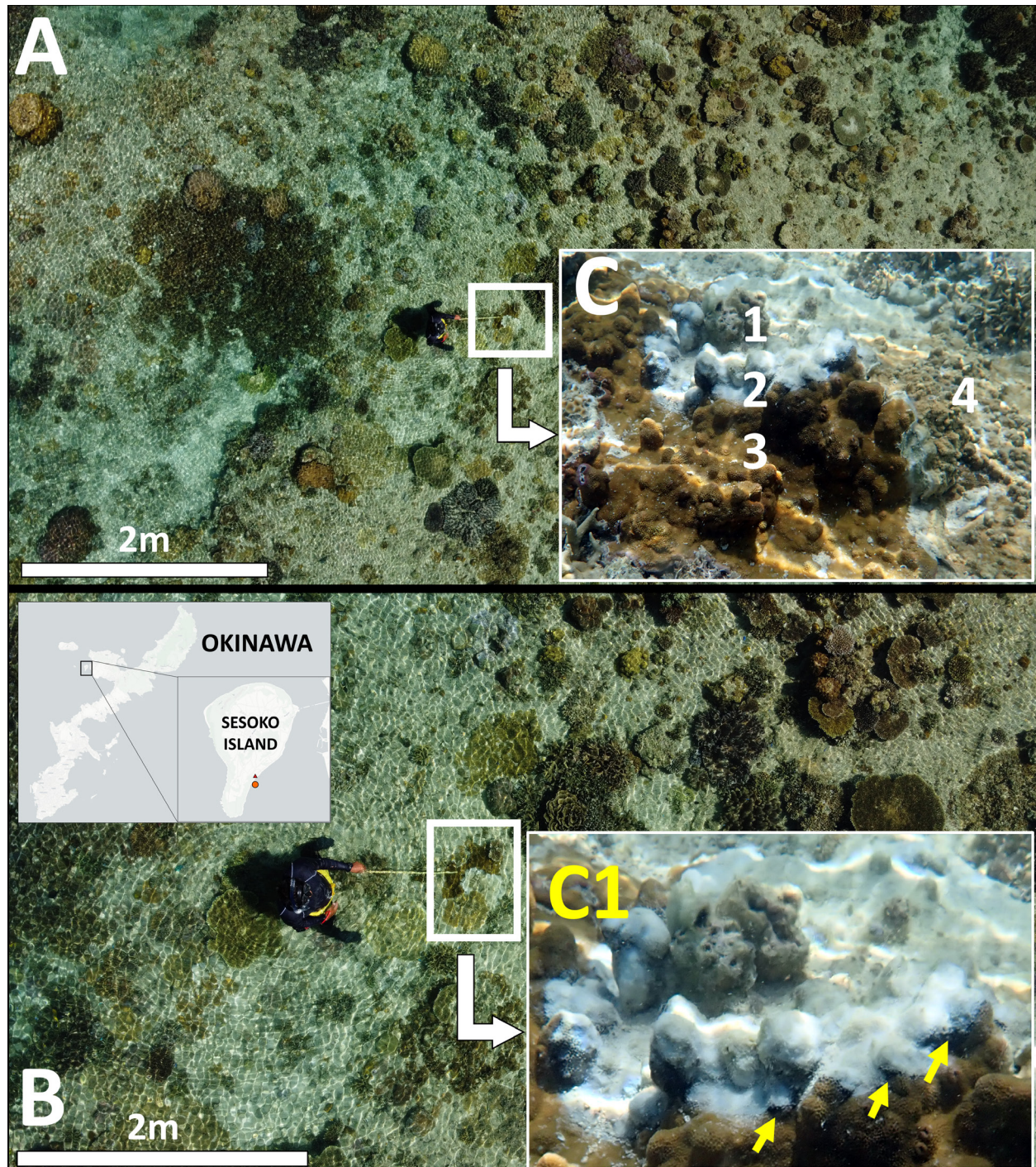
In this study, a total number of 148 images (4000 x 2250 pixels) and four videos (1920 x 1080 p; obtained between 9000 h to 1600 h) were taken from different heights between 30 to <100 m.

Aerial photographs were converted from initial .jpg to .tiff format. Three photographs were specifically chosen based on clarity and low sea surface reflection (sun glint), and in each of them a 5x5m quadrat was delineated. Additionally, wherever possible, in-situ images of the infected colonies were taken during reef walking/snorkeling (Olympus TG-5 camera/PT-058 housing). The drone images were then uploaded into open-sourced Coral-Net software (Beijbom et al. 2012) for benthic analyses. The benthic components were classified as tabular, encrusting, foliose, massive/submassive hard corals, dead coral, hard substrate/sand, and others. A total of 175 randomly generated points were created in each of the three 5x5 quadrats and were categorized accordingly. The categories were then identified visually based on the above-mentioned classification. Automatic annotation was not applicable since a minimum of twenty images are required to train the AI-based classification algorithm in Coral Net to provide satisfactory results (Chen et al. 2021).

## RESULTS AND DISCUSSION

Benthic analyses revealed encrusting hard corals had the highest percentage cover within quadrats ( $12.57\% \pm 5.72$  SD), followed by massive/submassive ( $6.09\% \pm 4.05$  SD) and tabular corals ( $2.66\% \pm 1.19$  SD) (Supplementary Table 1). Soft corals along with other benthos accounted for  $23.05\% \pm 4.01$  SD, while dead corals accounted for  $2.48\% \pm 2.16$  SD. A total of six encrusting *Montipora* colonies with BBD were identified through these drone images and videos. The dead white bare skeleton along



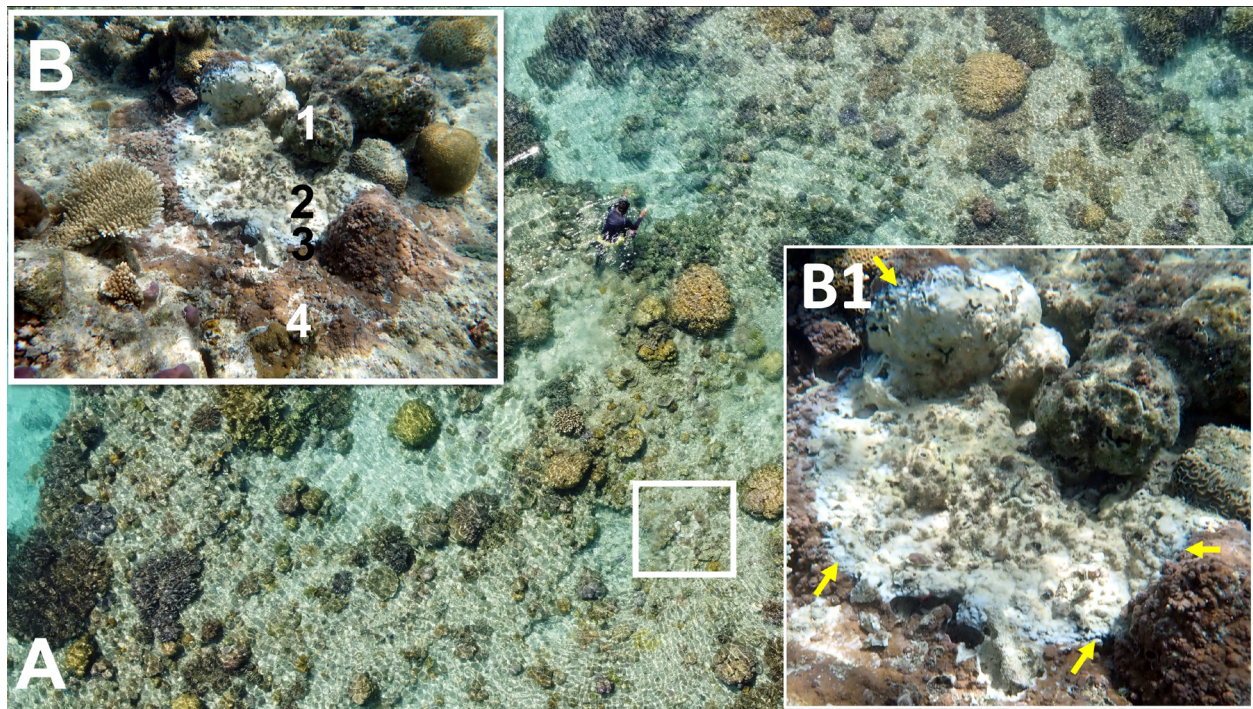


**Image 1.** A—Aerial view of the study area with a BBD-infected encrusting *Montipora* colony (white arrow) | B—Close-up image with BBD colony (White box) | C—In situ image of BBD infected colony (1—Recently dead coral skeleton | 2—Progressing BBD | 3—Healthy coral | 4—Healthy *Montipora* colony competing for space) | C1—Close-up image, yellow arrows indicating progressing black band. The black band is not as thick and wide probably due to low tide and direct exposure to sunlight. © Image A and B: PT-K; Image C and C1: RRD.

with neighboring healthy tissue was clearly visible through the high-resolution images (Image 1A–C, C1; 2A, B, B1). To our knowledge, this is the first study where cyanobacterial BBD affected areas were surveyed through drones.

Globally, coral reef health has been successfully monitored through the usage of commercial drones. Within the Persian Gulf, commercial drones have been used to map coral reefs, revealing coral mortality and bleaching (Kabiri et al. 2020). In a similar study at





**Image 2.** A—Aerial view of the study area with BBD colony | B—Close up of the same colony (white box) via Olympus TG5 camera (1—Dead coral skeleton overgrown by turf algae | 2—Recently dead coral skeleton | 3—Progressing BBD | 4—Healthy coral) | B1—Further zoomed image of (B), arrows indicating the progressing black band. © Image A: PT-K; Image B and B1: RRD.

Kaneohe Bay, Hawaii, coral bleaching was observed using drones (Levy et al. 2018). In the current study, we specifically focused on a readily observable disease (BBD) within a specific study area, combined with simultaneous in situ verification.

A fully developed BBD has a thick microbial consortium, generally black/dark in color, the dark coloration from the dominant cyanobacterium *Roseofilum reptotaenium* (Hutabarat et al. 2018). Wada et al. (2017), in research at Akajima, Okinawa (~40 km from Sesoko Is.), further showed four different types of BBD patterns; black bands; grey bands; mottled black bands, and an absence of bands, all affecting encrusting *Montipora*. At greater depths of >30m, BBD appears purple-black due to the lack of sulfide oxidizing (SO) bacteria *Beggiatoa* sp. (Kubomura et al. 2018). These SO bacteria form a major population within shallow water BBD bacterial mats and are thought to be responsible for the whitish coloration of BBD during the night (Richardson 1996).

In future studies, there remains a necessity to focus on more diverse coral genera which are affected by numerous other diseases and pathogens. Thus, this work demonstrates the potential of incorporating drones while concurrently doing field observations under appropriate conditions (low wind, low surface sunlight reflection, etc.). Additionally, drone usage will be very

effective if diseases have reached epizootic levels within a given reef. Further, recurrent observations of the same reef could also provide time-series datasets. Additionally, we were able to cover a large reef area in much shorter time than when compared to snorkeling/free swimming methods. The drone was flown over an area of 7,000 m<sup>2</sup> with approximately 75 m<sup>2</sup> (n = 3 of 5 X 5 m quadrats) was considered for analyses of coral percent coverage. It can be argued that drone-based observations are only limited to shallow reef ecosystems, but reefs in such shallow waters are often diverse and are among the most threatened by anthropogenic factors (Richards et al. 2015). We suggest the use of similar methodology to understand other forms of coral diseases, such as the coral-killing sponge *Terpios hoshinota* ("Black Disease"), which threatens intertidal reefs in many areas of southern Japan (e.g., Reimer et al. 2010, 2011) and elsewhere in the Indo-Pacific (e.g., Montano et al. 2015; Das et al. 2020).

Finally, we provide a few considerations and suggestions for utilizing drones to monitor coral reefs. Drone flight is feasible only under optimal environmental conditions, with successful flights and good-quality image acquisition hampered by strong winds, rain, or even too much sunshine. Additionally, even if drones can cover large areas, it is possible to overlook colonies



that have just begun to exhibit early signs of infection. For instance, BBD initially develops as a cyanobacterial patch (Sato et al. 2009), and because these early phases may not have considerable tissue loss, such colonies can be easily overlooked. Similar issues may exist in the case of other diseases and should be considered before research planning. Another issue is that drones can only clearly view very shallow or intertidal reefs, and to reach deeper reefs, submersible drones would be necessary (Das et al. 2022b). Finally, if there is a large population of marine birds in the survey region, drone flights should be carefully monitored or should be flown at a suitable height.

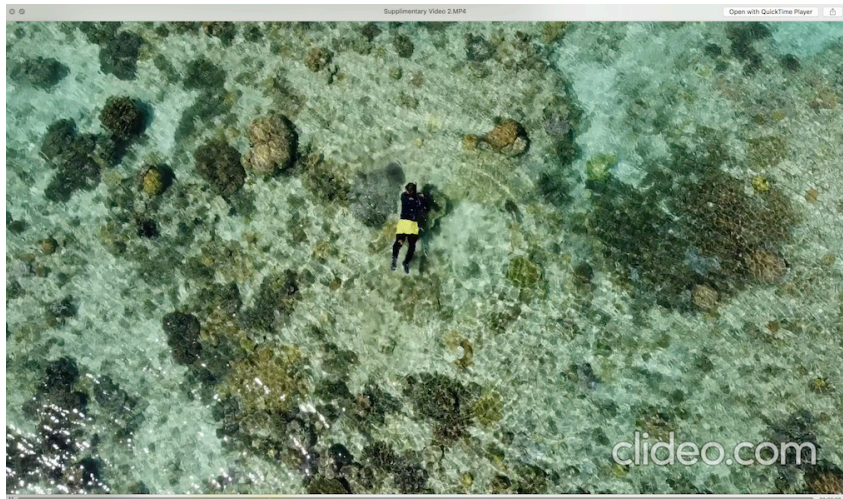
Therefore, based on these limitations and recommendations, we conclude that drones can be regarded as tools to be included when or if the weather conditions are suitable. Such technologies can significantly improve the efficiency of surveys of coral diseases and aid in creating effective management strategies for the preservation of the coral reef ecosystems.

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Supplementary Table 1. Percent coverage of benthic categories obtained from CoralNet.

Image ID	Image name	Annotation status	Points	51_tabular_hard_coral	Hard Coral (encrusting)	Hard Coral (foliose)	Hard Coral (massive/submassive)	Dead coral	Hard Substrate/Sand	Other
2293735	Drone-Q1-RRD-Sesoko-5x5-DJI_0385.JPG	Confirmed	175	4	18.286	1.143	1.714	4	47.429	23.429
2293736	Drone-Q2-RRD-Sesoko-5x5-DJI_0346.JPG	Confirmed	175	2.286	12.571	0	6.857	0	59.429	18.857
2295906	Drone-Q3-RRD-Sesoko-5x5-DJI_0451.JPG	Confirmed	175	1.714	6.857	0	9.714	3.429	51.429	26.857
		Average		2.666666667	12.57133333	0.381	6.095	2.4763333	52.76233333	23.048
		SD		1.189592087	5.714500007	0.659911358	4.05406993	2.163488	6.110100927	4.0136

Supplementary Video 1. In situ observation of BBD-infected encrusting *Montipora* colony.Supplementary Video 2. In situ observation of BBD-infected encrusting *Montipora* colony.

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