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NOTE

Observations of coral and cryptobenthic sponge fluorescence and recruitment on autonomous reef monitoring structures (ARMS)

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Abstract Fluorescence imaging of benthic communities is a widely used tool for determining the rate of hard coral recruitment in tropical reefs. Whilst fluorescent proteins are well-studied in scleractinian corals, less is understood about their distribution and function in other sessile reef invertebrates. This short study examines fluorescence images of benthic communities on Autonomous Reef Monitoring Structures (ARMS) from a remote and protected Indian Ocean reef system. We compare the abundance of adult and juvenile hard corals across three sites and between the topside and underside of ARMS recruitment plates. We also discuss observations of skeletal fluorescence in sponges, as well as uneven green fluorescent protein (GFP) concentrations across adult coral colonies. Our findings provide an insight into the recovery of shallow reefs previously hit by severe bleaching events and highlight the potential of ARMS fluorescence imaging for the analysis of cryptobenthic communities.

Keywords Fluorescence · Recruitment · Coral · Sponge · GFP · Autonomous reef monitoring structures

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Introduction

Recording the success and rate of the recruitment of reef benthic organisms can give us vital insights into coral reef health, recovery, and diversity. With high mortality bleaching events increasing in frequency across tropical reefs, and in the face of bleak climate predictions (IPCC 2021), it is necessary to monitor recruitment patterns if we are to predict and understand the state of future reefs and implement useful management plans. This is especially true of reefs which have experienced severe bleaching events in the past and can now be said to be at high risk from further large-scale climate-induced mortality events (Sheppard et al. 2020).

Reef sessile invertebrates, such as hard corals, soft corals, sponges, ascidians, tube-forming worms and bivalve molluscs, shape or are anchored to the reef matrix. Cryptic surfaces and crevices within the reef matrix often harbour highly diverse communities of non-hard coral invertebrates and provide shelter for young coral recruits (Kornder et al. 2021). Studying the recruitment of both hard corals and non-coral invertebrate recruitment in-situ can be complicated, as cryptic spaces are often inaccessible for sampling or photography. Autonomous Reef Monitoring Structures (ARMS) are artificial recruitment devices used for the collection and study of diversity usually found in cryptic reef spaces (Carvalho et al. 2019; Pearman et al. 2020). Composed of 9 stacked PVC plates, with alternating gaps between each layer, each ARMS provides recruitment surfaces and varied microhabitats for cryptobenthic fauna. These devices are now employed around the world to study these communities using a mix of standardised genetic and image analyses.

Fluorescence imaging is a popular census technique for identifying coral recruits on artificial tiles or in-situ reef surfaces (Baird et al. 2006; Zweifler et al. 2017). This method allows the capture of fluorescent pigments within organisms

such as green fluorescent proteins (GFPs), other fluorescent proteins (FPs) and photosynthetic pigments such as chlorophyll. Fluorescence imaging of hard coral recruits on artificial recruitment tiles has been widely used, but no study has yet used it to look at sessile communities on ARMS devices. In this study, we present results from high-resolution fluorescence images of ARMS devices deployed across the Chagos Archipelago, a remote and protected Indian Ocean reef system. We record abundances of hard coral adult colonies and juvenile recruits from sites previously impacted by severe bleaching events (Head et al. 2019) and present observations of the distribution of coral fluorescent pigments across ARMS. Observations of fluorescence from the skeletal elements of several sponge specimens are also presented and discussed.

Materials and methods

Fluorescence images were taken of Autonomous Reef Monitoring Structures (ARMS) in April 2021 in the Chagos Archipelago Marine Protected Area (MPA) as part of a wider research project on shallow reef benthic communities. Triplicate ARMS devices were retrieved from a depth of approximately 5–12 m across three sites across the northern atolls, including two exposed ocean-facing reefs (Ile Anglaise, 5°20′04.7"S 72°12′48.5"E, and Moresby, 5°14′00.3"S 71°49′50.3"E) and one sheltered lagoonal reef (Ile du Coin, 5°27′04.5"S 71°46′30.8"E). ARMS plates photographed for this article were retrieved and processed following standardised Global ARMS NOAA protocols after a 36-month deployment (Leray et al. 2015).

A Sony RX100 MkII camera with a Nightsea 450 nm barrier filter and two Inon Z240 UV strobe lights with Nightsea fluorescence excitation filters were used to capture sessile fluorescence on 153 plate faces across 9 ARMS devices (17 plate faces per device), from three shallow reef sites (Fig. 1). Photographs were taken at night inside a shaded bin with filtered seawater. Some plates were photographed multiple times to enhance resolution.

Images were processed using Adobe Lightroom Classic (for cropping, merging and enhancing brightness and contrast). The number of hard corals was counted in each image; individuals smaller than 15 mm were recorded as juveniles, whilst larger individuals were recorded as adults (Sheppard et al. 2017).

Counts of corals were then plotted using the '*ggplot2*' package in R (v3.3.5) and negative binomial generalised linear models were used to test for differences in abundance between sites and ARMS (as the data is count-based and does not follow a normal distribution), using the '*MASS*' and '*vegan*' packages (v7.3.54 and v2.5.7, respectively)(Venables & Ripley, 2002; Wickham, 2016; Oksanen et al. 2020).

Results and discussion

Hard coral recruitment across sampling sites and ARMS microhabitats

A total of 268 hard corals were counted on ARMS devices across the three sampling sites, with an average of 35 individuals per m^2. Juvenile recruits (< 15 mm) were consistently more abundant than adult colonies, with 57 juveniles and 34 adults found on average on each ARMS unit, and a density of 22 juvenile and 13 adult individuals per m^2 (Fig. 2). No significant differences in the abundance of adult or juvenile corals were observed between sampling sites, suggesting uniform recruitment patterns across sampled reefs.

The abundance of juvenile corals was found to be equal between the underside and topside of ARMS plates across all sites. Adult coral abundance was also equal between plate faces in Ile Anglaise and Moresby but was significantly

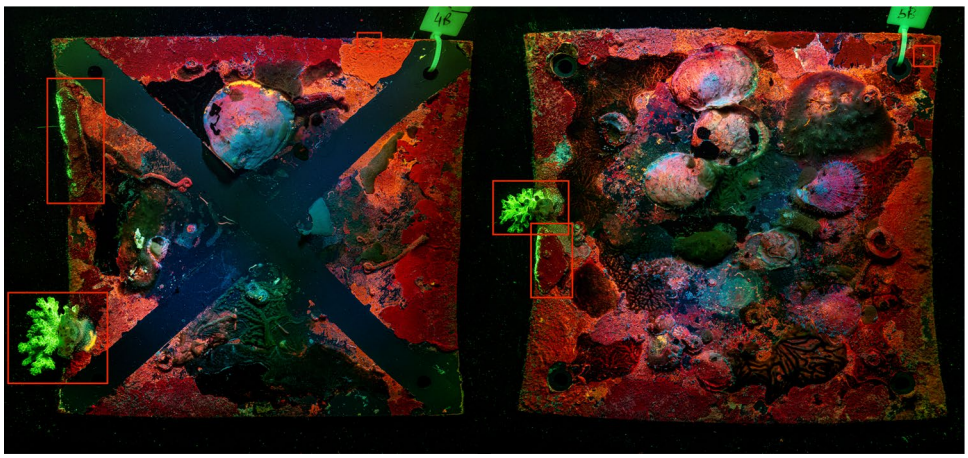

Fig. 1 Fluorescence images of two Autonomous Reef Monitoring Structure (ARMS) recruitment plates (23 cm × 23 cm). Red boxes highlight scleractinian coral adult colonies and juvenile recruits. The left-hand image is of a 'closed' surface, where PVC bars were placed across this plate and its adjoining neighbour to create four distinct recruitment surfaces, and the right-hand image is the equivalent with no PVC bars

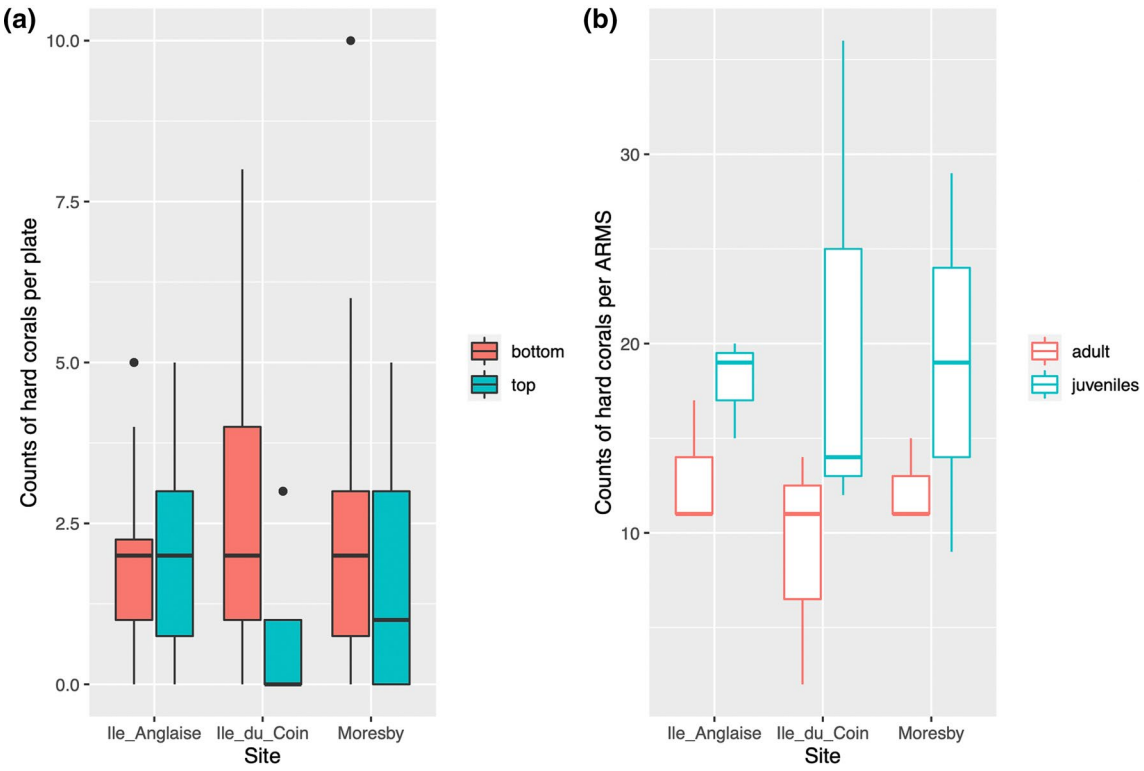

Fig. 2 Boxplots displaying count abundances of (**a**) all hard corals across top and bottom ARMS plate face images and of (**b**) juvenile recruits and adult coral colonies on ARMS devices across sampling sites

higher on plate undersides in Ile du Coin ($p < 0.001$***). This reef site is sheltered from prevailing currents and higher sedimentation has been observed in-situ compared to the two exposed reef sites. Juvenile recruit survival may be lower on the topside of ARMS plates at this site due to sediment loading.

The surface area of cryptic reef cavities has been shown to exceed that of exposed benthos by up to a factor of eight (Scheffers et al. 2010), meaning exposed surfaces represent only a portion of available recruitment space on the reef matrix. ARMS provide ideal refugia for non-photosynthesising cryptobenthic invertebrates but inadvertently also provide a desirable settlement surface for hard corals. Similar patterns of coral recruitment have been observed in other studies using artificial settlement tiles, with higher juvenile coral recruitment recorded in cryptic and grooved ridges than on flat exposed surfaces (Mallela 2018). Tight gaps between ARMS plates likely provide protection from grazers and physical damage (e.g. from loose rubble), resulting in coral colonies growing across sampling sites. Our results provide new insights into coral recruitment across reefs which are still recovering from back-to-back high-mortality bleaching events (Sheppard et al. 2020) and highlight the importance of investigating both exposed and hidden surfaces when assessing coral recruitment rates.

The highly standardised format of ARMS makes them an advantageous tool for studying coral recruitment with minimal disturbance to the natural matrix, as well as monitoring in-situ coral density of cryptic surfaces. However, whilst previous work has shown ARMS-based communities are comparable to those found across dead coral heads (Plaisance et al. 2011), further work is now required to determine whether ARMS's PVC plates bias the attachment and survival of hard coral juveniles compared to exposed natural reef surfaces. Fluorescence imaging allows for a quick scan of adult and juvenile recruits across ARMS plates but may overestimate counts due to increased signal to noise ratio or underestimate them due to genetic variability or the fact that shaded ARMS surfaces may lead to minimal or absent recruit fluorescence. Further work investigating coral recruitment on ARMS could include both daylight and fluorescence counts to allow for meaningful comparisons with similar studies.

Observations of fluorescence concentration across colonies

Fluorescent proteins are ubiquitous in scleractinian corals, but their functional role has often been a highly debated topic. Green fluorescent proteins (GFP) have been suggested

to play multiple roles including photoprotection (Smith et al. 2013), internal light regulation in mesophotic corals (Smith et al. 2017), immune response regulation (Palmer et al. 2009), and to attract symbionts (Field et al. 2006; Aihara et al. 2019).

Several adult hard coral colonies displayed uneven concentrations of green fluorescence across ARMS plates (Fig. 3). Encrusting colonies were observed to emit the brightest fluorescence closer to and along the exposed edges of ARMS recruitment plates (Fig. 3a, b and c). Furthermore, polyps along the topside of an adult *Pocillopora sp.* colony growing off the side of an ARMS plate (i.e. facing towards surface light) were shown to emit brighter fluorescence than polyps found on the underside of this colony (i.e. facing reef benthos) (Fig. 3d).

ARMS plates are stacked closely together with only 1-2 cm gaps between each plate face; this likely blocks a large amount of daylight from reaching the centre of each device. Patterns of fluorescence concentration across hard coral colonies were consistent with areas of ARMS plates

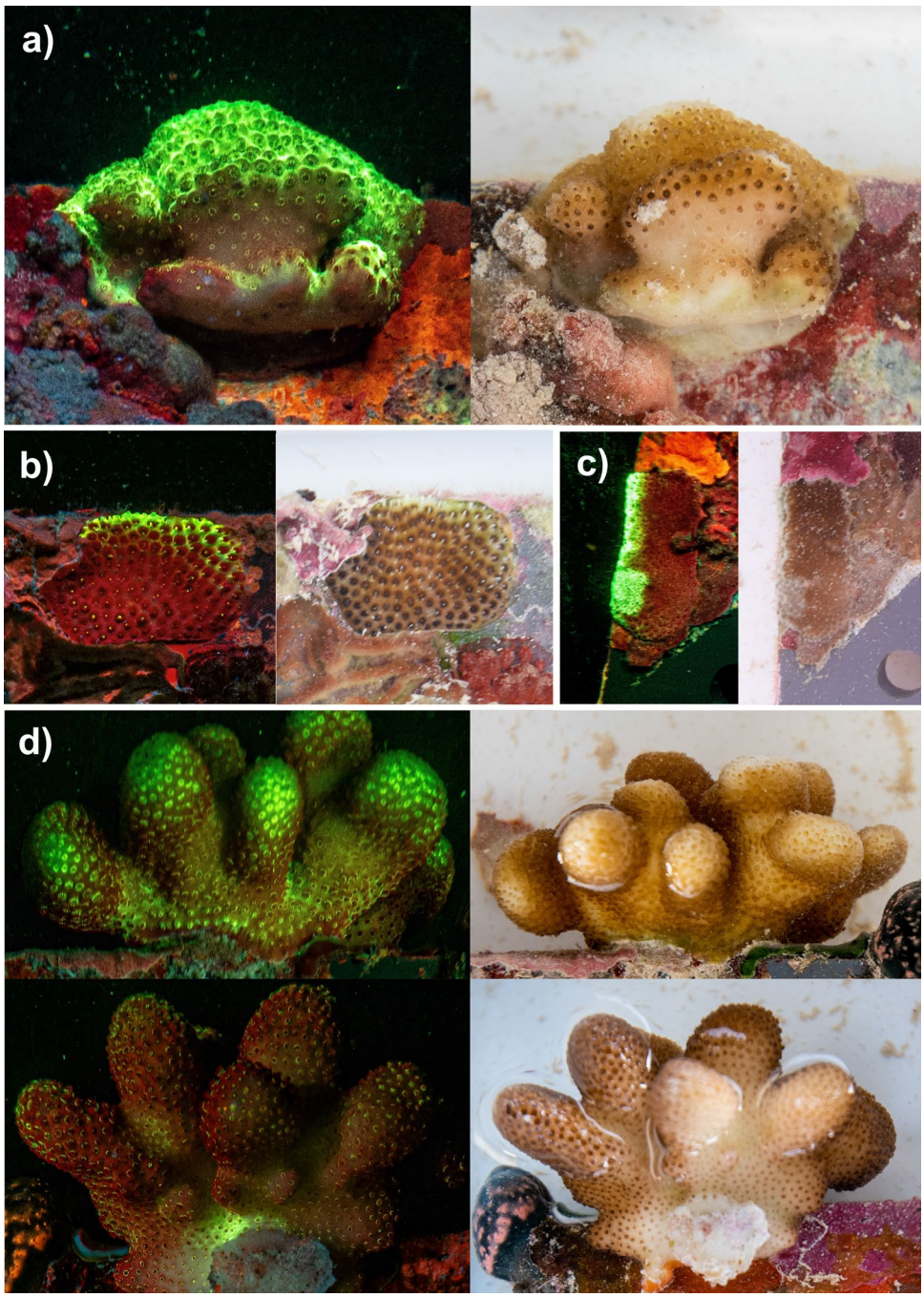

Fig. 3 Images of the same scleractinian coral colonies under UV light and daylight, where differences in GFP concentration can be observed across colonies close to ARMS plate edges (**a**, **b** and **c**) and between the topside (top left and right-hand images) and underside (bottom left and right-hand images) of the same *Pocillopora spp.* colony (**d**)

most exposed to direct sunlight (i.e. the outer edge of plates). Our results support similar findings from other studies analysing coral fluorescence patterns in response to controlled (D'Angelo et al. 2008; Smith et al. 2013) or in-situ (Bollati et al., 2020) light conditions.

Fluorescence patterns in sponges and other cryptobenthic taxa

Green and orange fluorescence was also observed across the skeletal elements of a few sponge specimens, from ARMS in the exposed Ile Anglaise site (Fig. 4). Deep-sea glass sponge spicules have been shown to have fibre-optical features (Sundar et al. 2003), and the close association between green algae and the siliceous spicules of demosponge *Tethya seychellensis* has been hypothesised, and since confirmed, to serve as a natural pipeline for light (Gaino and Sara 1994). This in turn likely benefits the metabolic activity of phototrophic organisms and their associated sponge host. We hypothesise that fluorescence observed here likely originates from algae which developed in close association with sponge skeletal elements, and ongoing genetic and microscopy work will determine specimen taxonomy. Sponge fluorescence on coral reefs is poorly documented or understood and has not previously been reported from sponge specimens in the Chagos Archipelago. Furthermore, to our knowledge, in-situ images of fluorescing tropical reef sponges of this kind have not previously been published.

Fluorescence was also observed from other sessile invertebrates across ARMS plates, such as solitary ascidians (yellow-green fluorescence), limpets (red fluorescence), and from the opercula of serpulid calcareous worms (green fluorescence). Almost all research on fluorescence from coral reef benthic communities focuses on

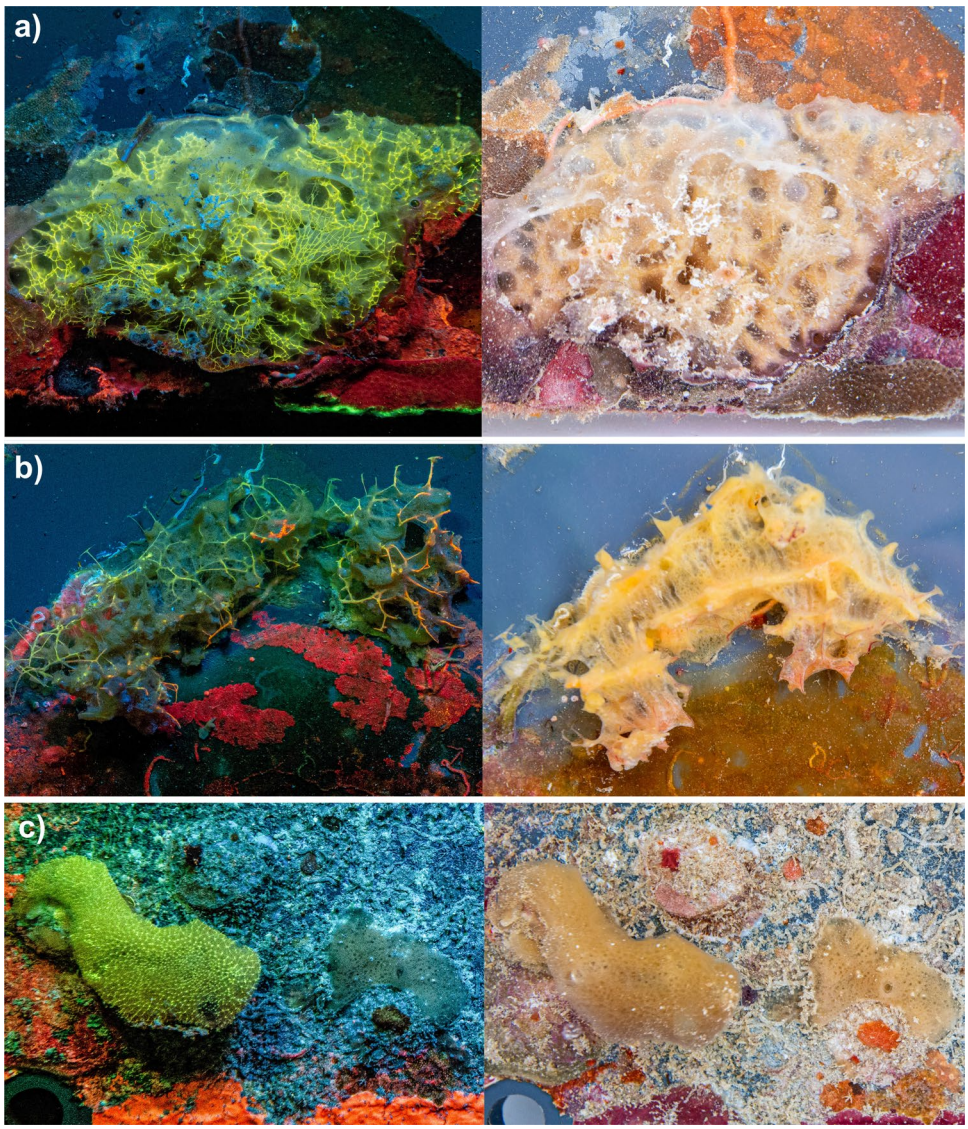

Fig. 4 Images of sponge specimens on ARMS recruitment plates under both UV light (left-hand side) and daylight (right-hand side)

hard corals, and little is known about the presence and role of fluorescent proteins in non-coral sessile reef invertebrates (Zawada and Mazel 2014). Multicolour fluorescence is observed across ARMS plates (Fig. 1), and we recommend that future ARMS studies could use fluorescence imaging to extract quantitative information from cryptobenthic communities.

Benthic communities found on ARMS devices have been shown to be highly diverse (Carvalho et al. 2019; Pearman et al. 2020), with both genetic and image analysis methods required to determine community composition and diversity patterns (Pearman et al. 2016). Analysis of ARMS plate images has so far been conducted under white light, followed by a random point count approach to determine the recruitment cover and composition of sessile communities (David et al. 2019). This approach is ideal for determining overall functional composition but is likely inaccurate for recording coral recruit abundance. Our study of ARMS demonstrates how fluorescence imaging of these devices could be an ideal standardised tool for studying the in-situ recruitment of hard corals as well as the presence and patterns of fluorescent proteins in cryptobenthic invertebrates.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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