



## Please mind the gap – Visual census and cryptic biodiversity assessment at central Red Sea coral reefs



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### ABSTRACT

Coral reefs harbor the most diverse assemblages in the ocean, however, a large proportion of the diversity is cryptic and, therefore, undetected by standard visual census techniques. Cryptic and exposed communities differ considerably in species composition and ecological function. This study compares three different coral reef assessment protocols: i) visual benthic reef surveys; ii) visual census of Autonomous Reef Monitoring Structures (ARMS) plates; and iii) metabarcoding techniques of the ARMS (including sessile, 106–500  $\mu\text{m}$  and 500–2000  $\mu\text{m}$  size fractions), that target the cryptic and exposed communities of three reefs in the central Red Sea. Visual census showed a dominance of Cnidaria (Anthozoa) and Rhodophyta on the reef substrate, while Porifera, Bryozoa and Rhodophyta were the most abundant groups on the ARMS plates. Metabarcoding, targeting the 18S rRNA gene, significantly increased estimates of the species diversity ( $p < 0.001$ ); revealing that Annelida were generally the dominant phyla (in terms of reads) of all fractions and reefs. Furthermore, metabarcoding detected microbial eukaryotic groups such as Syndiniophyceae, Mamiellophyceae and Bacillariophyceae as relevant components of the sessile fraction. ANOSIM analysis showed that the three reef sites showed no differences based on the visual census data. Metabarcoding showed a higher sensitivity for identifying differences between reef communities at smaller geographic scales than standard visual census techniques as significant differences in the assemblages were observed amongst the reefs. Comparison of the techniques showed no similar patterns for the visual techniques while the metabarcoding of the ARMS showed similar patterns amongst fractions. Establishing ARMS as a standard tool in reef monitoring will not only advance our understanding of local processes and ecological community response to environmental changes, as different faunal components will provide complementary information but also improve the estimates of biodiversity in coral reef benthic communities. This study lays the foundations for further studies looking at integrating traditional reef survey methodologies with complementary approaches, such as metabarcoding, which investigate other components of the reef community.

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### 1. Introduction

It has been estimated that one billion people benefit directly from coral reefs through a variety of services, including fisheries and tourism (Hughes et al., 2007). Furthermore, coral reefs provide substances for pharmaceutical application (Rocha et al., 2011) and protection of shores against the surge from storms and rising sea levels (Sheppard et al., 2005).

Coral reefs have been termed the “rainforests of the sea” (Reaka-

Kudla, 1997), as they are believed to host 25%–33% of marine biodiversity (Plaisance et al., 2011). Conservative estimates suggest that ~176,000 species populate coral reefs globally (Glynn and Enochs, 2010), but considering limitations in sampling reef associated habitats, up to one million species may reside in coral reefs (Reaka-Kudla, 1997; Glynn and Enochs, 2010). Recent reports show that the majority of the reef biodiversity is comprised of small invertebrates, which are cryptic (Dennis and Aldhous, 2004). Cryptic habitat space, or small cavities within the coral limestone in which organisms can inhabit, has been estimated at 30–75% of the reef habitat (Ginsburg, 1983; Scheffers et al., 2003). Exploration of these cryptic spaces has been achieved by using techniques that employ endoscopy of cavities in the limestone revealing, for example,

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abundant sponge communities in Red Sea coral reefs (Richter et al., 2001). Hence, a large cohort of biodiversity nested within coral reefs may remain unreported.

Coral reefs of the Red Sea have been previously described as biodiversity hotspots (Roberts et al., 2002), as well as refuge for potentially more temperature tolerant coral reef species, which might better endure the rise in sea surface temperature (Fine et al., 2013). Reef surveys following standard protocols assessing the exposed benthos will typically identify scleractinian corals as the dominant component of coral reefs, with them being vital elements in the structuring of the habitat. Any deviation from the dominance of scleractinian corals in tropical coral reefs may indicate an ongoing process of shifting ecosystem stages (Bruno et al., 2009). In the Red Sea, there are around 260 species of scleractinian coral (Dubinsky and Stambler, 2013), including 21 endemic hard coral species (DeVantier et al., 2000). A similar degree of biodiversity was observed for non-scleractinian cryptic taxa with 254 species being observed in reef cavities in the Gulf of Aqaba (Wunsch et al., 2000). Furthermore, reef survey protocols may target macroinvertebrates that play key roles in hard coral dominated reefs (e.g. sea urchins, crown-of-thorns starfish) but cryptic species, which could account for a large proportion of biodiversity in coral reefs, are not usually assessed. Cryptic species may include sessile, encrusting, and mobile species, which find habitat and ecological niches in the cryptic spaces. Species assessment on hidden substrata may double species biodiversity and illustrates the limits of the visual census of the exposed benthos.

The evaluation of current biodiversity baselines is important to better understand species richness in the marine realm, the economic value of biodiversity, and alterations of ecosystems services due to changes in community structures in the Anthropocene. Like other marine habitats, coral reefs are jeopardized by multiple factors on a local, regional and global scale that will alter biodiversity and ecosystem services during the 21st century (Hughes et al., 2007; Sandin et al., 2008; Kennedy et al., 2013; Salvat, 2015). Indeed, different biological components may respond differently to environmental pressures, and thus neglecting a relevant component may lead to less efficient conservation initiatives. Even within the cryptic component, different colonization and succession patterns may be observed between sessile and vagile epibenthic assemblages (Moura et al., 2008).

Recently, the ARMS (Autonomous Reef Monitoring Structure) sampling tool has been introduced to provide estimates of the cryptic reef biodiversity (Leray and Knowlton, 2015). ARMS units provide a standardized method that has been utilized at different locations around the globe since 2006 to conduct visual census of species abundance and molecular estimates of biodiversity (for more information, see [http://www.pifsc.noaa.gov/cred/survey\\_methods/arms/overview.php](http://www.pifsc.noaa.gov/cred/survey_methods/arms/overview.php)). The ARMS collection protocol includes the application of targeted metabarcoding of the community to estimate the expected biodiversity richness of both sessile and mobile fauna (sessile encrusting fauna; meiofauna, 106–500  $\mu\text{m}$ ; macrofauna, 500–2000  $\mu\text{m}$ ; large macrofauna, >2000  $\mu\text{m}$ ). The application of a standardized protocol that relies on amplicon sequencing provides an approach that is independent of the observer and worldwide datasets targeting the same gene regions can easily be combined and compared. Here, we present results from the standard visual benthic census of three Red Sea nearshore reefs (Jeddah, Kingdom of Saudi Arabia) and cryptic diversity assessments using ARMS. Results from three assessment methods, including 1) standard reef survey, 2) photo survey of ARMS plates, and 3) metabarcoding of ARMS units, were compared and analysed for similar patterns. We hypothesize that the different methods, which investigate different faunal components, will provide complementary information on the biodiversity pattern,

thus increasing the information available to detect responses to pressures affecting coral reef systems.

## 2. Material and methods

### 2.1. Study area

Three nearshore reefs were selected along the shoreline off Jeddah in order to represent a gradient of human pressure. One reef (JH) was located off of Jeddah harbour, the main harbour on the Saudi Arabian Red Sea coast, serving a city of over 3 million inhabitants. The other two reefs were located approximately 20 km (JS1 – in the vicinity of the most recent sewage treatment plant), and 40 km (JS2) south of JH (Table 1 and Fig. 1). Field permits for sampling were granted by the Saudi Arabian coastguard.

### 2.2. Standard reef benthic diversity surveys

#### 2.2.1. Strategy

Reef surveys were carried out in March 2014, just before the retrieval of ARMS units. In each reef, three replicate transects of 20 m each (5 m gap between transects) were assessed at 10 m depth. Along each transect, a photo (1 m<sup>2</sup>) was taken every 2 m. Quantification and identification of benthic categories were conducted using the Coral Point Count with Excel extensions (Kohler and Gill, 2006). Forty-eight points were randomly distributed within a total of 12 cells on each substrate image and the features underlying the points user-identified. In general, given the revisions on coral's taxonomy being undertaken worldwide, coral identification was limited to family or genus level, following Veron (2000).

Overall, benthic community composition was analysed in terms: 1) of number of morphological Operational Taxonomic Units (morphOTU; organisms with similar morphological characteristics being classified as a taxon); 2) cover of corals (both soft and scleractinian corals), sponges, hydroids and other invertebrates (e.g. bivalves, echinoderms), algae (subdivided into the following functional groups: macroalgae, turf, coralline algae); 3) cover of abiotic substrates (sand, rock, rubble); and 4) the occurrence of coral diseases and mortality.

### 2.3. Assessment of cryptic diversity: ARMS

#### 2.3.1. Deployment and sampling of ARMS units

Three replicate units of ARMS were deployed by scuba divers at three reef sites off Jeddah (Fig. 1) in April–May 2013 and were left undisturbed for one year. The ARMS were deployed on a flat rocky area at a depth of approximately 10 m. In order to characterize each reef area, water samples were collected and analysed for nutrients and chlorophyll *a* (see Pearman et al., 2016), for details on the analytical procedures). When the nine ARMS units were retrieved, they were covered by a 106  $\mu\text{m}$  net (to avoid the loss of mobile fauna), placed in individual boxes filled with filtered (106  $\mu\text{m}$ ) seawater, and transported to the laboratory for processing and analysis.

The ARMS samples were processed as described by Leray and Knowlton (2015). Briefly, the ARMS were dismantled in the transport box with each plate (9 plates in all per ARMS) being gently brushed to dislodge motile organisms from the plates into the filtered seawater. Individual plates were placed in labelled trays (top side up) and filled with filtered (0.2  $\mu\text{m}$ ) seawater. Pictures of the top and bottom sides of each plate were taken (Fig. 2), as well as close ups of each quarter of the plates. After the plates had been photographed, they were scraped clean to remove the sessile community. The sessile community from all plates (of a single

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