Comparison of cryptobenthic reef fish communities among microhabitats in the Red Sea (#21327)

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Comparison of cryptobenthic reef fish communities among microhabitats in the Red Sea

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Knowledge of community structure within an ecosystem is essential when trying to understand the function and importance of the system as well as when making management decisions related to this system. Within the larger ecosystem, microhabitats play an important role by providing inhabitants with a subset of available resources. On coral reefs, cryptobenthic fishes encompass many groups and make up an important proportion of the biodiversity. However, these fishes are relatively small and extremely cryptic, either behaviorally or visually and therefore are often overlooked. The largest family of cryptobenthic fishes is Gobiidae, a family currently containing more than 1600 species, although new species are continuously discovered. Many goby species are associated with a very specific microhabitat type, however most of this knowledge is limited to coral-dependent species. We examined the differences in fish community structure within three common reef microhabitats (live hard coral, dead coral rubble, and sand) using rotenone stations in the central Red Sea. Using a combination of morphological and genetic (COI barcoding) techniques, we identified 326 individuals representing 73 species spread across 17 families from the collections. The largest group collected was gobies, representing 232 individuals and 31 species. Goby assemblages in the three microhabitats were significantly different from each other – rubble microhabitats hosted the majority of collected gobies (69% of individuals), followed by live hard coral (20.6%), then sand (9.9%). These results provide essential baseline information about the ecology of understudied cryptobenthic fishes that can be used in future large-scale studies in the Red Sea region. Future reef assessments should also incorporate cryptobenthic fishes, as they are often ignored despite their potential functional importance.

Comparison of cryptobenthic reef fish communities among microhabitats in the Red Sea

Short title: Red Sea cryptobenthic fishes

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ABSTRACT

Knowledge of community structure within an ecosystem is essential when trying to understand the function and importance of the system as well as when making management decisions related to this system. Within the larger ecosystem, microhabitats play an important role by providing inhabitants with a subset of available resources. On coral reefs, cryptobenthic fishes encompass many groups and make up an important proportion of the biodiversity. However, these fishes are relatively small and extremely cryptic, either behaviorally or visually and therefore are often overlooked. The largest family of cryptobenthic fishes is Gobiidae, a family currently containing more than 1600 species, although new species are continuously discovered. Many goby species are associated with a very specific microhabitat type, however most of this knowledge is limited to coral-dependent species. We examined the differences in fish community structure within three common reef microhabitats (live hard coral, dead coral rubble, and sand) using rotenone stations in the central Red Sea. Using a combination of morphological and genetic (COI barcoding) techniques, we identified 326 individuals representing 73 species spread across 17 families from the collections. The largest group collected was gobies, representing 232 individuals and 31 species. Goby assemblages in the three microhabitats were significantly different from each other – rubble microhabitats hosted the majority of collected gobies (69% of individuals), followed by live hard coral (20.6%), then sand (9.9%). These results provide essential baseline information about the ecology of understudied cryptobenthic fishes that can be used in future large-scale studies in the Red Sea region. Future reef assessments should also incorporate cryptobenthic fishes, as they are often ignored despite their potential functional importance.

INTRODUCTION

Habitat has frequently been shown to influence species abundances and distribution patterns in a range of ecosystems (Venier & Fahrig, 1996; Warren et al., 2001). Within global tropical regions, coral reefs provide complex habitats that support around 25% of marine fish species, despite occupying only 0.1% of ocean area (Spalding et al., 2001). On coral reefs, benthic composition and coral cover may influence the associated fish assemblages (Messmer et al., 2011; Chong-Seng et al., 2012; Komyakova, Munday, & Jones, 2013), with live coral habitats having a direct effect on many species through the provision of shelter and food (Bell & Galzin, 1984; Buchheim & Hixon, 1992; Cole, Pratchett & Jones, 2008; Coker, Wilson & Pratchett, 2014). In addition to biotic habitats, the physical structure and internal complexity of the reef has been shown to influence a number of reef fishes (Graham & Nash, 2013). Within coral reefs, different types of microhabitats exist, such as areas dominated by living hard corals, soft corals, rubble patches, macroalgae, or sandy areas. These habitats can offer a range of resources such as food and shelter for small fishes (Beukers & Jones, 1997; Depczynski & Bellwood, 2004, Brooker, Munday & Ainsworth, 2010). For example, corals provide refuge spaces within the branches (Robertson & Sheldon, 1979), while the colony itself can provide shelter underneath for larger fishes (Kerry & Bellwood, 2012). In addition, degraded and structurally deteriorated reefs provide additional habitats that are inhabited by species that are better adapted to these habitat features (Ahmadia, Pezold & Smith, 2012; Coker, Graham & Pratchett, 2012). It is therefore expected that small and benthic-associated fishes would be influenced greatly by available habitats, and therefore understanding microhabitat requirements is essential for reef fishes.

On coral reefs, there are many groups of fishes (blennies, gobies, triplefins, etc.) that are

relatively small (< 50 mm), have a close association with the substrate, and have a cryptic nature (Depczynski & Bellwood, 2003). These fishes, termed "cryptobenthic reef fishes", can be behaviorally cryptic by seeking out cracks and crevices in the reef in which to hide, or they can be visually cryptic, having coloration that matches the substrate where they live (Depczynski & Bellwood, 2003). Fast-growing and with naturally short lifespans, these fishes are an important functional group on coral reefs, contributing greatly to the transfer of energy through the food chain. For example, in the Great Barrier Reef up to 8% of the cryptobenthic fish population are consumed by predators every day (Depczynski & Bellwood, 2006). Despite their functional importance, they are difficult to sample due to their cryptic nature and therefore vastly understudied worldwide. Previous visual surveys have not been able to account for the majority of cryptobenthic fishes on a reef (Ackerman & Bellwood, 2000; Robertson & Smith-Vaniz, 2008) and most surveys ignore them due to logistical and taxonomic difficulties. Thus, more targeted surveying techniques have recently been employed in order to gain a more accurate count of cryptobenthic reef fishes. Chemical ichthyocides, such as rotenone and clove oil, have proven to be an effective tool to collect small fishes and have been shown to reveal more cryptic species when compared to traditional visual surveys (Brock, 1982). Many groups of fish are considered to be cryptobenthic but within this group the largest (most speciose) is the family Gobiidae, which currently contains over 1600 species spread across 200 genera (Thacker, 2003; Tornabene et al., 2013). Gobies occupy a variety of habitats including corals, seagrass, sand, and rubble (Munday, Jones & Caley, 1997; Munday et al., 2002; Patzner et al., 2011). The majority of gobies are epibenthic or cryptobenthic, living on or just above the substrate. Gobies are small fish, with most attaining an adult size of less than 3 cm (Patzner et al., 2011). They will also have small home ranges, often no more than 0.25 to 2 m^2 (Depczynski & Bellwood, 2004). Their

small size, coupled with a small home range, has enabled gobies to make use of a multitude of different microhabitats. Many species of goby are habitat specialists and coral-obligate gobies have been highly studied (e.g., Munday, Jones & Caley, 1997, 2001; Munday, 2002). Other groups of cryptobenthic fishes should be studied to determine more broadly understand the importance of various habitats.

Globally, knowledge of cryptobenthic fishes is still limited. A majority of studies have arisen from well-studied areas, such as the Great Barrier Reef and the Caribbean (see Ackerman & Bellwood, 2000, 2002; Harborne et al., 2012), but an important region that is greatly understudied when it comes to cryptobenthic fishes as well as conspicuous ones (Berumen et al., 2013), is the Red Sea. The Red Sea is a unique environment, with higher average temperatures and salinity compared to other regions. It is recognized as a biodiversity hotspot with an estimated 14% of fish species in the Red Sea endemic to the region (DiBattista et al., 2016). Recent studies in this region show that cryptobenthic fish communities differ latitudinally and with distance from shore, and that habitat may be a driving factor (Coker et al., 2017).

This study aims to examine differences among the community of cryptobenthic reef fishes associated with three common reef microhabitats. Live hard corals, rubble patches, and sandy areas all represent habitats that cryptobenthic fishes are known to utilize (Depczynski & Bellwood, 2004; Ahmadia et al., 2012), and these habitats may also be representative of different stages of reef degradation. It is hypothesized that cryptobenthic fish assemblages will significantly differ between these three microhabitats. This information will help us better understand abundance and distribution patterns within the region and provide baseline data about understudied cryptobenthic fishes and their functional role in the Red Sea.

MATERIALS AND METHODS

Study site

Fishes were sampled in May 2017 during daylight hours from the southern portion of a reef situated near the edge of the continental shelf (Al Fahal, 22° 13.6558 N, 38° 58.1853 E) (Fig. 1 A). The specific study site was located on the eastern side of the reef and thus is protected from the typical north-western wave patterns. Al Fahal was chosen as a sample site because it is a relatively large reef that hosts the variety of habitat types used in this study. This single reef (and wave exposure type) was selected to minimize any potential environmental variables, such as temperature, wave energy, current, and turbidity that could be introduced if sampling in varying parts of the reef (e.g., sides exposed to or sheltered from dominant wave action).

Microhabitat definitions

Three microhabitat types were sampled: live hard coral, dead coral rubble, and sand (Fig. 1 B-D). A hard coral microhabitat was defined as having at least 70% of the quadrat covered with hard coral; tabular *Acropora* corals were targeted. A rubble microhabitat was defined as a flat or gently sloping area (<10º) containing at least 50% coverage of dead hard coral rubble, rocks, or empty shells. A sand microhabitat was defined as a flat or gently sloping area (<10º) containing at least 90% sand coverage containing little or no rubble. For each of the three microhabitat types, five replicate quadrats were sampled, resulting in a total of 15 quadrats.

Percent cover of each microhabitat category was calculated using a grid point system. A 10 x10 grid (i.e., 100 total intercepting points) was overlaid onto a digital image of each quadrat. Each point in the image was categorized into the above microhabitat type. From these tallies, a total percent cover for each microhabitat type was estimated for each quadrat.

Rugosity of the sample quadrat was measured as a linear distance using a 1m chain (sensu Risk, 1972), wherein the chain is draped in a straight line through the quadrat following the vertical contour of the substrate. Subsequently, the 'overhead' view (i.e., two-dimensional horizontal length) of the chain is measured, and the rugosity value is calculated by dividing the length of the chain by the length of the 'overhead' view of the draped chain. A value of 1 would thus represent a flat surface, while higher values indicate a more three-dimensionally complex habitat. This method was repeated three times within each quadrat to provide a mean rugosity for each quadrat.

Fish community sampling

An ichthyocide (rotenone) was used to collect fish due to its high success rate in targeting cryptic species (Robertson & Smith-Vaniz, 2008). A rotenone mixture was prepared by mixing 500g of 4% rotenone powder (Consolidated Chemical Company) with 100ml 96% ethanol, 250ml liquid dishwashing detergent, and 100ml water (adapted from Ackerman & Bellwood, 2000).

Quadrats (1 m^2) constructed from PVC pipe (25mm diameter, see Fig. 1) were placed at 10-15m depth onto a flat or gently sloping surface (<10º) of the reef in one of the three targeted microhabitats. A 4mm mesh net weighted down with chain was placed around the quadrat, enclosing the whole area. The rotenone mixture was gently squirted into the netted area until the whole area was covered. After waiting about five minutes for the rotenone to take effect, the net was removed and fishes were collected using handnets and tweezers (following Ackerman & Bellwood, 2000). Because some fish were observed escaping the enclosed quadrat (through the reef matrix) while the rotenone took effect, all deceased fishes directly around the sample area

were collected for consistency. After collecting the visible fishes, three divers intensively searched within each quadrat by lifting up any rubble or debris where hidden fishes could have settled. The quadrats were searched until no new fishes were found for a period of five minutes. Larger predatory fishes that came too close to the quadrat were chased away before they could consume any of the asphyxiated fishes..

Immediately after the dive, collected fishes were placed into an ice slurry to preserve coloration for photography (within ~ 2 hrs). Once photographed, total body length and standard length measurements (to the nearest mm) were taken. Fishes were then placed into individual labeled vials containing a solution of 96% ethanol for preservation. A tissue sample (pectoral or caudal fin) was collected from each individual for genetic analysis.

Fish sampling was done in accordance with the guidelines and procedures approved under the auspices of the King Abdullah University of Science and Technology (KAUST) Institutional Animal Use and Care Committee (IACUC) under approval number 17-04-004.

Genetic fish identification

Tissue samples were cleaned with 96% ethanol and gently patted dry before placed into 96-well plates containing 100µl 50mM NaOH. DNA was extracted from the samples using the HotSHOT protocol (95ºC for 20 minutes, 4ºC for 10 minutes) (Meeker et al., 2007). After extraction, 10µl of 1M Tris-Hcl (10%) was added to each well and mixed with pipettes.

80µl from each sample was transferred to a new plate for DNA amplification. A polymerase chain reaction (PCR) Qiagen Multiplex Mastermix containing Taq polymerase, dNTPs, $MgCl_2$, and reaction buffers was added to each well. Primers COI Universal Fish R2 5' ACT TCA GGG TGA CCG AAG AAT CAG AA 3' and F2 5' TCG ACT AAT CAT AAA GAT

ATC GGC AC 3' (Ward et al., 2005) were used to amplify the COI region of DNA. Each PCR well contained 6.25µl MasterMix, 4.25µl nuclease free water, 0.5µl forward primer, 0.5µl reverse primer, and 1µl DNA, for a total volume of 12.5µl. Thermocycling occurred with an initial denaturation period of 15 minutes at 95 °C, followed by 35 cycles of 30 seconds at 94 °C, 60 seconds at the annealing temperature of 45 °C, and 60 seconds at 72 °C, followed by a final extension period of 10 minutes at 72 °C. PCR products were visualized using a QIAxcel system. Following PCR amplification, 2.14µl ExoStar was added to each well to clean the PCR product. The mixture was incubated in the thermocycler for 60 minutes at 37 °C, then 15 minutes at 85 °C. Cleaned PCR products were sequenced via Sanger sequencing via ABI 3730xl sequencers in the KAUST Bioscience Core Lab.

In the event that a sample sequence did not yield a long enough strand of base pairs to be entered into a database (~500 bp), DNA was extracted again using a more precise Qiagen DNeasy Blood and Tissue kit. The PCR process was then repeated on those samples.

After sequencing was completed, sample sequences were checked against several sequence databases for potential matches. The National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Barcode of Life Data System (BOLD) (http://www.boldsystems.org) were used as public databases. A custom (in-house) Red Sea fish sequence database was also checked (see Coker et al., 2017; DiBattista et al., 2017; Isari et al., 2017). A sequence was considered to be a good match for a species in the database if the matched sequence was 98% similar or higher. Sequence matches were then checked against visual guides and morphological keys to double-check identity. In the event of a non-matching sequence, a sample was identified to species level, or as close as possible, with the use of keys that examined morphological features (see next section). When a species was unable to be

identified using the aforementioned methods, it was assigned an operational taxonomic unit (OTU) so as to still be included in the data analysis. OTUs were named for previous uploaded sequences in the databases if there was a match. In the event a sequence did not match a previously assigned OTU within one of the databases, a new OTU was assigned.

Morphological fish identification

With the use of keys, fishes that could not be identified genetically were assigned to a species, genus, or family level. The typical morphological characters that were useful in discriminating taxa included: counts of fin spines and soft rays, pelvic fin structure, cephalic pore counts, lateral line scale counts, as well as general meristics. Morphological characters were assessed using light dissection microscopy and an online image analysis tool for measuring morphometrics (Froese & Pauly, 2017). For a list of keys used, see Appendix S1.

Community analysis

For each quadrat, several metrics were calculated. Species richness was defined as the number of species or OTUs identified from each quadrat. Abundance was determined by the total number of individuals collected in each quadrat. Diversity (Shannon's diversity index, H') was calculated using the following formula: $H' = -\sum p_i \ln(p_i)$ (Shannon & Weaver, 1963), and then subsequently averaged within each microhabitat type. The fish communities present at each microhabitat type were plotted using non-metric multidimensional scaling (nMDS) using a Bray-Curtis resemblance matrix. Analyses were conducted using R version 3.4.0 (R Core Team, 2017) and the vegan package (Oksanen et al., 2017). Statistics used included analysis of variance (ANOVA) and permutational analysis of variance (PERMANOVA).

RESULTS

Fish communities

A total of 326 individuals representing 73 species and 17 families were collected from three microhabitat types (Appendix S2). The total number of all fishes collected at each quadrat ranged from 1 (Quadrat sand_4) to 65 (Quadrat rubble_3). Rubble quadrats had the highest average numbers of fish abundances (one-way ANOVA $F_{2,12}$=11.59, p=0.002), species richness ($F_{2,12}$=6.78, p=0.011), and diversity ($F_{2,12}$=2.80, p=0.100), followed by coral quadrats, and then sand quadrats (Fig. 2) and fish communities in all three microhabitats differed significantly (PERMANOVA $F_{2,12}$=2.61, p<0.001) (Fig. 3). Out of the 326 fish collected, the family Gobiidae comprised the majority, with a total of 232 individuals (71.1%) collected representing 31 species. The next most common families were Pseudochromidae, with 33 individuals (10.1%) collected representing 12 species, and Pomacentridae with 16 individuals (4.9%) representing 7 species.

Goby communities

The number of individual gobies collected at each microhabitat type followed patterns similar to that of all collected fishes. Abundance ranged from 0 gobies (Quadrat sand_4) to 46 gobies (Quadrat rubble_3). The most abundant goby was *Trimma avidori,* with a total of 44 individuals. This goby was collected in greatest abundance in both coral and rubble microhabitats. The second most abundant goby collected was *Callogobius bifasciatus* with 29 individuals collected in mainly rubble quadrats (Fig. 4). Of 31 species of goby collected, 11 species (35.4%) were represented by only a single individual, and the majority of collected species (64.5%) had less than 5 individuals collected.

Out of the coral quadrats, the most abundant goby collected was *T. avidori.* Both *C. bifasciatus* and *A. semipunctata* were the most abundant species found in rubble, and *Istigobius decoratus* was the most abundant species found in the sand microhabitat quadrats.

On average, rubble communities had the highest levels of goby abundance (one way ANOVA $F_{2,12}$=24.61, p<0.001), species richness ($F_{2,12}$=14.95, p<0.001), and diversity ($F_{2,12}$=5.10, p=0.025), followed by coral communities, and then sand communities (Fig. 2). Overall, goby community composition significantly differed among microhabitats (PERMANOVA $F_{2,12}$=3.67, p<0.001) (Fig. 3).

Of all the fish collected, 25.7% could not be confidently assigned to a species using either morphological or genetic techniques, so they were given an OTU. Several candidates for new species were identified from these OTUs; some of these are possibly Red Sea endemics. COI sequences for new OTUs have been deposited to GenBank under accession numbers MG583518-MG583524, as well as sequences from *Bryaninops natans, Gobiodon reticulatus,* and *Trimma flavicaudatum,* all which currently have no COI sequence information on GenBank.

Habitat composition

Rugosity was highest on average in coral microhabitats (Fig. 1 E). Rubble and sand microhabitats were less rugose, with rubble quadrats having higher rugosity, on average, than sand (one-way ANOVA, $F_{2,10}$ = 19.86, p<0.001). Percent cover of each microhabitat type was highest for sand quadrats, with sand quadrats averaging 97.6% (± 0.66 SE) cover of sand. Coral microhabitat quadrats averaged 85.3% (± 4.83 SE) cover of all hard corals, with tabular *Acropora* averaging 56.2% cover (± 9.56 SE) within the coral microhabitat quadrats. Rubble microhabitat quadrats averaged 65.6% cover (± 4.43 SE) of rubble (Fig. 1 F).

DISCUSSION

Cryptobenthic fish communities, including gobies, are vastly understudied compared to other, more conspicuous groups, especially in the Red Sea. Fish communities from live hard coral, rubble, and sand microhabitats differed from each other by having differing levels of abundance, species richness, and diversity. Research is still just scratching the surface of cryptobenthic fish ecology and this study found that habitat is a strong indicator of what kind of cryptobenthic fish assemblages will be present on a reef in the Red Sea. Importantly, these results provide a framework for future studies in the region to examine additional microhabitats in additional locations.

The rubble microhabitats sampled yielded the highest levels of fish abundance, richness, and diversity. A possible explanation for this preference is the sediment load that is deposited onto the rubble. Runoff and detritus from more productive areas on a reef will eventually settle onto the substrate below, delivering more nutrients to the benthos (Crossman et al., 2001; Wilson et al., 2003; Depczynski & Bellwood, 2004; Goatley, González-Cabello & Bellwood, 2016). Cryptobenthic fishes will primarily feed upon microscopic prey such as epibenthic invertebrates, algae, or detritus (Depczynski & Bellwood, 2003), and this food is readily available on the substrate. Indeed, in the rubble microhabitats sampled, there was a presence of epilithic algae on many of the large pieces of rubble. *Asterropteryx semipunctata,* one of the most abundant species collected from the rubble microhabitats, is a known detritivore (Depczynski & Bellwood, 2003). Because cryptobenthic fishes are sedentary and will not forage far from their home areas (Depczynski & Bellwood, 2004), they must live in places that have a steady supply of food.

Coral microhabitats also yielded high levels of fish abundance, richness, and diversity. Coral quadrats sampled were characterized by a large percentage of live hard coral cover, with a

298 majority of the coral cover represented by tabular *Acropora*. These coral habitats also exhibited
299 the highest measures of rugosity. There is a positive correlation between habitat complexity and
300 fish abundance in both conspicuous fishes (Caley & St John, 1996; Friedlander & Parrish, 1998)
301 as well as cryptobenthic fishes (Depczynski & Bellwood, 2004). Complexity offers shelter from
302 predators as well as lowered mortality rates for juveniles (Beukers & Jones, 1997). One of the
303 most abundant fish collected from coral microhabitats was *Trimma avidori*. This goby is known
304 to inhabit small overhangs or caves underneath coral structures (Herler & Hilgers, 2005), and it
305 is likely that the large tabular *Acropora* present in the coral quadrats provided adequate shelter
306 for these fishes.

307 Sand microhabitats exhibited the lowest levels of fish abundance, richness, and diversity.
308 These areas were characterized by a lack of biotic structures and were overall quite barren. The
309 sand quadrats were extremely flat with low rugosity and no complexity, and this may have been
310 a contributing factor to the low levels of abundance, species richness, and diversity found within.
311 Structural complexity has long been shown to influence fish abundance and diversity (Risk,
312 1972; Luckhurst & Luckhurst, 1978). Cryptic fishes rely on hiding places to avoid being preyed
313 upon and unlike the coral habitats, the sand microhabitats had little, if any, areas to hide.
314 However, sand habitats are not completely devoid of life. Many cryptobenthic specialists are
315 able to thrive in sandy habitats due to less competition from other fishes and the ability to form
316 internal tunnels within the substrate. Flatfish, such as flounders and soles, are able to blend in
317 perfectly with the coloration and the extreme flatness of sand, and many wrasses are known to
318 burrow underneath it to escape predation. These microhabitats may be further used by
319 cryptobenthic fishes for foraging at night. Our daytime sampling regime precludes us for
320 assessing this possibility.

The microhabitat types used in this study represent microhabitats nearly universal to coral reefs that are important to fish assemblages. However, it is important to note that the hard coral, rubble, and sand microhabitats in the Red Sea may not be functionally equivalent to those worldwide. For instance, coral species will vary from region to region, rubble size and detrital material can vary, and sand sediment grain size and composition may vary as well. Because the three microhabitats used in this study were found to be different from each other, it provides a good foundation for future research in this region. Future studies may address the coarseness of sand grains across multiple sand microhabitats or the size of rubble across multiple rubble microhabitats to determine if there are finer-scale differences that may determine microhabitat use among cryptobenthic fishes. Additional factors not considered here, such as exposure to wave action or distance from shore (Depczynski & Bellwood, 2005; Goatley, González-Cabello & Bellwood, 2016) may also warrant further investigation in the Red Sea.

Cryptobenthic fishes are an understudied group; hence there is a lack of public sequence information available for many species, especially gobies. For 232 collected gobies, using the 98% similarity threshold, only 128 (55.2%) gobies were able to be identified genetically using COI barcoding. The remaining 104 (44.8%) could not be identified using genetic methods, and were either identified using morphological characteristics or given an OTU. This gap in publicly available cryptobenthic fish sequences is a hindrance for identification that relies on genetic data. These difficulties with identifying cryptobenthic fishes highlight the need for a comprehensive sequence library that includes reference images.

The Red Sea is a known biological hotspot with high rates of endemism for many groups of species. An estimated 14% of Red Sea fishes are endemic (DiBattista et al., 2016), however these estimates may under-represent cryptic species. Out of 73 fish species, a total of 17 (23.2%)

endemic species from the Red Sea and Arabian Peninsula were found in this study. Four of those endemic species were gobies (see DiBattista et al., 2016 for checklist). It is also extremely likely that some of the unnamed goby OTUs are undescribed species, and these could possibly be endemic to the region. However, endemic status could only be more confidently asserted if supported by more sampling in adjacent regions. With the potential number of undiscovered cryptobenthic fishes in the region, the number of known endemics can be expected to increase in time.

CONCLUSIONS

This study provides baseline information on cryptobenthic fish communities in the central Red Sea and their microhabitat preferences. Three distinct microhabitats were found to host different communities of fishes, with different abundance, richness, and diversity levels. Rubble microhabitats were found to have the highest levels of abundance, richness, and diversity overall, followed by hard coral microhabitats, and finally sand microhabitats. We are only just beginning to understand the ecology of cryptobenthic fishes and such fine-scale habitat partitioning is likely to contribute to the high diversity found on a reef. On a single reef, there can be distinct microhabitat communities that are within meters of each other, yet support different fish assemblages. More data is needed to accurately quantify the cryptobenthic biodiversity of the region. Future larger-scale projects may find more evidence of microhabitat associations in the Red Sea. Because cryptobenthic fishes are estimated to compose up to half of all fishes on a reef and previous studies have found only a fraction of that proportion, more microhabitat types, reef parts, and depths should be sampled. By expanding the parameters of a cryptobenthic fish study, it is likely that knowledge of these cryptic fishes in the region will increase.

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Figure 1

Microhabitat characteristics of sampled quadrats

Examples of 1m² quadrats sampled in the central Saudi Arabian Red Sea to assess cryptobenthic fish assemblages in each of three microhabitat types: coral (indicated by purple), rubble (orange), and sand (teal). Quadrats were sampled using rotenone at 10-15m depth. (A) Map of study site, with reef habitat indicated in grey. (B) Coral quadrat. (C) Rubble quadrat. (D) Sand quadrat. (E) Average rugosity for each microhabitat type. A value of 1 indicates a completely flat surface while higher values represent greater vertical complexity. (F) Percent cover of the target substrate for each quadrat for each microhabitat type. Lighter colors in the coral quadrats represent the proportion of the coral cover comprised by tabular *Acropora* (visible in panel B). (Photographs taken by EMT. Map created in ArcMap, version 10.3, by Michael Campbell using various mapping sources freely available through ESRI.)

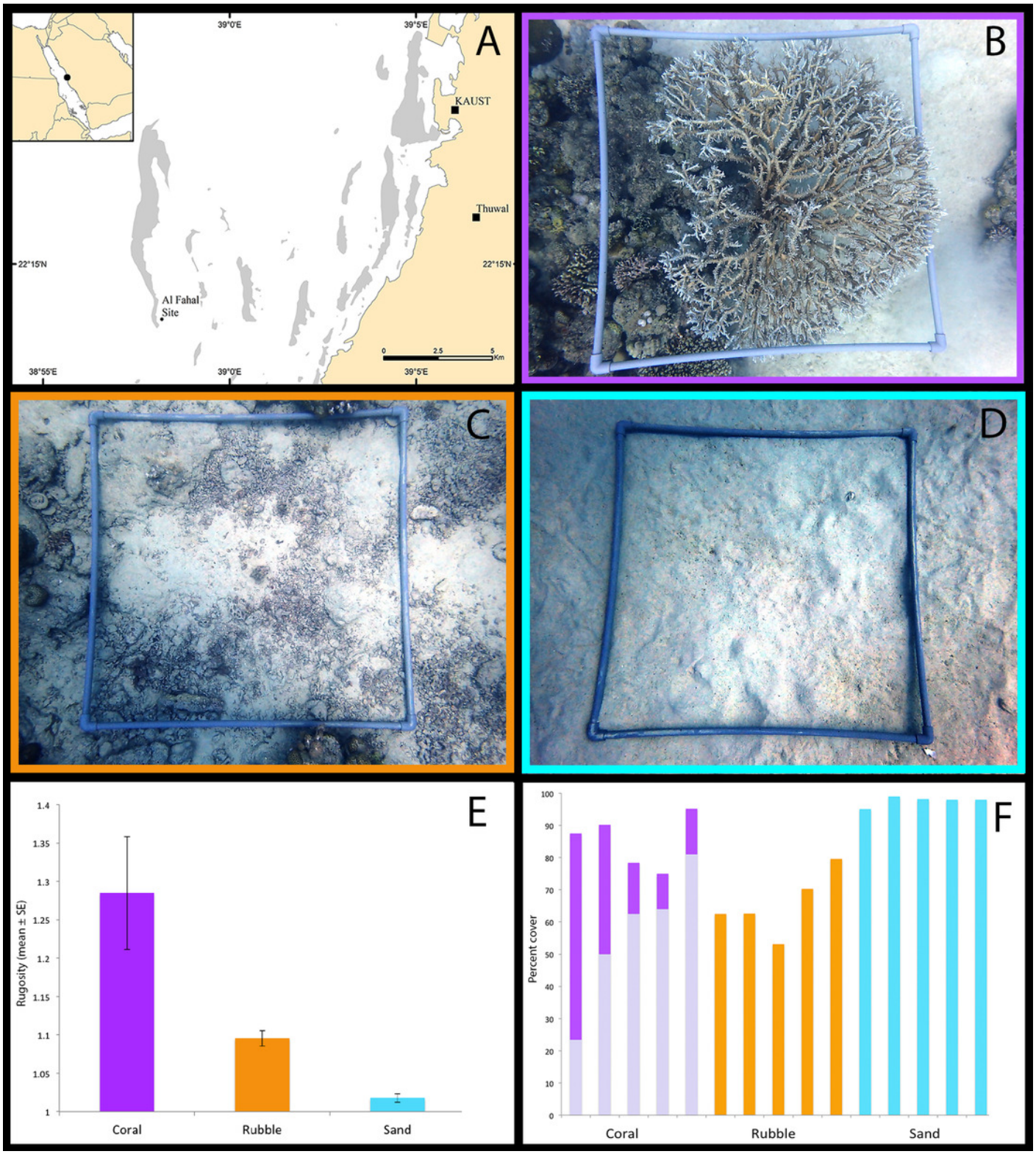

A
39°0'E
39°5'E
KAUST
Thuwal
22°15'N
Al Fahal Site
38°55'E
0 2.5 5 Km
B
C
D
E
Rugosity (mean ± SE)
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
Coral
Rubble
Sand
F
Percent cover
100
90
80
70
60
50
40
30
20
10
0
Coral
Rubble
Sand

Figure 2

Bar chart of community indices

Abundance (mean number of individuals per m^2 ± SE), species richness (mean number of species per m^2 ± SE), and diversity (H') (mean value per m^2 ± SE) for all collected fish species and for family Gobiidae for each of the three microhabitat types sampled using rotenone stations ($1m^2$ quadrats, n = 5 quadrats per microhabitat type) in the central Saudi Arabian Red Sea.

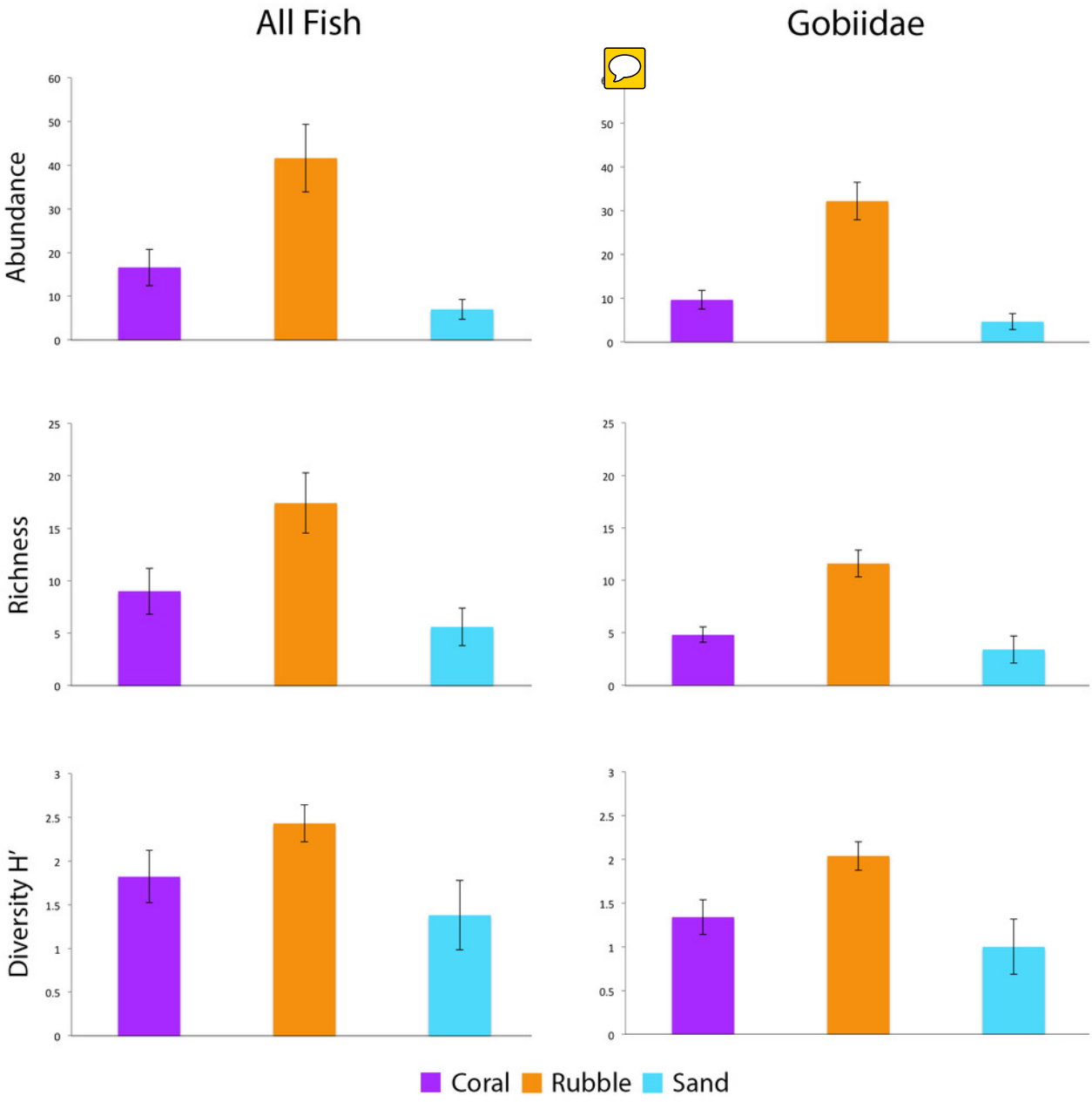
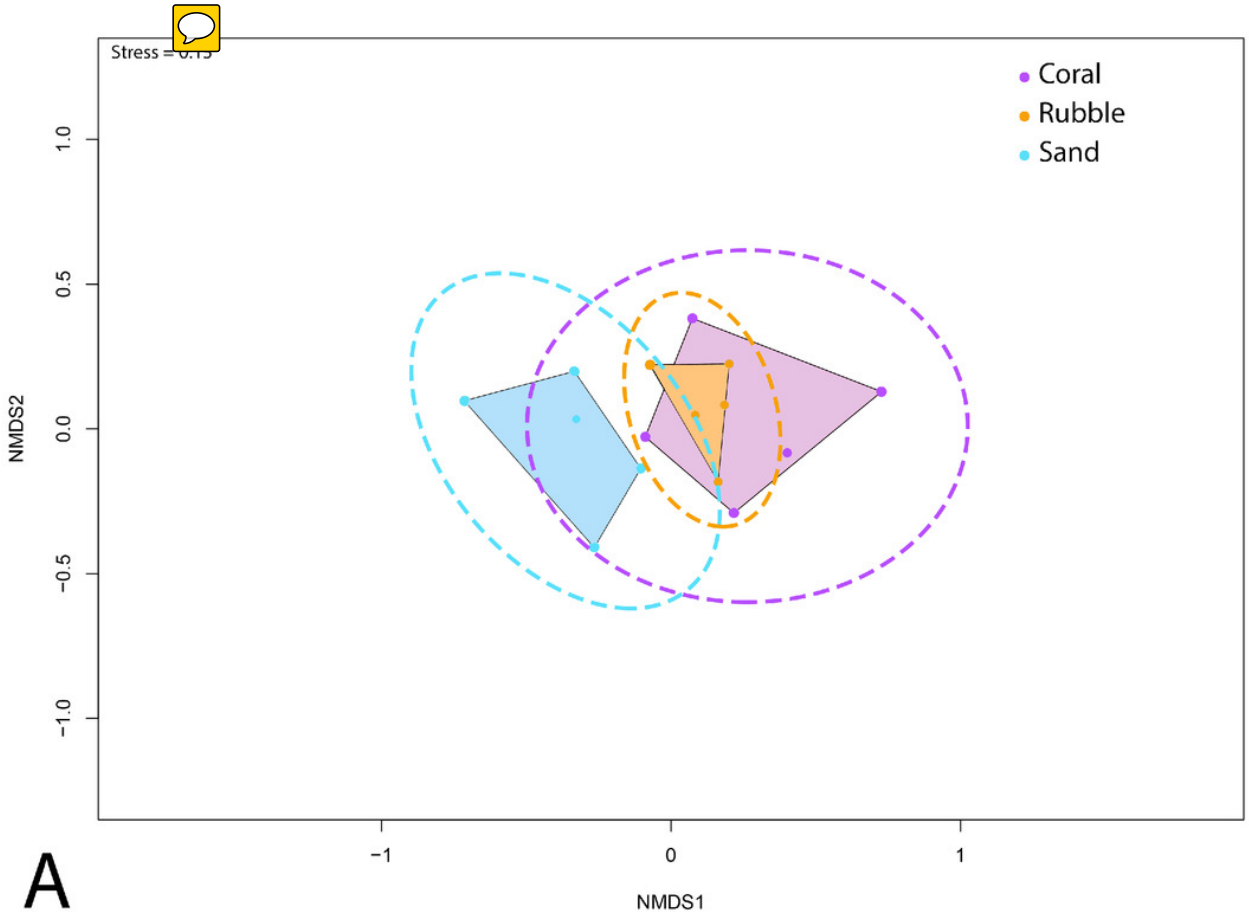

All Fish
Gobiidae
Abundance
Richness
Diversity H'
Coral
Rubble
Sand

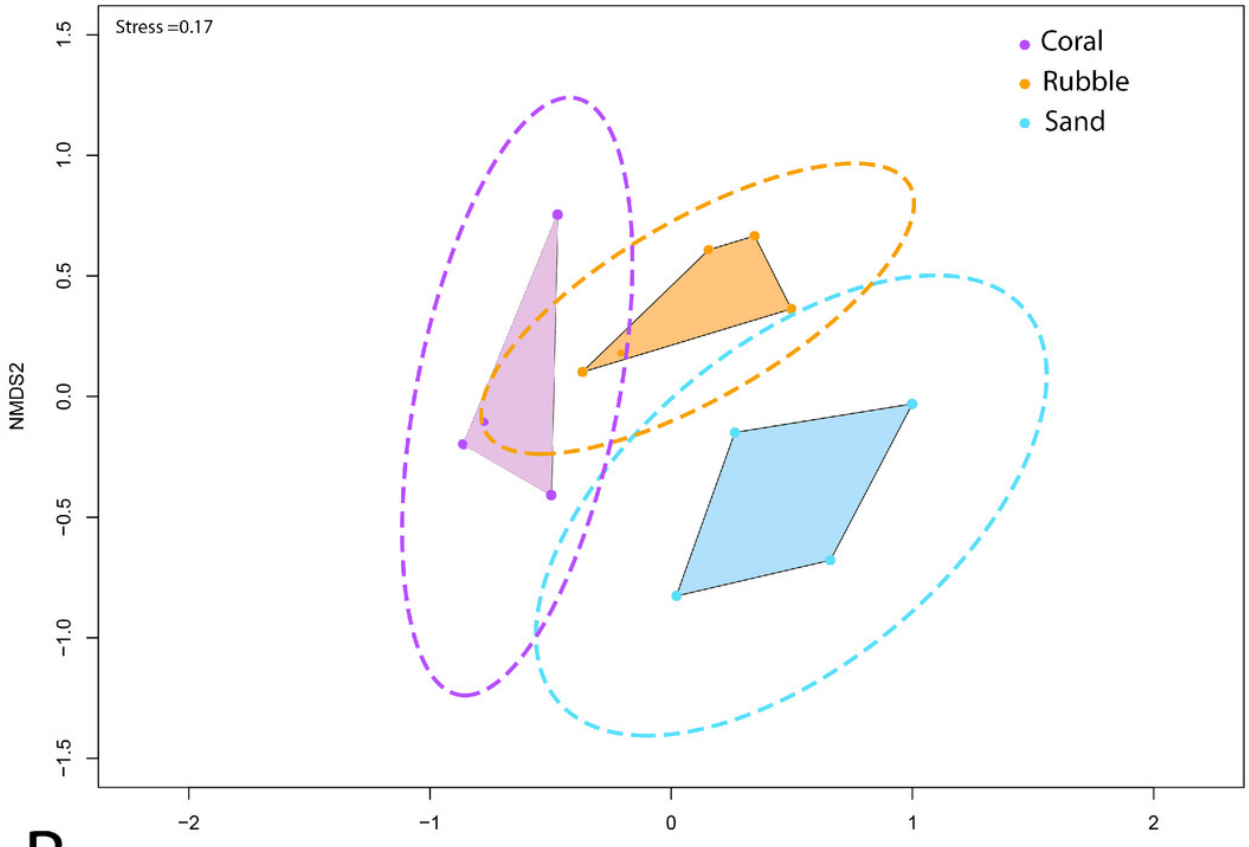
Figure 3

nMDS plots of community similarities

nMDS plots using Bray-Curtis similarity of (A) all fish communities (i.e., relative abundances of all collected species) and (B) goby communities (i.e., relative abundances of individuals from the family Gobiidae) sampled using rotenone stations in each of the three microhabitat types in the central Saudi Arabian Red Sea. Solid points represent individual quadrats from each microhabitat type, which define the shaded minimum convex polygons for each habitat type. (Note that one quadrat from coral microhabitat and one quadrat from sand microhabitat were excluded from the goby nMDS due to an absence of gobies in these quadrats.) Ellipse size represents 95% confidence limits for each microhabitat type. Overall, total fish community composition significantly differs for each microhabitat (PERMANOVA $F_{2,12}$=2.61, p<0.001) and goby community composition significantly differs for each microhabitat (PERMANOVA $F_{2,12}$=3.67, p<0.001).


Stress =0.15
Coral
Rubble
Sand
NMDS2
NMDS1
1.0
0.5
0.0
−0.5
−1.0
−1
0
1

A


Stress =0.17
Coral
Rubble
Sand
NMDS2
NMDS1
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2
−1
0
1
2

B

Figure 4

Goby species abundances

Rank-abundance plot of goby species sampled in the central Saudi Arabian Red Sea using 1m^2 rotenone stations in three microhabitat types (color coded). Vertical bars represent the total combined number of individuals sampled in all quadrats (n = 5 quadrats per microhabitat type) for all 31 goby species found in this study. Coloration of the vertical bars indicates the portion of individuals of each species found in the three microhabitat types.