Barcoding and Metabarcoding the Cryptofauna of the Northern Red Sea

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Abstract

Cryptofauna (small hidden organisms) are difficult to survey with traditional dive collection methods (Plaisance et. al, 2009), but by using autonomous reef monitoring systems (ARMS), these invertebrates can be collected with relative ease. To better understand the biodiversity in the understudied Gulf of Aqaba (northern Red Sea), DNA barcoding and DNA metabarcoding (Yu et. al, 2012) were used to identify different sized organisms collected via ARMS. It was found that the biodiversity of organisms 500 µm - 2 mm in size is far greater than the biodiversity of organisms larger than 2 mm.

An ARMS sits on the ocean floor in the Gulf of Aqaba. Photo courtesy of Matthieu Leray.

Methods

Two types of samples were processed:

Individual specimen (organisms larger than two mm) were processed with DNA barcoding:

- Each specimen was tissue sampled and DNA was extracted from each subsample
- PCR was used to isolate and amplify a portion of the mitochondrial CO1 gene
- The partial CO1 genes were sequenced and used for individual identification
- Taxonomic identifications were made using NCBI Basic Logical Alignment Search Tool (BLAST, 98% similarity cutoff) and Barcode Of Life Database Systems (BOLD, 98% similarity cutoff)

Bulk samples (material containing organisms 500 µm – 2 mm in size) were processed with DNA metabarcoding:

- Samples were blended into a homogenous mixture
- A subsample of the mixture was taken and DNA from the organisms present was extracted
- Universal primers were used in the PCR process to isolate and amplify a portion of the CO1 gene from all DNA present
- The partial CO1 genes were sequenced using a pyrosequencing platform
- Taxonomic identifications were made using NCBI BLAST (98% similarity cutoff), BOLDSystems (98% similarity cutoff), and Statistical Assignment Package (SAP, 80% and 95% posterior probability)

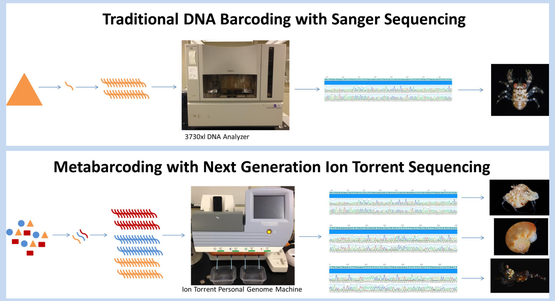
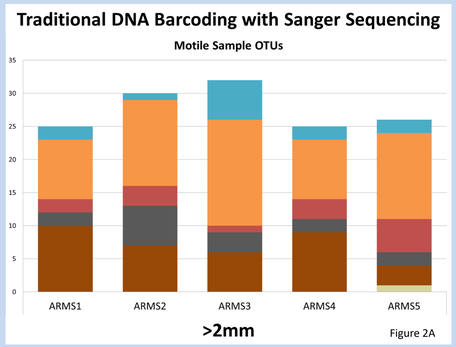
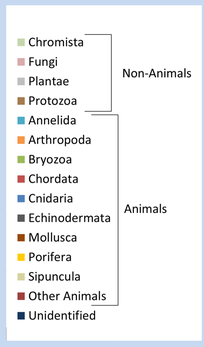


Figure 1A and 1B: Diagrams illustrating the traditional barcoding and metabarcoding processes

Motile organisms, from left to right: crab (Brachyura), spaghetti worm (Terebellidae), sea star (*Aquilonastra yairi*). Photos courtesy of Matthieu Leray.

Results





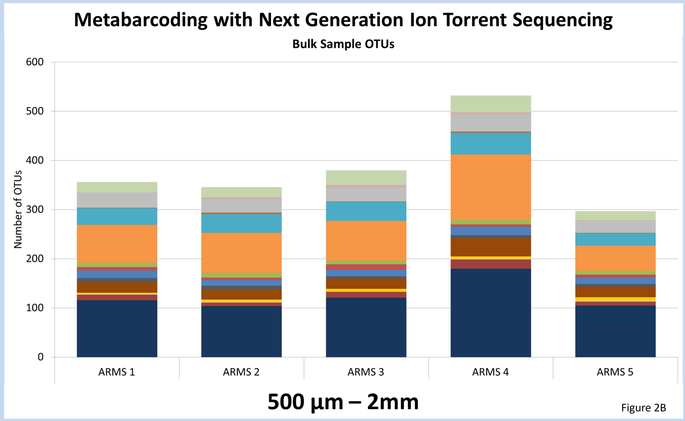


Figure 2A and 2B: Number of operational taxonomic units (OTUs) for both 500µm-2mm and >2 mm size classes, separated according to the ARMS they were found on. The legend above is provided for both Figures 1 and 2. "Other" contains Brachiopoda, Entoprocta, Nematoda, Nemertea, Platyhelminthes, Tardigrada, and Xenacoelomorpha.

As seen in Figure 2A and 2B, the individual samples (organisms > 2mm) contained far fewer operational taxonomic units (OTUs) than the bulk samples (organisms 500 µm - 2 mm). Both types of samples included large proportions of Arthropoda and similar proportions of Chordata and Annelida.

25.30% of individual samples (organisms > 2mm) could be matched to reference sequences present in NCBI BLAST and BOLDSystems (>98% similarity). Morphology was used to provide taxonomic assignments to unidentified individual samples.

Only 3.13% of sequences from the 500 µm - 2 mm bulk samples were successfully matched to a reference sequence in NCBI BLAST or BOLDSystems (>98% similarity). An additional 58.49% were identified using a phylogenetic approach, but 38.38% of sequences could not be confidently assigned to a higher taxonomic level.

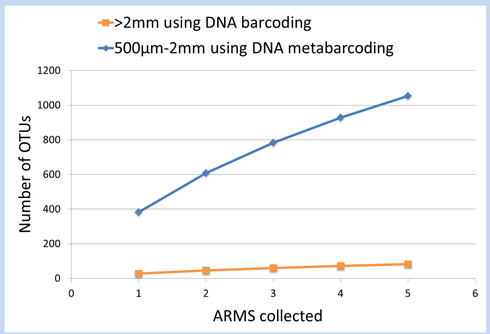


Figure 3: The total number of OTUs collected plotted against the number of ARMS collected shows the trend of how many organisms of each size class can be found by increased sampling.

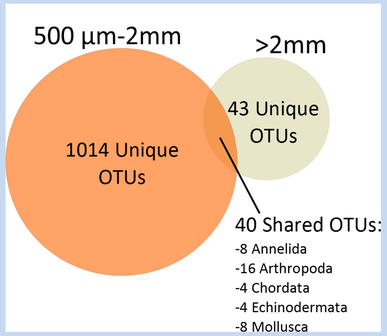


Figure 4: Number of operational taxonomic units (OTUs) found in each sample, with shared OTUs indicated by overlap.

As more ARMS were collected, the overall number of OTUs collected increased for both types of samples (Figure 3). However, as the number of ARMS surveyed increased, the number of OTUs in the bulk samples (500 µm-2mm) increased more drastically than the number of OTUs in the individual samples (>2 mm). Thus by increasing the sample size (collecting additional ARMS), a greater representation of the full biodiversity can be collected, particularly for very small organisms.

Metabarcoding of bulk samples yielded a far greater total number of OTUs (1054) than traditional barcoding of individual samples (83) (Figure 4). Only 40 OTUs were shared between the individual and bulk samples.

Discussion

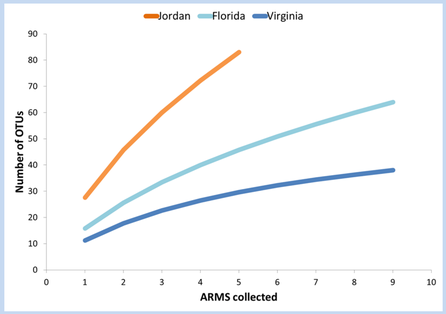


Figure 5: Number of operational taxonomic units collected in individual samples (>2mm) from ARMS at different sites.

ARMS provide a standardized method of examining coral reef communities, and thus can be used to compare reefs around the world, as well as different locations within one reef, in a variety of ways. Still, there are limited data available to compare these Gulf of Aqaba findings with different locations. Since samples of 500 µm – 2 mm are not usually collected and studied, only the motile samples (>2mm) can be compared to those of different reefs. As seen in Figure 5, the number of OTUs found in ARMS from the Gulf of Aqaba is greater than the number of OTUs found in ARMS from Florida and Virginia (TMON ongoing research). Since the biodiversity of the bulk samples was so much greater than the individual samples in the Gulf of Aqaba, a similar pattern would be expected in other regions.

Conclusions and Future Work

- Within the Gulf of Aqaba, the biodiversity of organisms 500 µm – 2 mm in size (bulk samples) is far greater than the biodiversity of organisms > 2mm in size
- Metabarcoding is able to detect many OTUs that are overlooked in samples of large organisms identified by traditional barcoding, but many sequences obtained from bulk samples do not match sequences in existing databases
- Since the number of OTUs found in the Gulf of Aqaba is greater than those found in Florida and Virginia for motile samples, it is likely the number of OTUs found in organisms 500 µm – 2mm in size is also greater than what is present in Florida and Virginia
- Future data collection should include organisms 500 µm – 2mm in size to provide a more in-depth look at the entire community

Big Picture

- Metabarcoding allows for the detection of organisms that have been previously overlooked due to their small size, which opens up possibilities for a variety of new biodiversity studies

Acknowledgments

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REU Site, EAR-1062692

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