Passive eDNA collection enhances aquatic biodiversity analysis

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Motivation

Environmental DNA (eDNA) Metabarcoding

● Novel method for assessing biodiversity wherein samples are taken from the environment via water, sediment or air from which DNA is extracted, and then amplified using general or universal primers in polymerase chain reaction and sequenced using next-generation sequencing to generate thousands to millions of reads.

● Metabarcoding removes the need for multiple taxonomic experts by automatically matching the DNA samples to a taxonomic identity from an existing database.

● Because the identity matching is automated, the limitations come purely from how many samples can be metabarcoding.
Motivation

Room for Improvement?

- Main objective $\rightarrow$ more samples
- Increase the amount of samples that one can collect by switching from active filtering to passive filtering

Active Filtering

- Collect water samples (1 L - 20 L, depends on the environment) and actively pump the water through membranes to collect eDNA samples
- Extremely time and energy intensive, requiring specialized equipment
Design and Implementation

Their Approach

- They present the alternative of switching from the active pump system to a passive membrane collection system
- Using two membranes:
  - **positively charged nylon** to catch eDNA particles by charge attraction
  - **non-charged cellulose ester** to catch eDNA particles by entrapment
- They attached these membranes to an oyster aquaculture frame with mesh pockets and submerged underwater
Evaluation

Varying Climate

● They tested their system in two different climates
  ○ Tropical Waters in the Ashmore Reef
  ○ Temperate Waters around Daw Island

Varying Collection Time

● They tested how varying the particle collection time affected the overall taxa identification

Compared Against Active Filtering in all Cases

● In all tests at both locations they compared against active filtering (using 9 L of water) as their ground truth
Sample Post Processing

Standard Methods Used for all eDNA Sequencing

● One-step quantitative polymerase chain reaction (qPCR) were performed with each sample and a universal primer
  ○ Primer is picked based on the fact that they’re targeting fish taxa
● PCR outputs also include controls
  ○ Positive Control: DNA sample of a fish that should not be in the environment that they collected, but should be identified with 100% accuracy
    ■ All identified the known fish with 100% accuracy
    ■ Minimum reads in the positive control was 36, therefore a conservative cutoff for their application was 40 reads
  ○ Negative Control: use deionized water instead of DNA sample
    ■ No sample produced more than 5 reads for any species
● They compare these outputs against a database of known fish (different for each region)
  ○ < 80% match: sample discarded
  ○ 80 % < match < 90 % : family
  ○ 90 % < match < 97 % : genus
  ○ > 97 % : species
### Supplementary Data and Results - Ashmore

#### Table 1: Data detected at Ashmore Reef

| Family name | Taxon name | Passive filtration | Non-charged | Active filtration |
|-------------|------------|---------------------|-------------|-------------------|
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | Charged 4h | 8h | 12h | 24h | Non-charged 4h | 8h | 12h | 24h | Active filtration 4h | 8h | 12h | 24h |

#### Table 1 (continued)

| Family name | Taxon name | Passive filtration | Non-charged |
|-------------|------------|---------------------|-------------|
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |

#### Table 1 (continued)

| Family name | Taxon name | Passive filtration | Non-charged |
|-------------|------------|---------------------|-------------|
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |

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*Note: This table details selected data extracted from the Ashmore Reef dataset. Additional information is available in the full report.*

#### Table 2: shouted at Ashmore Reef

| Family name | Taxon name | Passive filtration | Non-charged |
|-------------|------------|---------------------|-------------|
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |
|             |            | Charged 4h | 8h | 12h | 24h | 4h | 8h | 12h | 24h |

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*Note: This table includes selected data from the Ashmore Reef dataset. Additional information is available in the full report.*
### Table 2: Taxa detected at Daw Island.

| Family name | Taxon name | Passive filtration | Active filtration |
|-------------|------------|--------------------|-------------------|
|             |            | Charged            | Non-charged        |
|             |            | 4 h  6 h  12 h  24 h 34 h  4 h  6 h  12 h  24 h 34 h |

#### Table 2 (continued)

| Family name | Taxon name | Passive filtration | Active filtration |
|-------------|------------|--------------------|-------------------|
|             |            | Charged            | Non-charged        |
|             |            | 4 h  6 h  12 h  24 h 34 h  4 h  6 h  12 h  24 h 34 h |

#### Table 2 (continued)

| Family name | Taxon name | Passive filtration | Active filtration |
|-------------|------------|--------------------|-------------------|
|             |            | Charged            | Non-charged        |
|             |            | 4 h  6 h  12 h  24 h 34 h  4 h  6 h  12 h  24 h 34 h |

#### Table 2 (continued)

| Family name | Taxon name | Passive filtration | Active filtration |
|-------------|------------|--------------------|-------------------|
|             |            | Charged            | Non-charged        |
|             |            | 4 h  6 h  12 h  24 h 34 h  4 h  6 h  12 h  24 h 34 h |
## Results - Analyzing Mean Values

### Mean Taxa Detected

|                | Ashmore | Daw   |
|----------------|---------|-------|
| **Charged**    | 3       | 8     |
| **Non-charged**| 10      | 11    |
| **Active**     | 42      | 17    |

### Mean Taxa Detection Based on Submersion Time

|                | Ashmore | Daw   |
|----------------|---------|-------|
| **After 4 Hours** | 2       | No significant differences between any of the filters, including active |
| **After 8 Hours** | 5       |       |
Results - Analyzing Taxa Community

Ashmore Reef
a) Charged(45) Non-Charged(100)
5  5  25
4  31 39
63
Active Filtration(137)

Daw Island
Charged(59) Non-Charged(59)
5  8  5
4  42 4
3
Active Filtration(53)

b) Charged - Non-Charged - Active Filtration
Conclusion

Their results show **promising evidence** that it could be used to properly collect eDNA and **significantly expand the amount of environmental metabarcoding** that can be done and biodiversity that can be analyzed.

The passive solution is

- Inexpensive and scalable  
  - Eliminates any need for active / manual collection and filtration
- More appropriate for temperate, but still acceptable for tropical environments
- Easily replicable for more analysis on viability as well as implementation  
  - Different membrane materials, understanding physical limitations of membranes, understanding implications of varying environments