# 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 → **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

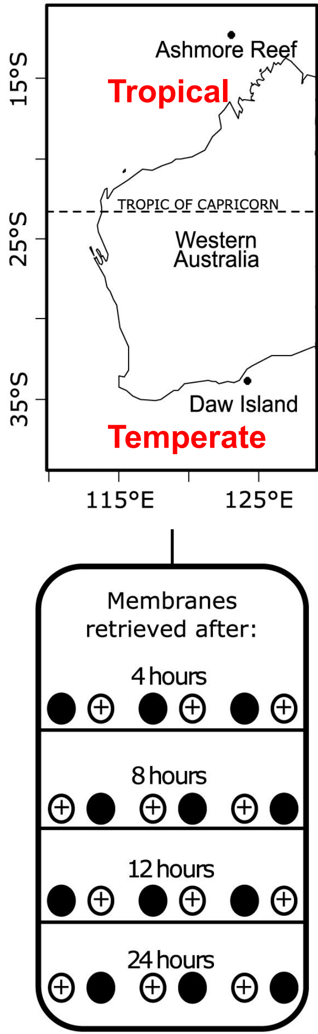
- They tested their system in two different climates
    - **Tropical Waters** in the Ashmore Reef
    - **Temperate Waters** around Daw Island

## 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 Taxa detected at Ashmore Reef.**

| Family name | Taxon name | Passive filtration | | | | | | | | Active filtration |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Charged | | | | Non-charged | | | | |
| | | 4 h | 8 h | 12 h | 24 h | 4 h | 8 h | 12 h | 24 h | |
| Acanthuridae | *Acanthurus blochii* | | | | | | | B | B | B |
| | *Acanthurus lineatus* | | | | | | | | | A |
| | *Acanthurus triostegus* | | B | B | B | B | B | B | B | B |
| | *Acanthurus xanthopterus* | | B | B | B | B | B | | B | B |
| | Ctenochaetus binotatus | | B | | B | | B | B | | B |
| | *Ctenochaetus striatus* | | B | | B | | B | B | | B |
| | *Naso annulatus* | | | | | | | | | A |
| | *Naso brachycentron* | | | | | | | P | | |
| | *Naso unicornis* | | | | | | | | | A |
| | *Zebrasoma* sp. | | | | | | | | | A |
| | *Zebrasoma scopas* | | | | | | | | | A |
| Albulidae | *Albula glossodonta* | | | | | B | B | B | | B |
| Apogonidae | *Apogon* sp. | | | | | | | B | B | B |
| | *Jaydia* sp. | | | | | | | B | | B |
| Arripidae | *Arripis georgianus* | | | | | P | | | | |
| Atherinidae | *Hypoatherina temminckii* | | | | B | B | B | | B | B |
| Balistidae | *Balistapus undulatus* | | | | | P | | P | P | |
| | Balistidae—unknown 1 | | | | | B | B | B | B | B |
| | *Melichthys niger* | | | | | | B | | | B |
| | *Rhinecanthus aculeatus* | | B | | B | B | B | B | B | B |
| | *Rhinecanthus rectangulus* | | | | | | | | | A |
| | *Rhinecanthus verrucosus* | | | | | | | | P | |
| Belonidae | *Platybelone argalus* | | | | | | | | | A |
| | *Strongylura incisa* | | | B | | | B | B | | B |
| | *Tylosurus crocodilus* | | B | | | B | B | B | B | B |
| Blenniidae | *Aspidontus taeniatus* | | | | | | | | | A |
| | *Atrosalarias holomelas* | | | | | | | | | A |
| | *Blenniella periophthalmus* | | | | B | B | | | B | B |
| | *Cirripectes* sp. | | | | | | | P | | |
| | *Salarias fasciatus* | | | | | | | B | B | B |
| Carangidae | *Caranx ignobilis* | | | B | B | B | | | | B |
| | Carangidae—unknown 1 | | B | | | | | B | B | B |
| Chaetodontidae | *Chaetodon auriga* | | | | | | | P | | |
| | *Chaetodon vagabundus* | | | | | | B | | | B |
| | *Chaetodon* sp. | | | | | | | | | A |
| Cirrhitidae | *Paracirrhites forsteri* | | | | | | | | | A |
| Clupeidae | *Amblygaster sirm* | | | | | | B | | | B |
| | *Spratelloides delicatulus* | B | B | B | B | B | B | B | B | B |
| | *Spratelloides gracilis* | | | | | | | | P | |
| Congridae | *Gnathophis* sp. | | | | | | | | | A |
| Ephippidae | *Platax orbicularis* | P | | | | P | | | | |
| | *Platax teira* | | B | B | B | | B | B | B | B |
| Fistulariidae | *Fistularia commersonii* | | | | | | | | | A |
| Gadidae | *Micromesistius* sp. | | | P | | | | | | |
| Gobiidae | *Bryaninops* sp. | | | | | | | | | A |
| | *Eviota* sp. 1 | | | | | | | | | A |
| | *Eviota* sp. 2 | | | | | B | B | B | | B |
| | *Exyrias* sp. | | | | | | | | | A |
| | *Gobiodon* sp. | | | | | | | | | A |
| | Gobiidae—unknown 1 | | | | | | | | | A |
| | Gobiidae—unknown 2 | | | | | | | | | A |
| | Gobiidae—unknown 3 | | | | | | P | | | |
| | Gobiidae—unknown 4 | | | | | | | | | A |
| | *Paragobiodon* sp. | | | | | | | | | A |
| | *Vanderhorstia ornatissima* | | | | | | | | | A |
| Haemulidae | *Plectorhinchus chaetodonoides* | | | | | B | B | B | B | B |
| Hemiramphidae | *Hyporhamphus* sp. | | | | | | | | B | B |
| Holocentridae | *Myripristis botche* | B | | | | B | | | B | B |
| | *Myripristis murdjan* | | | | | | | | | A |
| | *Sargocentron rubrum* | | | | | | | | | A |
| Kuhliidae | *Kuhlia* sp. | | | | | | | | | A |
| Kyphosidae | *Kyphosus* sp. | | | | | B | B | B | B | B |

**Table 1 (continued)**

| Family name | Taxon name | Passive filtration | | | | | | | | Active filtration |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Charged | | | | Non-charged | | | | |
| | | 4 h | 8 h | 12 h | 24 h | 4 h | 8 h | 12 h | 24 h | |
| Labridae | *Anampses* sp. | | | | | | | | | A |
| | *Chlorurus* sp. | | | | | | | | | A |
| | *Choerodon schoenleinii* | | | | | | | | | A |
| | *Cirrhilabrus exquisitus* | | | | | | | | | A |
| | *Cymolutes praetextatus* | B | B | | | B | B | B | B | B |
| | *Epibulus* sp. | | P | | | | | | | |
| | *Gomphosus varius* | | | | | | P | | | |
| | *Halichoeres hortulanus* | | | | | | | | | A |
| | *Halichoeres melanurus* | | | | | | | | | A |
| | *Halichoeres nebulosus* | | | | | | | | | A |
| | *Halichoeres trimaculatus* | | B | B | | B | B | B | B | B |
| | *Hipposcarus longiceps* | | B | | | | B | B | | B |
| | *Labrichthys unilineatus* | | B | B | | B | | | | B |
| | *Novaculichthys taeniourus* | | | B | | | | | B | B |
| | *Pseudocheilinus evanidus* | | | | | | | | P | |
| | *Pseudocheilinus hexataenia* | | | | | | | | B | B |
| | *Scarus dimidiatus* | | | | | | | | | A |
| | *Scarus niger* | | | | | | | | | A |
| | *Scarus psittacus* | | | | | | | | | A |
| | *Scarus* sp. | | | | | | | | | A |
| | *Stethojulis bandanensis* | | | | | | | B | B | B |
| | *Stethojulis strigiventer* | | | | | | | | B | B |
| | *Stethojulis* sp. | | | | | | | B | B | B |
| | *Stethojulis trilineata* | | | | | P | | | | |
| | *Thalassoma amblycephalum* | | | | | B | | | | B |
| | *Thalassoma lunare* | | | | | | B | | B | B |
| | *Thalassoma* sp. | | | | | | B | | B | B |
| Lethrinidae | *Lethrinus nebulosus* | | | | | | | | | A |
| | *Lethrinus obsoletus* | | | | | | | | | A |
| | *Lethrinus* sp.1 | | | | | B | B | | B | B |
| | *Lethrinus* sp.2 | | | | | | | | | A |
| | *Lethrinus variegatus* | | | | | | | | | A |
| | *Monotaxis grandoculis* | | | | | | | | | A |
| Lutjanidae | *Aprion virescens* | B | | | | B | B | B | | B |
| | *Caesio caerulaurea* | | | | | | | P | | |
| | *Caesio xanthonotus* | | | | | | | | | A |
| | *Lutjanus bohar* | | B | B | | B | B | B | B | B |
| | *Lutjanus decussatus* | | | | | | | | | A |
| | *Lutjanus fulvus* | | | | | | | P | | |
| | *Lutjanus kasmira* | | | | | | | | B | B |
| | *Lutjanus* sp. 1 | | B | B | | B | B | B | B | B |
| | *Lutjanus* sp. 2 | B | | | | B | B | B | B | B |
| | *Pterocaesio* sp. | B | B | | | B | | | | B |
| | *Pterocaesio tile* | | | | | | | | | A |
| Monacanthidae | *Cantherhines dumerilii* | | | | | P | P | | | |
| | *Monacanthus chinensis* | P | | | | | | | | |
| Mugilidae | *Crenimugil crenilabis* | | | | | | P | | | |
| | *Ellochelon vaigiensis* | | P | | | | | P | | |
| | *Mulloidichthys vanicolensis* | | | | | B | B | | B | B |
| | *Parupeneus barberinus* | | | | | | | | | A |
| | *Parupeneus chrysopleuron* | | | | | P | | | | |
| | *Parupeneus multifasciatus* | | | | | | | | | A |
| | *Parupeneus* sp. | B | B | | | | B | | B | B |
| | *Upeneus tragula* | P | | | | | | | | |
| Muraenidae | *Echidna nebulosa* | | | | | | B | B | | B |
| | *Gymnothorax buroensis* | | | | | | | | | A |
| | *Gymnothorax flavimarginatus* | | | | | | | | | A |
| | *Gymnothorax* sp. | | | | | B | B | B | | B |
| Myctophidae | *Diaphus watasei* | | | | | | | | | A |
| | Myctophidae—unknown 1 | | | | | | | | | A |
| Myliobatidae | *Aetobatus ocellatus* | B | B | B | B | B | B | B | B | B |
| Platycephalidae | *Sunagocia otaitensis* | | | | | | P | | | |
| | *Plesiops* sp. | | | | | | B | B | | B |

**Table 1 (continued)**

| Family name | Taxon name | Passive filtration | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Charged | | | | Non-charged | | |
| | | 4 h | 8 h | 12 h | 24 h | 4 h | 8 h | 12 |
| Pomacentridae | *Abudefduf sexfasciatus/vaigiensis* | | | | | B | B | B |
| | *Acanthochromis* sp. | | | | | | | |
| | *Amblyglyphidodon curacao* | | | | | | | |
| | *Chromis atripectoralis* | | B | | | | | |
| | *Chromis atripes* | | | | | | | |
| | *Chromis lepidolepis* | | | | | | P | |
| | *Chromis ternatensis* | | B | | | | | |
| | *Chromis* sp. | | | | | B | | B |
| | *Chromis viridis* | | | | B | | | |
| | *Chrysiptera glauca* | | B | | B | B | | |
| | *Chrysiptera rex* | | | | | | | |
| | *Chrysiptera* sp. | | B | | | | B | B |
| | *Dascyllus aruanus* | | | | | | | B |
| | *Dascyllus reticulatus* | | | | | P | P | |
| | *Dascyllus trimaculatus* | | | P | | | P | |
| | *Dischistodus prosopotaenia* | | | | | | | |
| | *Hemiglyphidodon plagiometopon* | | | | | | | |
| | *Neopomacentrus* sp. | | | | | | | |
| | *Plectroglyphidodon lacrymatus* | | | | | | | |
| | *Plectroglyphidodon leucozonus* | | | | | B | | |
| | *Pomacentrus bankanensis* | | | | | | | |
| | *Pomacentrus lepidogenys* | | | | | | | B |
| | *Pomacentrus pavo* | | | | | B | | B |
| | *Pomacentrus* sp.1 | | | | B | | | B |
| | *Pomacentrus* sp. 2 | | | | | | | |
| | *Stegastes fasciolatus* | | | | | | | |
| | *Stegastes nigricans* | | | | B | | | B |
| Pseudochromidae | *Pseudochromis* sp. | | B | | | B | | |
| Rhynchobatidae | *Rhynchobatus* sp. | | P | | | P | P | P |
| Schindleriidae | *Schindleria* sp. | | | | | | | |
| Scombridae | *Auxis* sp. | | | | | | | P |
| | *Euthynnus* sp. | | | | | | | |
| | *Scomberomorus commerson* | | | | | | | |
| Scorpaenidae | *Scorpaenodes guamensis* | | | | | | | |
| Serranidae | *Aethaloperca rogaa* | | | | | | | B |
| | *Cephalopholis argus* | | | | | B | | |
| | *Cephalopholis leopardus* | | | | | | P | |
| | *Epinephelus* sp. | | | | | | B | |
| | *Plectropomus laevis* | B | B | | B | | | B |
| | *Pseudogramma polyacanthus* | | | | | | | |
| | *Variola louti* | | | | | | B | B |
| Soleidae | *Pardachirus pavoninus* | | | | | | | P |
| Synodontidae | *Trachinocephalus myops* | | | | | | | |
| Tetraodontidae | *Arothron mappa* | | | | | | | |
| | *Arothron stellatus* | | | | | | | |
| Xiphiidae | *Xiphias gladius* | | P | | | | | |

Taxa at Ashmore Reef detected by passive filtration treatment and submersion time (h) compared to those detected by active filtration. An A indicates tax
those detected by passive filtration only and B indicates taxa detected by both methods.

# Supplementary Data and Results - Daw

**Table 2 Taxa detected at Daw Island.**

| Family name | Taxon name | Passive filtration: Charged 4 h | Charged 8 h | Charged 12 h | Charged 24 h | Charged 34 h | Non-charged 4 h | Non-charged 8 h | Non-charged 12 h | Non-charged 24 h | Non-charged 34 h | Active filtration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aplodactylidae | *Aplodactylus* sp. | | | B | B | B | B | B | B | B | B | B |
| Arripidae | *Arripis georgianus* | B | B | B | B | B | B | B | B | B | B | B |
| | *Arripis* sp. | B | B | B | | | B | B | B | B | | B |
| Aulopidae | *Latropiscis purpurissatus* | | B | | B | | B | | | | | B |
| Berycidae | *Centroberyx* sp. | P | | | | | | | | | | |
| Bothidae | *Lophonectes* sp. | | | | | | | | P | | | |
| Callionymidae | *Repomucenus calcaratus* | P | | | | | P | | P | P | | |
| Carangidae | *Pseudocaranx* sp. | B | B | B | B | | B | B | B | B | | B |
| | *Pseudocaranx wrighti* | | B | | | | | | | | | B |
| | *Seriola lalandi* | | B | B | | B | B | | B | | B | B |
| | *Trachurus* sp. | | | | B | | | | | | | B |
| Cheilodactylidae | *Cheilodactylus* sp. | | | | | | P | | | | | |
| | Cheilodactylidae—unknown 1 | P | P | | | | P | P | | P | | |
| | *Nemadactylus valenciennesi* | | | | | | | | | P | | |
| Chironemidae | *Chironemus georgianus* | P | | P | | | P | P | | | P | |
| | *Chironemus maculosus* | | B | B | B | | | | B | B | | B |
| Clinidae | *Heteroclinus adelaidae* | B | | | B | | | | B | B | | B |
| | *Heteroclinus eckloniae* | | | | | | B | | | | | B |
| Clupeidae | Clupeidae—unknown 1 | B | B | | | | | B | | | | B |
| | *Sardinops sagax* | B | B | B | B | B | B | B | B | B | B | B |
| Congridae | *Gnathophis longicauda* | | | P | | | | | | | | |
| Dasyatidae | *Bathytoshia brevicaudata* | | | B | B | | | | | | | B |
| Dinolestidae | *Dinolestes lewini* | | | | | | | | | | | A |
| Dussumieriidae | *Etrumeus jacksoniensis* | B | | B | B | | B | B | | B | | B |
| Engraulidae | *Engraulis australis* | B | | | B | | | B | B | B | | B |
| Enoplosidae | *Enoplosus armatus* | | B | B | | | | | | B | | B |
| Gerreidae | *Parequula melbournensis* | | | | | | | B | | B | | B |
| Hemiramphidae | *Hyporhamphus melanochir* | B | | B | | B | B | B | B | B | B | B |
| Isonidae | *Iso rhothophilus* | | | | | | | | | | | A |

**Table 2 (continued)**

| Family name | Taxon name | Passive filtration: Charged 4 h | Charged 8 h | Charged 12 h | Charged 24 h | Charged 34 h | Non-charged 4 h | Non-charged 8 h | Non-charged 12 h | Non-charged 24 h | Non-charged 34 h | Active filtration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kyphosidae | *Girella* sp. | | B | B | B | | B | B | B | B | B | B |
| | *Kyphosus gladius/sydneyanus* | B | B | B | B | | B | B | B | B | B | B |
| | *Scorpis* sp. | B | B | | B | B | B | B | B | B | | B |
| Labridae | *Achoerodus* sp. | B | B | B | B | | B | B | B | | B | B |
| | *Austrolabrus maculatus* | B | B | | | | B | B | B | B | | B |
| | *Bodianus* sp. | | | | P | | P | | | | | |
| | *Eupetrichthys angustipes* | B | B | B | B | | B | B | B | B | | B |
| | *Halichoeres brownfieldi* | B | B | B | B | | B | B | B | B | B | B |
| | Labridae—unknown 1 | | | | B | | | | B | | | B |
| | *Notolabrus fucicola* | | B | | | | B | | | | | B |
| | *Notolabrus parilus* | | B | B | | | B | | B | B | B | B |
| | *Ophthalmolepis lineolata* | | | B | B | | B | | | B | B | B |
| | *Pictilabrus laticlavius* | B | B | B | B | | B | B | B | B | B | B |
| Lamnidae | *Carcharodon carcharias* | | P | | | | | | | | | |
| Monacanthidae | *Acanthaluteres* sp. | B | B | | | | | | B | B | B | B |
| | Monacanthidae—unknown 1 | | | B | | | B | B | B | | B | B |
| | *Nelusetta ayraudi* | P | | P | | | | | P | | | |
| | *Scobinichthys granulatus* | | | | | | | | B | | | B |
| Moridae | *Lotella rhacina* | B | | | | | | | | | | B |
| | *Pseudophycis barbata* | | | | | | | | | | | A |
| Mullidae | *Upeneichthys* sp. | B | | | | B | | | B | B | B | B |
| | *Upeneichthys stotti* | B | B | B | B | | B | B | B | B | | B |
| Myliobatidae | *Myliobatis australis* | | | B | B | | B | B | B | | | B |
| Odacidae | *Heteroscarus acroptilus* | B | | B | | | B | | B | B | | B |
| | *Olisthops cyanomelas* | B | B | B | | | B | B | B | B | B | B |
| | *Siphonognathus* sp. | B | B | | B | | B | B | B | B | | B |
| Pempheridae | *Parapriacanthus elongatus* | B | B | B | B | | B | B | B | B | | B |
| | *Pempheris* sp. | B | B | B | B | | B | B | | | B | B |
| Pinguipedidae | *Parapercis haackei* | B | | B | | | | B | | | | B |
| | *Parapercis ramsayi* | | P | P | | | | | | | | |



**Table 2 (continued)**

| Family name | Taxon name | Passive filtration: Charged 4 h | Charged 8 h | Charged 12 h | Charged 24 h | Charged 34 h | Non-charged 4 h | Non-charged 8 h | Non-charged 12 h | Non-charged 24 h | Non-charged 34 h | Active filtration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Platycephalidae | *Leviprora inops* | P | | | | | | | | | | |
| | *Platycephalus grandispinis* | B | | | B | | | B | B | | | B |
| Pomacentridae | *Chromis* sp. | P | | | P | | P | | | | P | |
| | *Parma microlepis* | B | B | | | | B | B | B | B | | B |
| Scombridae | Scombridae—unknown 1 | | | | | | B | | | | | B |
| | *Scomber* sp. | B | B | | B | | B | B | | B | | B |
| Scorpaenidae | Scorpaenidae— | | | | | | P | | | | | |

# Results - Analyzing Mean Values

## Mean Taxa Detected

Ashmore -

- Charged: 3
- **Non-charged: 10**
- Active: 42

Daw -

- Charged: 8
- **Non-charged: 11**
- Active: 17

## Mean Taxa Detection Based on Submersion Time

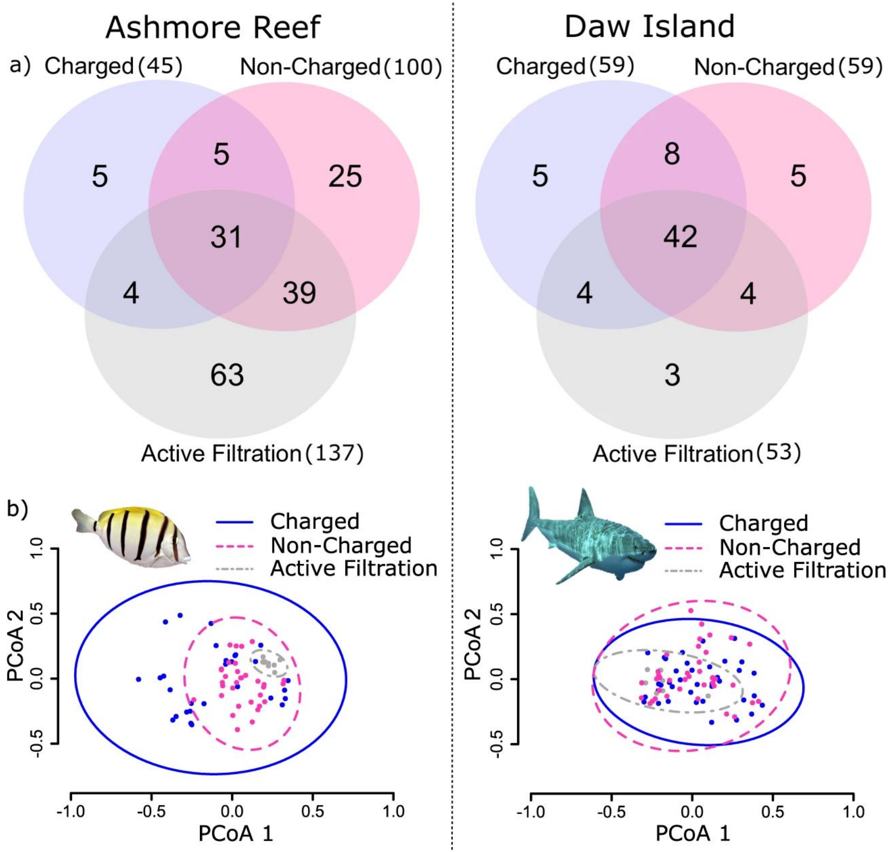
Ashmore -

- After 4 Hours: 2
- After 8 Hours: 5

Daw -

- No significant differences between any of the filters, including active

# Results - Analyzing Taxa Community



# 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