Progress Report

Biomonitoring and bioassessment of fish community structure in a marine protected area with environmental DNA metabarcoding: a pilot EDNAM project

1. Synopsis

On a global scale, human impacts on the natural world are now driving a sixth mass extinction event, and accumulating evidence indicates that biodiversity is not being effectively conserved. Within marine spatial planning frameworks, marine protected areas (MPAs) are important tools to promote ocean health, and to build social, ecological and economic resilience (i.e., 'Blue Economy'). Reel Science Coalition (RSC; a non-profit organisation) has been implementing a shore-based research program within the Helderberg Marine Protected Area (HMPA) for assessing and monitoring surf-zone fish community composition and species abundance with seine net and tag-and-release surveys. However, the structure of fish assemblages accounted for by different sampling methods (e.g., net types, visual surveys) can differ significantly, even among similar ones, in terms of species composition, functional groups (ecological and trophic guilds), and fish size distribution. Consequently, a cross-validation of fish sampling methodologies is necessary to ensure standardization and comparability of monitoring methods. Environmental DNA (eDNA) metabarcoding is a novel, non-invasive method of monitoring and 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 high-throughput sequencing to generate thousands to millions of reads. The proposed project aims to apply marine eDNA metabarcoding, at a temporal and spatial scale, as an alternative approach for the monitoring and assessment of fish biodiversity in the HMPA, to get a near-comprehensive snapshot of community structure and site occupancy rates in the HMPA. Moreover, a comparative analysis of eDNA metabarcoding with conventional sampling methods will allow us to understand the strengths and limitations of each sampling approach. It is envisaged that eDNA metabarcoding will empower the informed management actions necessary for the conservation of marine ecosystems, and to protect and sustain the Blue Economy.

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2. Team and deliverables description

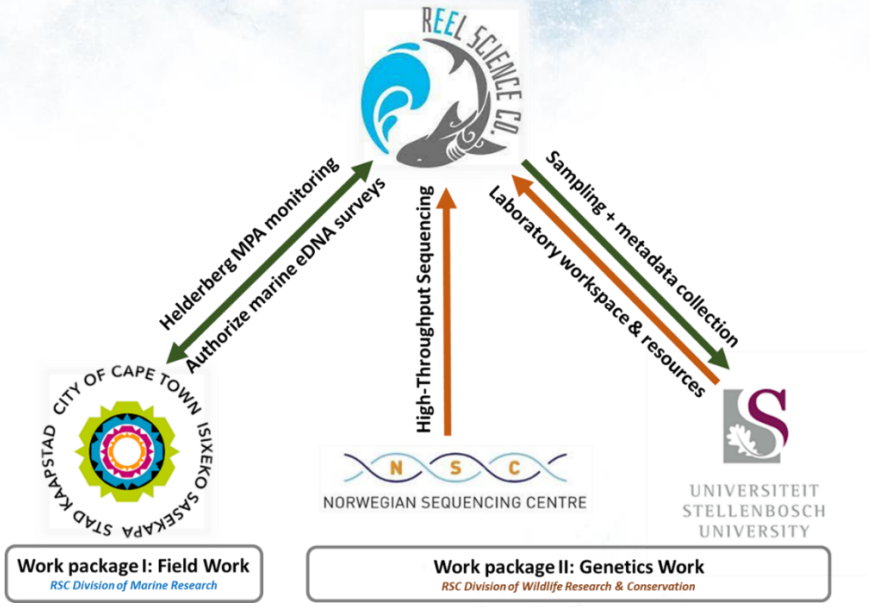


Figure 1 Project organization within Reel Science Coalition (RSC) and co-operating academic (NSC and Stellenbosch University) and government (City of Cape Town) institutions. Green arrow – services RSC provides (single-arrowhead) or commissioned to provide through agreements (double-arrowhead); orange arrow – services provided by collaborators to the RSC project.

Work package I: Field Work (Figure 1)

Manager: Dr Soekoe

1.1. Dr Michelle Soekoe (Founding Director & Research Scientist, Division of Marine Research, Reel Science Coalition; RSC) and Prof. Maduna – sampling design.

1.2. Dr Soekoe – assemble a fieldwork team, collect seawater and filter water samples from HMPA in the field/lab.

Work package II: Genetics Work (Figure 1)

Manager: Prof. Maduna

2.1. RSC Lab technician – perform wet laboratory work with guidance from Prof. Maduna.

2.2. Prof. Aletta Bester-van der Merwe, Stellenbosch University – provide the laboratory space and general resource for lab work, and other non-project specific laboratory equipment.

2.3. Technicians at the Norwegian Sequencing Centre who will help with DNA library preparation and generating high-throughput sequencing data with PacBio HiFi sequencing.

Work package III: Scientific Reporting and Outreach

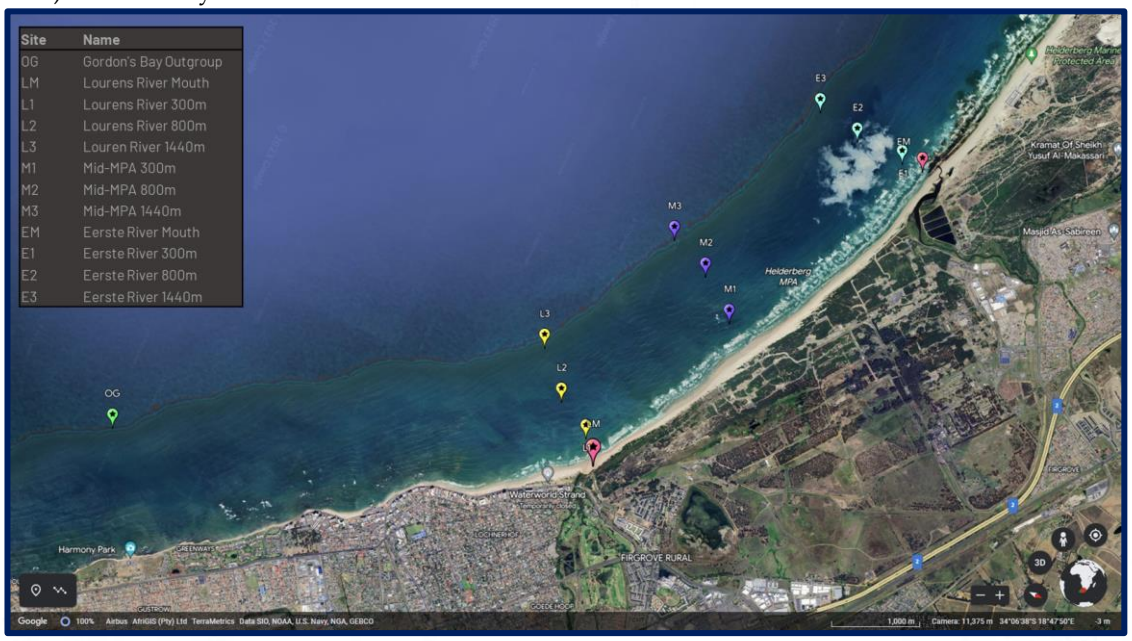
Manager: Prof. Maduna

3.1. RSC Personnel - Conducting scientific outreach in the form of public lectures, podcasts, blogs and social media posting to inform the public on our project, which will take place over the duration of the project.

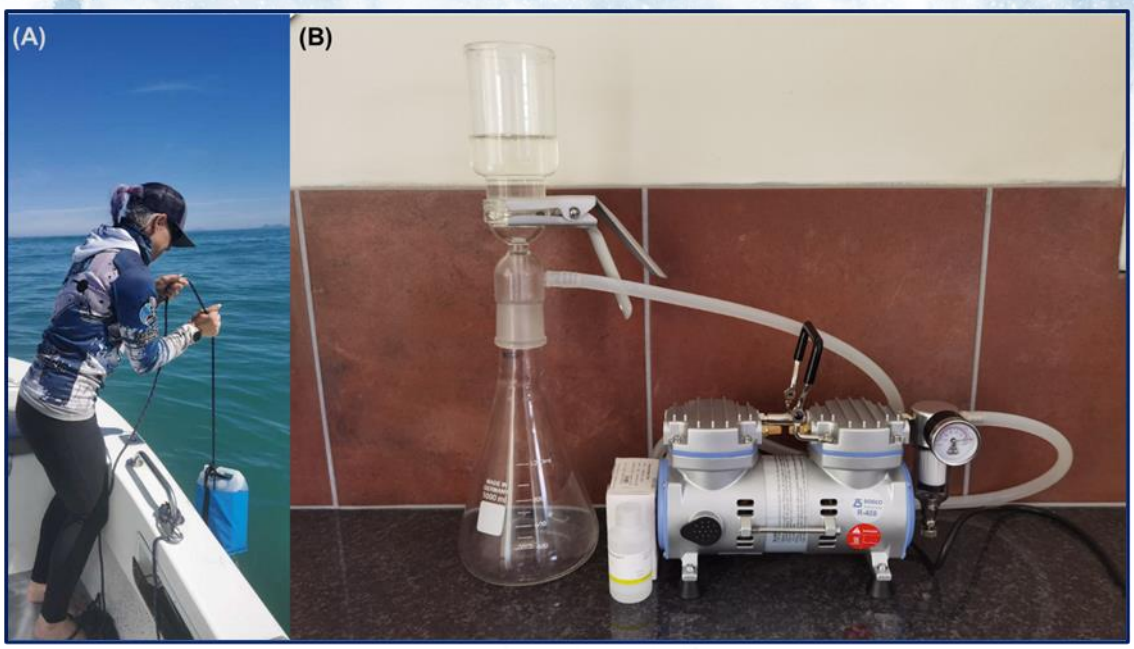
3.2. Dr Soekoe and Prof. Maduna – Scientific reporting and producing education material for various academic levels on the use of eDNA metabarcoding in conservation and management of biodiversity.

3. Milestones

- ***Spatiotemporal Sampling Design:*** Our sampling design has been completed. We designed a spatial sampling strategy that included both horizontal and vertical transects. Here, we classified the horizontal transect based on the division of the MMPA into three partitions from east to west; its start at Lourens River, middle equidistant point from start to end, and its end at the Eerste River. Whereas the vertical transects was based on the distance from the shoreline, where we also included sampling points from the respective river mouths (**Figure 2**). We also included an outgroup sampling site located outside but adjacent to the HMPA at Gordon's Bay, and later, opportunistically added two additional outgroup sites from Witsand (White Sands) and Stilbaai (Still Bay) on the south-east of the Western Cape province. We then obtained temporal samples for our spatial sampling points in the HMPA, where we sampled summer, autumn/fall, and winter months of the project calendar year spanning July 2022- June 2023. However, we could not obtain samples over February-March 2023 due to an outbreak of harmful algal bloom (Red Tide) in our study area.



- **Figure 2** Study sampling design and location of marine water sampling sites in the Helderberg Marine Protected Area (HMPA) on the north-eastern side of False Bay, Western Cape, South Africa. Field view is from the coastline (north) to the seawaters (south).
- ***Water Sampling and Filtration:*** Our water sampling and filtration phase of the project has been achieved. We sampled seawater using sterilized 5L Jerry Cans as sample containers instead of the initially proposed sterile foil laminated plastic "Bag-in-the-Box" containers following field trails accounting for our sampling boats' resources (**Figure 3A**). Moreover, the portable vacuum pumps we initially proposed to use were not available and there were difficulties associated with shipment. We had to pivot and opted to buy a complete vacuum pump system with pipes and manifold (BOECO Vacuum Pump R-400) from Boeco Germany through a local vender in South Africa (**Figure 3B**); cost was a little over £1200 – additional costs incurred were covered by RSC. This phase of our pilot project was unexpectedly among the most time-consuming objectives.



- **Figure 3** Water sampling (A) and filtration (B) equipment. Dr Michelle Soekoe (Work package I manager) collecting seawater samples at the HMPA.
- ***Cataloguing of Fish Species in the HMPA Part I:*** We have completed the cataloguing of species in the HMPA using data from our internal surf-zone fish community composition monitoring project in the HMPA and from available data baited remote underwater video (BRUV) surveys.
- ***Reference Database for Metabarcoding Sequencing Data:*** Using information obtained from the above species catalogue we identified species that do not have any available sequence data for our metabarcodes of interests. We then obtained fin clip samples from our internal finclip biobank or conducted target sampling during our field trip to have a near-complete reference databases per metabarcode. This phase of the project is near-complete pending a few rounds of resequencing for validation purposes.
- ***DNA Extraction, Amplification, and Sequencing:*** The ordering of reagents took longer than expected, however, all reagents had arrived, and this phase of the project is ongoing.
- ***Data Analyses:*** We are busy finalizing our bioinformatics and ecoinformatics pipelines for processing the genetic and ecological metadata using publicly available data as mock data for our experimental design.
- ***Scientific Reporting and Outreach:*** This phase of the project is ongoing, and we have successfully created a soon-to-be-released webpage on our website where we will centralize the sharing of project and outreach documents and videos. We have been busy designing an introductory eDNA course that will cater various academic levels on the use of eDNA metabarcoding in conservation and management of biodiversity.

Yours sincerely,

Prof. Simo Njabulo Maduna | *M.Sc. cum laude (Genetics), Ph.D. (Genetics)*

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