

Microbial dynamics in freshwater pans of Khakea-Bray TBA using metagenomics techniques

Tafara Frank Bute 21B7647

MSc Zoology

Department of Zoology and Entomology

Rhodes University

Background of the study

Certain freshwater pan ecosystems are fed from underground water reserves and these systems are highly dynamic and support a range of aquatic biodiversity.

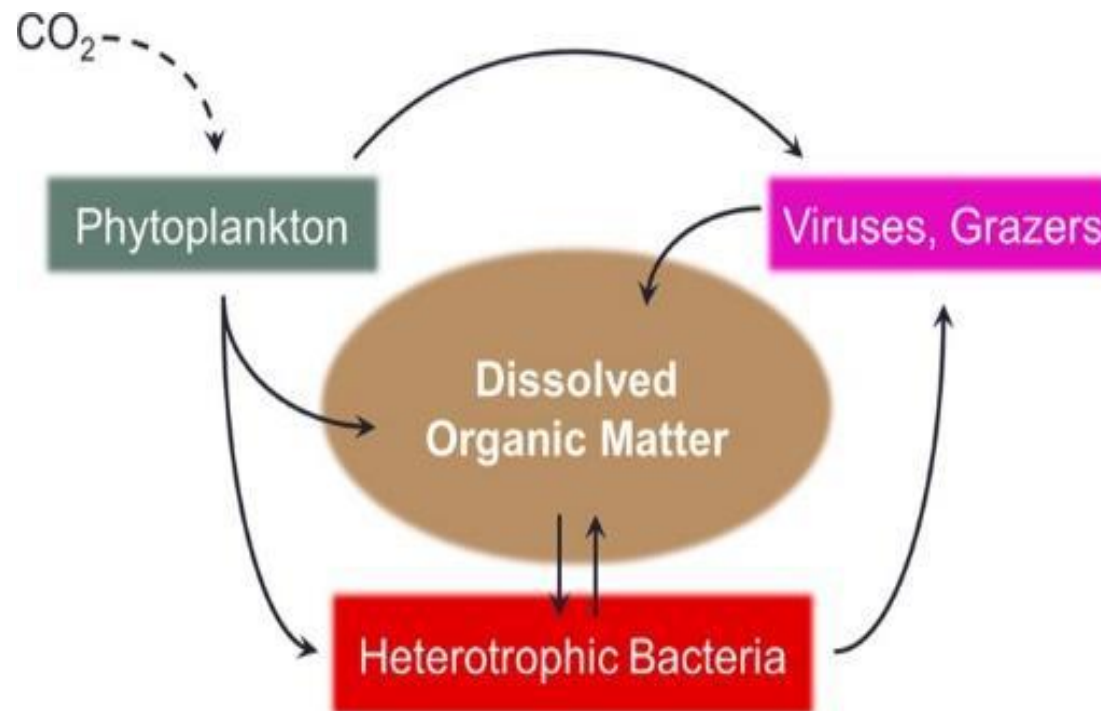


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Background continued....

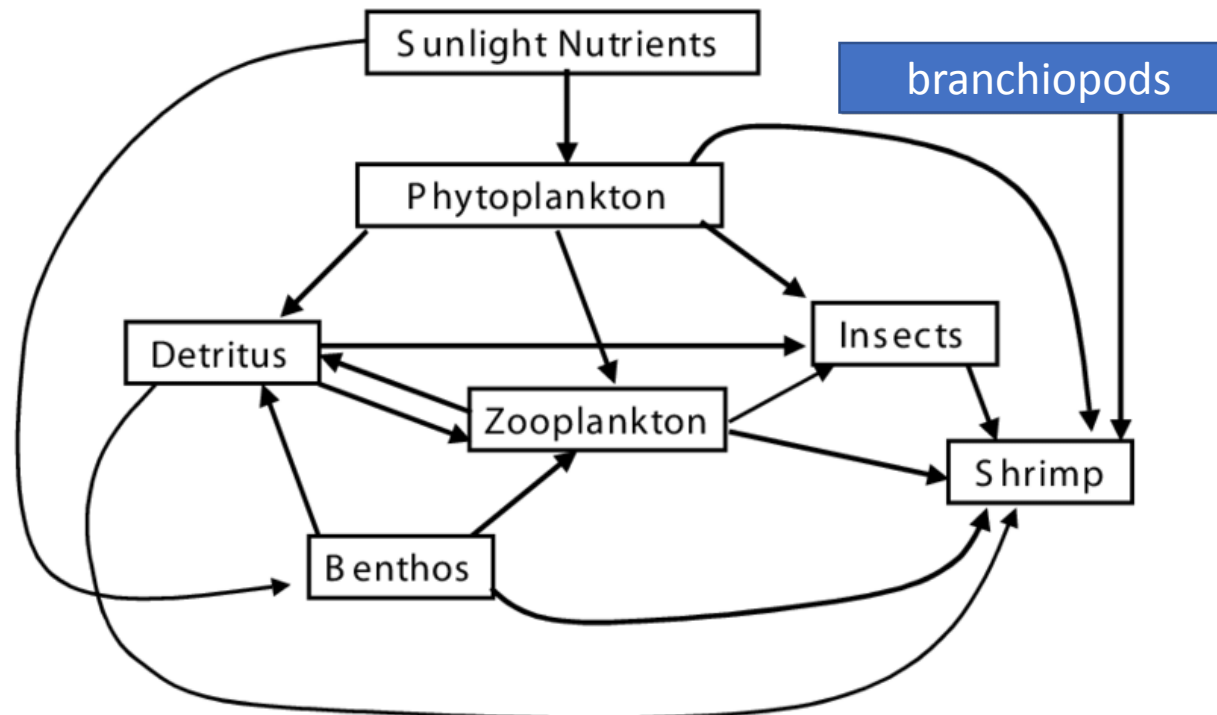
- In these systems, microbial communities contribute to ecological health and their interactions are essential-to food web dynamics.

The microbial loop



Background continued....

- During wet seasons large branchiopods occupy these freshwater pans and are major contributors to secondary productivity biomass of temporary wetland food webs.



Problem statement

- Over-exploitation of groundwater from the Khakea-Bray system for agriculture and domestic use has reduced the amount of water available to support biodiversity in the region, with implications for productivity dynamics
- If the pans are not filling up to their maximum capacity what biodiversity is being lost?

Justification

- There is lack of biodiversity data with reference to the composition of microbial communities found in freshwater systems in southern Africa, including pans of Khakea-Bray TBA.
- There is no knowledge on how loss in diversity of microbes will impact ecosystem functioning.

General Objective

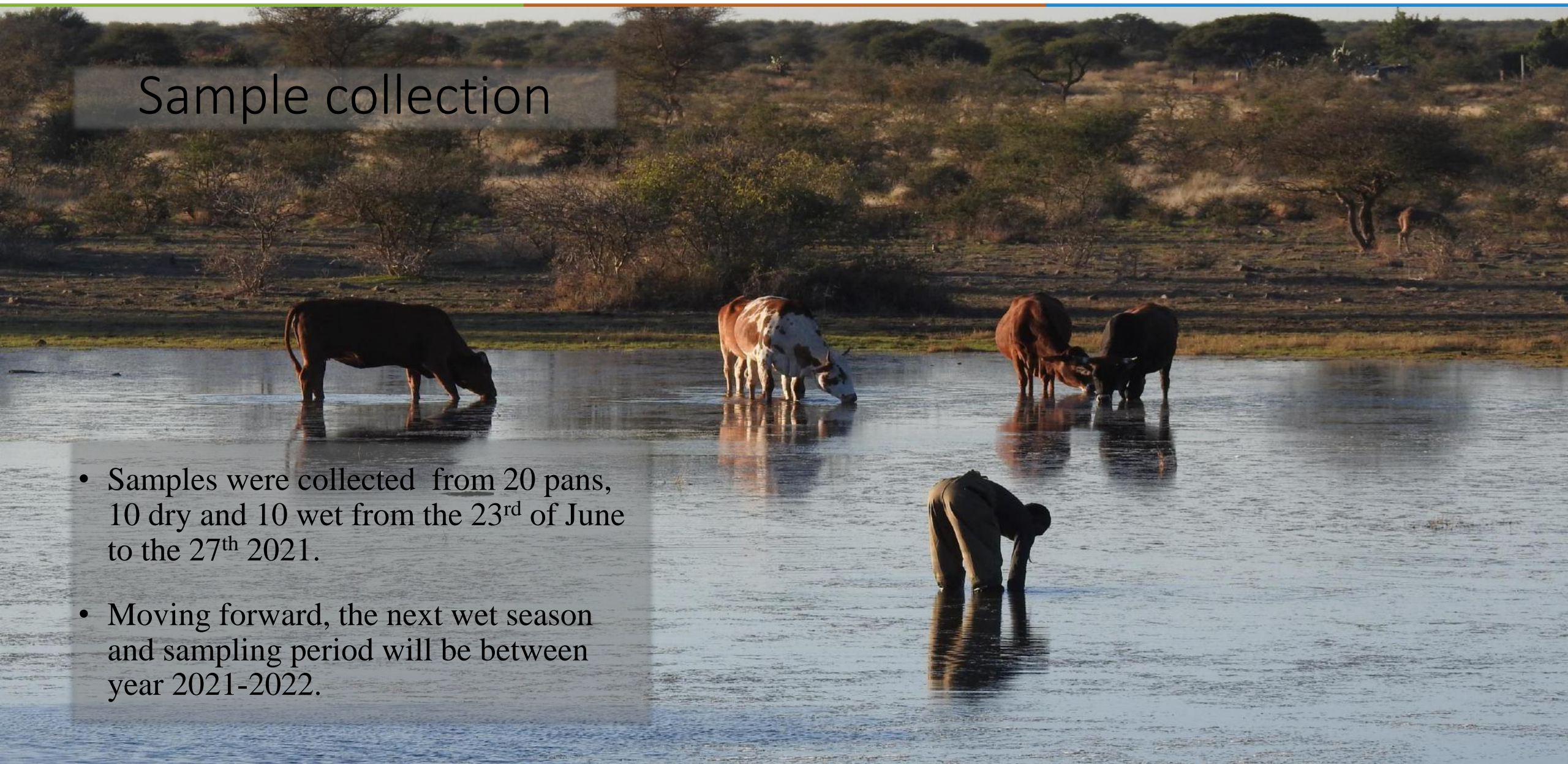
- The main objective of this study is to conduct an ecological assessment of microbial diversity of the freshwater pans in Khakea-Bray TBA, and its role in the diet of major secondary producer biomass.

Specific objectives

- To determine microbial diversity in benthic sediment using Illumina Sequencing technology.
- To determine extracellular polymeric substance (carbohydrate & protein) production associated with microbial activities.
- To assess the contribution of microbes to diet of large branchiopod crustaceans (major contributors to secondary productivity biomass) in pans.

Sample collection

- Samples were collected from 20 pans, 10 dry and 10 wet from the 23rd of June to the 27th 2021.
- Moving forward, the next wet season and sampling period will be between year 2021-2022.



Sample collection

- A transect was generated as highlighted from the centre/deep, middle and outside. Samples were extracted in replicates of 3 moving from the centre to the outside making a total of 9 per each pan.

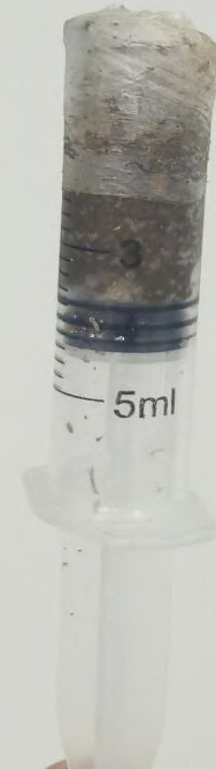


Student and Dr. Adam Wyness
collecting sample from the
outside point of a transect.



Collected sediment samples

- Benthic sediment samples were collected by targeting upper 2 cm using a sediment corer of 12 mm internal diameter for DNA and 20mm corer for benthic chlorophyll-a



Sediment analysis for extracellular polymeric substances (EPS)

- EPS is made up (mostly) of carbohydrates and proteins
- Carbohydrates are produced predominantly by microalgae
- Proteins are produced by bacteria
- The overall amount, and ratio between them will tell us how much microbial production there is, and who is responsible for it
- Carbohydrate concentration in sediment samples will be measured by the phenol-sulfuric acid assay (Underwood, 1995)
- Total protein (PRT) analysis will be measured using a modified Lowry procedure

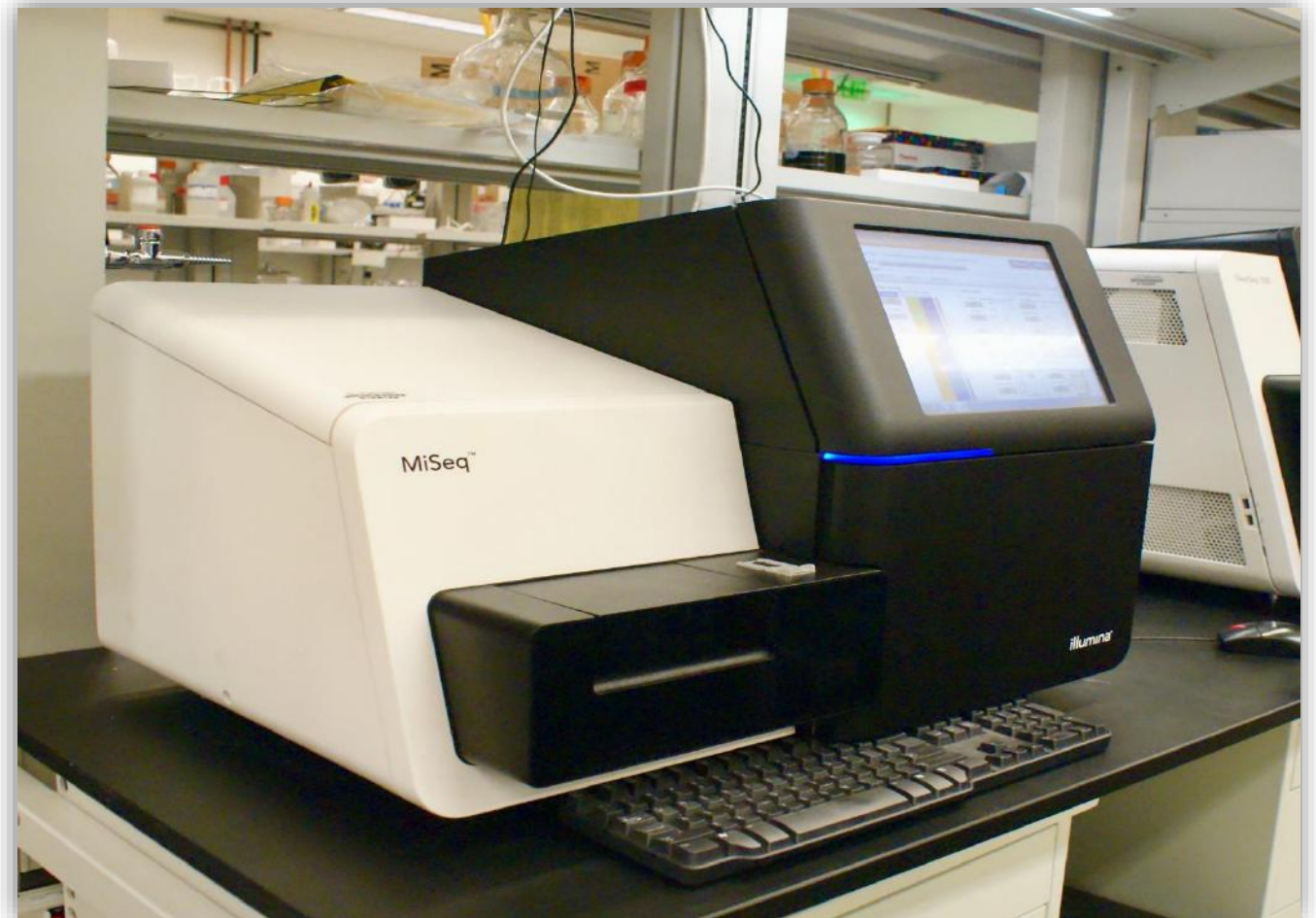
Wet phase

- Large branchiopods
- Secondary production
- Gut microbiota

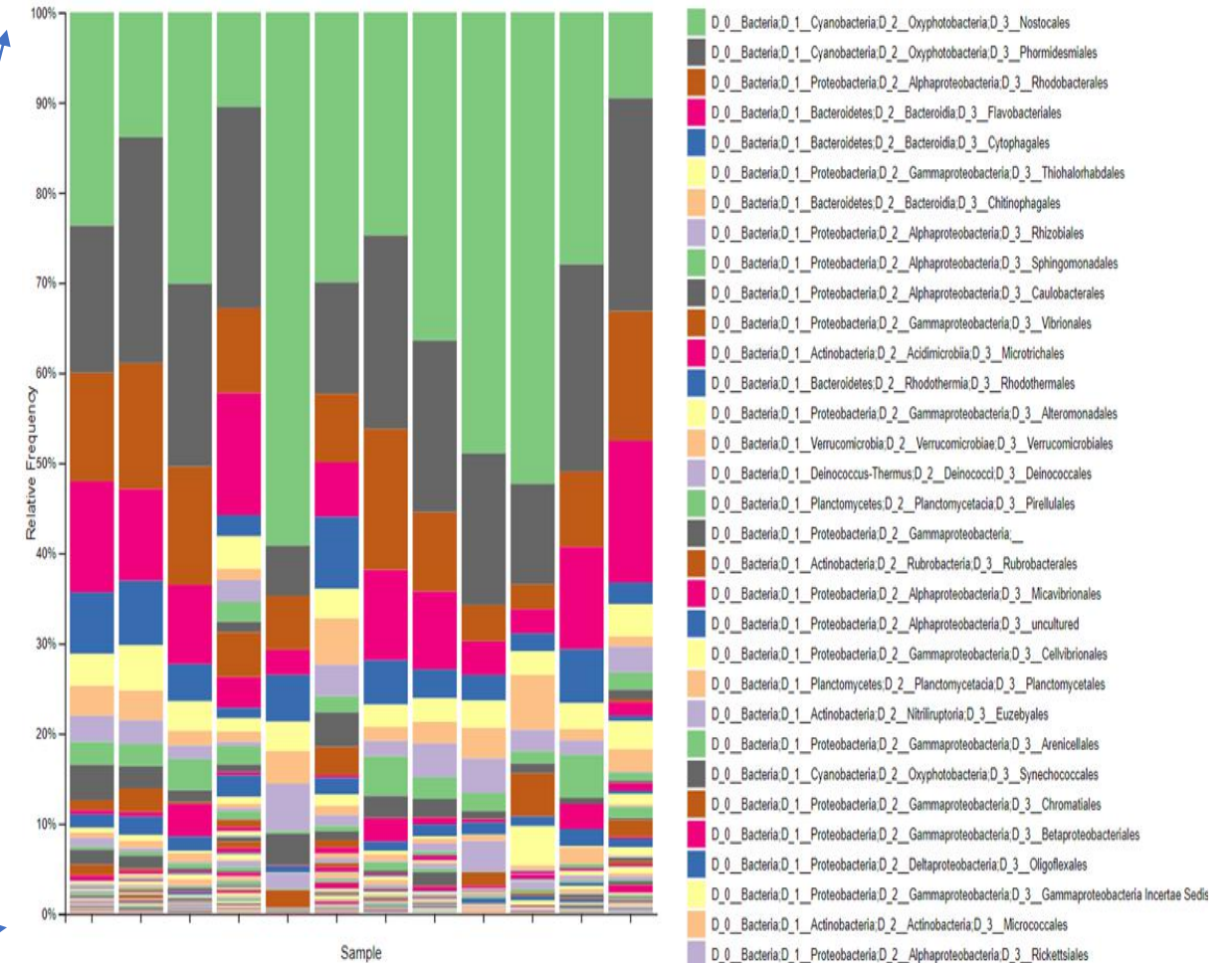
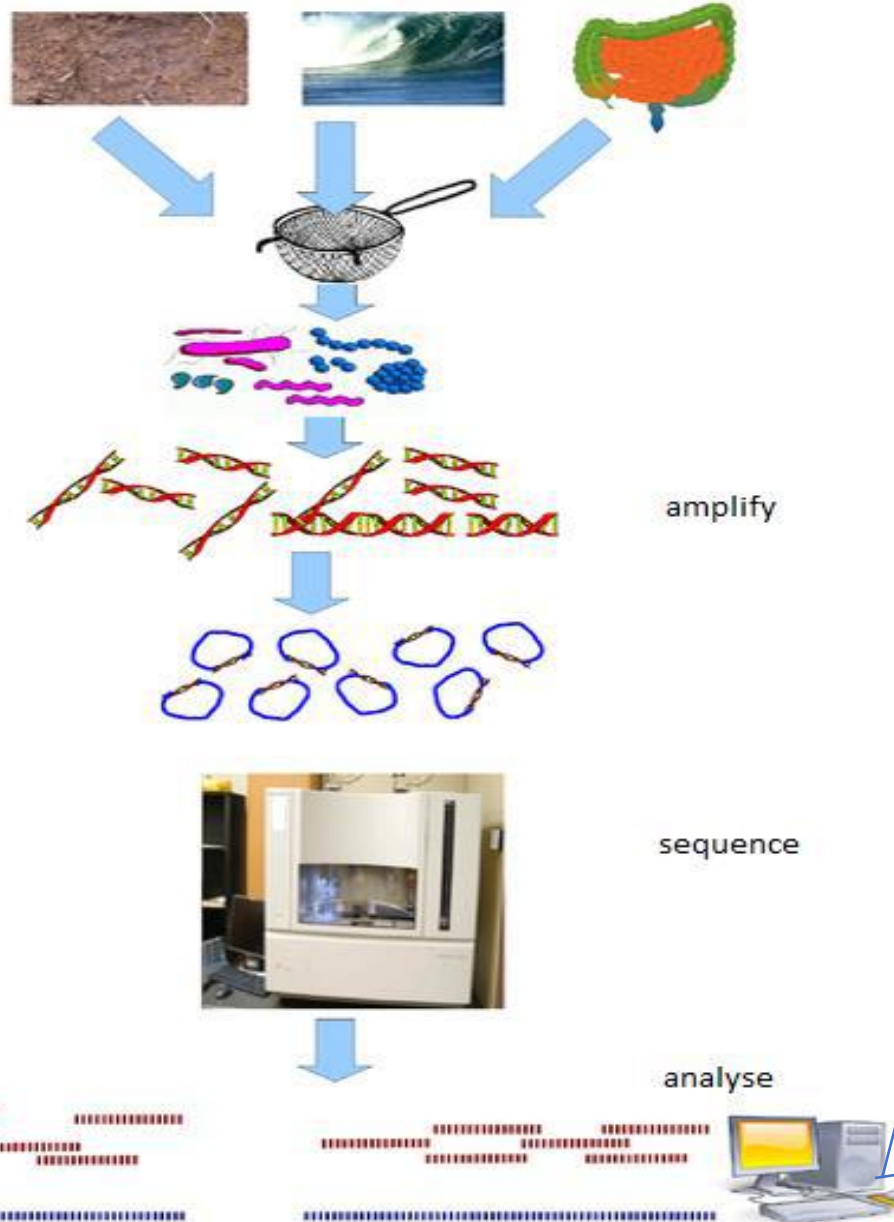


Sample analysis

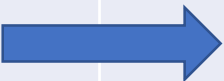
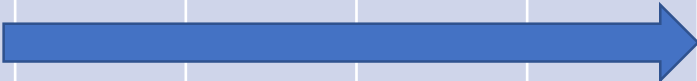

- Samples are going to be analysed at South African Institute for Aquatic Biodiversity (SAIAB). Illumina-MiSeq high-throughput sequencing is going to be used for analysing using meta-barcoding approaches
- Bacteria: 16S rRNA
- Eukaryotes: 23S rRNA



Metagenomics will be conducted using SAIAB platform.



Timeline

Activity	2021				2022				
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
Proposal writing									
Data collection									
Thesis writing									

The End