eDNA kit testing & sampling with local communities and stakeholders

Ocean Revolution Mozambique



Figure 1: Map of Inhambane bay and the MPAs

Ocean Revolution Mozambique (ORM) is a Non-Governmental Organisation that focuses on effective interactions between indigenous people and other stakeholders in Mozambique. ORM's work is centred around Inhambane bay and the Inhambane Bay Community Conservation Network (IBCCN), a unique community-led network of nine no-take marine protected areas (MPAs) encompassing important seagrass and mangrove habitats. These habitats are vital for supporting fish populations that provide a sustainable food source for the local communities, but they are also fragile and threatened by factors including coastal development, pollution from agricultural run-off, and over-exploitation. Overfishing has increased in recent years, coinciding with erosion of traditional rules on where and when to fish, which was historically determined by the village elders. Further details are given on the Marine Conservation Institute webpage.

Ocean Revolution works closely with the IBCCN to facilitate links between the communities themselves and scientists from the National University Eduardo Mondlane and elsewhere, combining local knowledge and modern science for effective community-led marine conservation. NatureMetrics first visited Inhambane in October 2019, and discovered a strong interest in improved monitoring tools on the part of both ORM and the communities themselves.

During Phase 1 of this project, NatureMetrics worked with ORM and the IBCCN to investigate the potential of eDNA data for helping the communities to better understand the distribution and abundance of fish inside and outside of the MPAs and to support the Community Fishing Councils in deciding how best to regulate fishing activity within the bay.

We aimed to address several key questions:

- Can fishermen and fisherwomen be trained to collect high quality eDNA samples, and can these skills easily be passed on?
- In what format should instructions be provided?
- Is there potential to empower women within the communities to help redress gender imbalance?
- How should the results from the eDNA analysis be reported to the communities so as to be informative and useful for decision-making?

Can fishermen and fisherwomen be trained to collect high quality eDNA samples, and can training easily be passed on?

Since NM could not visit Inhambane due to the COVID-19 pandemic, this was tested earlier than anticipated. The primary language spoken among the communities is Gitonga, so training was first passed from NM to ORM in Portuguese and then from ORM to the communities in Gitonga.

Once ORM received the sampling kits, NM staff trained ORM staff how to use them via live video. Overall, this worked well, even though the connection was poor out at sea.

Next, ORM passed on the training to community members through direct training workshops. Seven community champions were selected from the villages around Inhambane bay (Table 1).

Table 1: Community champions for eDNA selected from the IBCCN

Community	Champion(s)
Guiduane	Hortência Dércio Joaquim Anselmo António Inguane
Mucucune	Nilza Joaquim Hélio Pascoal
Nhampossa	Ilda Godido Cipriano Godido Cipriano Junior
Morrumbene-Jogó	Lúcia Amosse

Community champions are non-paid spokespersons that have two main roles: to collect samples and to share the results with the wider communities and the fishing councils so that evidence-based decisions can be made regarding what to fish.

In total, 29 eDNA samples were collected by ORM and the community champions in Inhambane bay. Half of the samples were collected in July 2020 and half in September 2020.

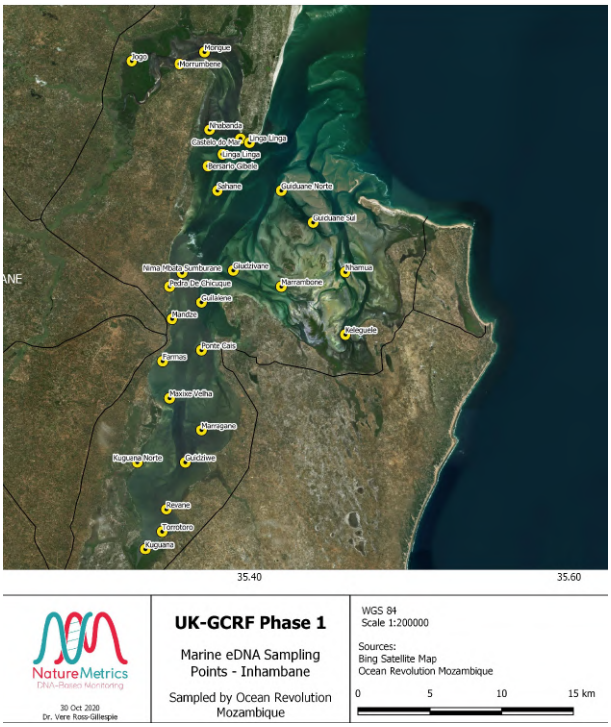

Figure 2: map showing the sampling locations in Inhambane Bay

Valuable feedback was obtained, which has led to modification of the sampling kits. The two main user concerns were:

1. The instability of the water collection bags, which often spilled or leaked while being handled and transported on boats. During phase 2 we will trial alternative collection devices that are more suitable for use when sampling from small boats.
2. The amount of plastic waste. We have now run trials to test whether we can reduce the amount of single-use packaging on the kit components. Results suggest that we can provide unwrapped components in kits going forward, reducing the environmental impact of our solution.



Community champion in action

Work outside Inhambane

Building on the work in Inhambane and the success of the remote training, several other stakeholders were remotely trained in eDNA sampling using the NatureMetrics kits. These included staff from the National Institute for Fisheries Research (IIP), the Wildlife Conservation Society (WCS) and the Natural History Museum in Maputo.

After receiving the training, IIP scientists collected 10 eDNA samples from a variety of lakes and rivers in Southern Mozambique, WCS staff collected 7 marine eDNA samples on the coast, and Erica Tovela at the Natural History Museum in Maputo collected 3 eDNA samples from a variety of coastal environments.

Table 2: details of the eDNA samples collected by stakeholders in Mozambique during Phase 1

NM ID no	Sampled by	Sample ID provided	Corrected Coordinates (DD)	Place	Sampler	Water type	Kit ID #	Sampling date	Volume filtered
8340	IIP	IIP_Chilembene		Chilembene	Luisa Simbine	Lake	WAT20-0291	31-Jul-2020	2000 ml
8332	IIP	IIP_Incomate_Manhica		Manhica	Luisa Simbine	River	WAT20-0290	31-Jul-2020	1800 ml
8343	IIP	IIP_Lago Bicene		Lago Bicene	Luisa Simbine	Lake	WAT20-0300	07-Aug-2020	1800 ml
8356	IIP	IIP_Maputo Especial Reserve		Maputo Special Reserve	Luisa Simbine	Lake	WAT20-0293	07-Aug-2020	2000 ml
8323	IIP	IIP_Massingir1		Massingir	Luisa Simbine	Lake	WAT20-0294	31-Jul-2020	800 ml
8334	IIP	IIP_Massingir2		Massingir	Luisa Simbine	Lake	WAT20-0296	31-Jul-2020	2000 ml
8360	IIP	IIP_Matola Rio		Matola	Luisa Simbine	River	WAT20-0302	29-Jul-2020	2000 ml
8354	IIP	IIP_Rio Maputo		Maputo	Luisa Simbine	River	WAT20-0289	04-Aug-2002	800 ml
8349	IIP	IIP_Sabie		Sabie	Luisa Simbine	River	WAT20-0299	03-Aug-2020	1800 ml
8345	IIP	IIP_Umbeluzi		Umbeluzi	Luisa Simbine	River	WAT20-0298	29-Jul-2020	2000 ml
8338	ORM_Sept	ORM_Morrumbene1	-23.7071, 33.3926	Morrumbene	Eugenio Tinga	Bay water	WAT20-0279	28-Sep-2020	1000 ml
8339	ORM_Sept	ORM_Sumburane	-23.8214, 35.3580	Nima Mbata Sumburane	Luiso	Bay water	WAT20-0125	28-Sep-2020	1000 ml
8355	ORM_Sept	ORM_Morrumbene2	-23.6907, 35.3564	Morrumbene	Domingos Mufemane	Bay water	WAT20-0281	28-Sep-2020	1000 ml
8348	ORM_Sept	ORM_Mandze	-23.8503, 35.3516	Mandze	Felix Liberato	Bay water	WAT20-0131	28-Sep-2020	1000 ml
8347	ORM_Sept	ORM_Guidzivane	-23.82, 35.39	Giudzivane		Bay water	WAT20-0270	28-Sep-2020	1000 ml
8359	ORM_Sept	ORM_Linga Linga	-23.7472, 35.3833	Linga Linga	Laurinda Mabalane	Bay water	WAT20-0124	28-Sep-2020	1000 ml
8341	ORM_Sept	ORM_Sahane	-23.77, 35.38	Sahane		Bay water	WAT20-0272	28-Sep-2020	1000 ml
8330	ORM_July	ORM-Jul_Bercario Gibele	-23.7545, 35.3741	Bersario Gibele	Toneco	Bay water	WAT20-0128	28-Sep-2020	1000 ml
8333	ORM_July	ORM-Jul_Guiduane Norte	-23.77, 35.42	Guiduane Norte		Bay water	WAT20-0268	28-Sep-2020	1000 ml
8320	ORM_July	ORM-Jul_Guiduane Sul	-23.79, 35.44	Guiduane Sul		Bay water	WAT20-0267	22-Jul-2020	1000 ml
8344	ORM_July	ORM-Jul_Guidziwe	-23.94, 35.36	Guidziwe		Bay water	WAT20-0276	22-Jul-2020	1000 ml
8327	ORM_July	ORM-Jul_Guilalene	-23.84, 35.37	Guilalene		Bay water	WAT20-0274	22-Jul-2020	1000 ml
8331	ORM_July	ORM-Jul_Castelo do Mar	-23.7376, 35.3944	Castelo do Mar	Horacio Jose	Bay water	WAT20-0133	22-Jul-2020	1000 ml
8350	ORM_July	ORM-Jul_Keleguele	-23.86, 35.46	Keleguele		Bay water	WAT20-0266	22-Jul-2020	1000 ml
8342	ORM_July	ORM-Jul_Kuguana Norte	-23.94, 35.33	Kuguana Norte		Bay water	WAT20-0280	22-Jul-2020	1000 ml
8352	ORM_July	ORM-Jul_Linga Linga	-23.74, 35.40	Linga Linga		Bay water	WAT20-0271	22-Jul-2020	1000 ml
8322	ORM_July	ORM-Jul_Marragane	-23.92, 35.37	Marragane		Bay water	WAT20-0275	22-Jul-2020	1000 ml
8326	ORM_July	ORM-Jul_Marrambone	-23.83, 35.42	Marrambone		Bay water	WAT20-0273	22-Jul-2020	1000 ml
8321	ORM_July	ORM-Jul_Maxixe Velha	-23.90, 35.35	Maxixe Velha		Bay water	WAT20-0278	22-Jul-2020	1000 ml
8358	ORM_July	ORM-Jul_Nhamua	-23.8210, 35.46	Nhamua		Bay water	WAT20-0265	22-Jul-2020	1000 ml
8318	ORM_July	ORM-Jul_Pedra De Chicuque	-23.83, 35.35	Pedra De Chicuque		Bay water	WAT20-0269	22-Jul-2020	1000 ml
8325	ORM_July	ORM-Jul_Ponte Cais	-23.87, 35.37	Ponte Cais		Bay water	WAT20-0277	22-Jul-2020	1000 ml
8319	ORM_Sept	ORM-Sep_Farmas	-23.8768, 35.3457	Farmas	Horacio Jose	Bay water	WAT20-0129	28-Sep-2020	1000 ml
8329	ORM_Sept	ORM-Sep_Kuguana	-23.9944, 35.3350	Kuguana	Toneco	Bay water	WAT20-0132	28-Sep-2020	1000 ml
8353	ORM_Sept	ORM-Sep_Torrotoro	-23.9835, 35.3454	Torrotoro	Luis Dozentos	Bay water	WAT20-0284	28-Sep-2020	1000 ml
8314	ORM_Sept	ORM-Sep_Mongue	-23.6833, 35.3719	Mongue	Luis Amosse	Bay water	WAT20-0282	28-Sep-2020	1000ml
8317	ORM_Sept	ORM-Sep_Nhabanda	-23.7319, 35.3750	Nhabanda	Horacio	Bay water	WAT20-0127	28-Sep-2020	1000 ml
8315	ORM_Sept	ORM-Sep_Jogo	-23.6889, 35.3264	Jogo	Sergio Ricardo	Bay water	WAT20-0283	28-Sep-2020	1000 ml
8324	ORM_Sept	ORM-Sep_Revane	-23.9695, 35.3480	Revane	Toneco	Bay water	WAT20-0130	28-Sep-2020	1000 ml
8357	WCS	WCS_Ponta do Ouro3		Ponta do Ouro	Jorge, Eleuterio, Naseeba	Open sea	WAT20-0304	06-Aug-2020	2000 ml
8351	WCS	WCS_Baia Mall	-25.9449674, 32.3200388	Baia Mall	Jorge & Naseeba	Bay water	WAT20-0292	29-Jul-2020	2100 ml
8346	WCS	WCS_Bairro dos pescadores	-25.9007891, 32.6548755	Bairro dos pescadores	Jorge & Naseeba	Estuary	WAT20-0295	29-Jul-2020	2100 ml
8336	WCS	WCS_Macaneta2	-25.73994895, 32.6787923	Macaneta	Jorge & Naseeba	Open sea	WAT20-0288	29-Jul-2020	2000 ml
8337	WCS	WCS_Macaneta1	-25.8237193, 32.6723356	Macaneta	Jorge & Naseeba	Open sea	WAT20-0286	29-Jul-2020	2100 ml
8328	WCS	WCS_Ponta do Ouro1		Ponta do Ouro	Jorge, Eleuterio, Naseeba	Open sea	WAT20-0303	06-Aug-2020	1500 ml
8335	WCS	WCS_Pouta do Ouro5		Ponta do Ouro	Jorge, Eleuterio, Naseeba	Open sea	WAT20-0287	06-Aug-2020	2000 ml
8316		NO FIELD SHEET					?		

Results of the stakeholder sampling

Samples collected by stakeholders were returned to the UK for analysis. Because of the delay in shipping the samples while we arranged logistics and permits for export and import, most of the samples were stored at ambient temperature in Mozambique for a period of approximately 3 months and there was a concern that the DNA may have degraded during that time, reducing the viability of the samples for analysis.

Fresh samples were collected from Inhambane at the end of September shortly before shipping to the UK to provide a comparison.

DNA was extracted, amplified for fish and other vertebrates, and sequenced. DNA quality was good and the analysis was successful, **detecting 303 fish species across the 41 samples**. These include a huge variety of groups, from tropical reef fish to herrings and anchovies, mackerel, jacks, gobies, sand-eels, seahorses, flying fish, flatfish and many more.

Table 3: Detection of fish species in each group of samples. This preliminary analysis only includes the samples that sequenced without any troubleshooting (41 out of 48 samples)

			Number of samples	Total species	Average species	Max species
Inhambane July	July	ORM	13	128	28.9	49
Inhambane Sept	Sept	ORM	14	155	33.1	50
Ponto do Ouro	July	WCS	3	58	24.7	33
Maputo Bay	July	WCS	3	55	35.0	42
Freshwater	July	IIP	8	58	14.8	27

Only 86 fish species (28%) could be identified to species level due to incompleteness of the DNA reference library, as discussed above. 172 species (57%) could be identified to genus level and 284 (94%) to family level. As the reference database grows, we will be able to revisit this dataset and update it to add new species names where they are available.

Even without species names, an NMDS chart can show the extent to which different sampling locations are characterised by different communities of fish.

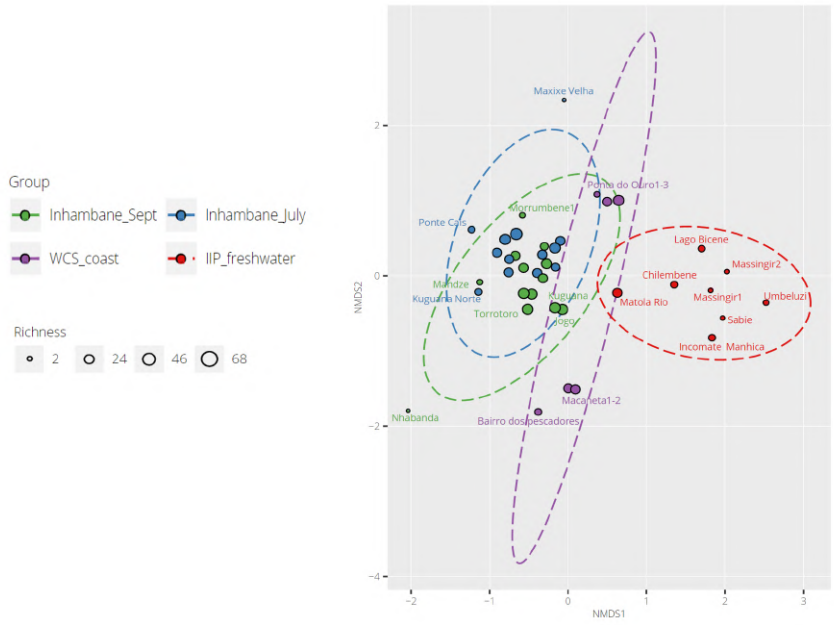

Figure 3: NMDS chart showing the 41 eDNA samples analysed. Samples are positioned according to the relative similarity of their fish communities. The closer together two points are, the more species they have in common. Points are coloured by location or sampler and the size of the points reflects the species richness of the sample.

The NMDS chart shows the relative similarity of the 41 samples in terms of the species they contained. This shows that:

- Samples collected in July and September in Inhambane contained a very similar set of species overall. This adds confidence to the overall approach by showing that the results are repeatable even when samples are collected several months apart.
- Samples collected in and around Maputo Bay (Bairro dos Pescadores and Macaneta) contained a similar set of species to one another, although the sample from Bairro dos Pescadores contained much fewer species. This sample was taken much closer to the centre of Maputo than the other two.
- The samples in red are the ones collected by IIP in a range of freshwater habitats. The sample from Matola Rio was the only one influenced by the marine environment as it was taken from close to the river mouth. This sample contained some marine species as well as freshwater ones, and therefore lies closest to the marine samples on the chart.
- The three different marine habitats (Inhambane Bay, Maputo Bay and Ponta do Ouro) can be distinguished from one another by their fish communities, which supports our hypothesis that biological communities derived from eDNA samples can be used to classify marine habitats. In Phase 2 we will sample much more extensively and sequence DNA from more species-rich groups (e.g. invertebrates and mircoorganisms). We expect this to further strengthen these

types of analyses and enable differentiate between healthy and degraded habitats of the same type.

In addition to the fish species, humpback whale was detected in all three samples collected by WCS from the Ponta do Ouro sites at the southern extent of the Mozambique coastline - a point that lies on their migration route. A dolphin species was also detected in these samples, and this species was also detected in several samples in Inhambane, both in July and in September.

The three-month storage time does not appear to have compromised the quality of the samples, which is further evidence of the robustness of our system. This is extremely helpful to know, since it is likely that in some sampling locations in Phase 2 it will not be possible to transport samples quickly back to Maputo for shipping or analysis.