Identification of fish species in Tanzanian and Kenyan coral reefs using eDNA

Garcia Zuluaga Mariana¹, Vervenne Lynn², Mtonga Cretus Joseph Philipo², Rumisha Cyrus², Bergacker Stephen², Huyghe Filip² and Kochzius Marc²

- Department of Biology, Ecology and Evolution, Université de Liège E-mail: mariana.garciazuluaga@student.uliege.be
- ² Marine Biology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium

The **East African coastline** harbours coral reefs with diverse fish communities. These marine habitats, along with the fish communities occurring on them, are particularly sensitive to climate change. The health of these fish communities along the East African coastline is extremely important to artisanal fisheries, as many families rely on them for food and income. It is thus important to survey the fish diversity in this region and to monitor possible changes to implement effective conservation measures.

Environmental DNA (eDNA) analyses have great potential as a tool in **fish diversity surveys**, since they are less time consuming than traditional techniques (*e.g.* visual surveys), allow for **species-level identification** while removing observer biases and are rapidly becoming more affordable (Kumar *et al.*, 2019; Wang, 2021). Despite its advantages, eDNA is not yet widely used as a reliable measure of diversity due to certain drawbacks. One of them, is uncertainty on the genetic marker to be used. The COI marker has been widely used for DNA barcoding and as a consequence a large reference database is available (Wang, 2021). Compared to DNA barcoding, shorter sequences need to be used when analysing eDNA because high-throughput sequencing techniques are limited to < 300 bp fragments. Shorter COI primers designed for eDNA analysis were recently shown to perform less good than **12S rRNA primers** when actinopterygian fishes are targeted (Collins *et al.*, 2019). Compared to COI, a good reference library for **Western Indian Ocean** (WIO) **fishes** was not readily available for 12S. However, such a library has been compiled recently, currently comprising of 98 species belonging to 32 families.

Here, we will test the accuracy of 12S as a marker for eDNA analysis targeting WIO reef fish using a dual approach. First we will compare two sequencing techniques, **Illumina Next Generation Sequencing**, the platform of choice so far, as well as **Oxford Nanopore Technologies**. The latter is a faster and cheaper method than Illumina but is thought to suffer from higher error rates (Egeter *et al.*, 2022). We will compare the reliability and efficiency of both sequencing techniques for fish eDNA diversity surveys using the newly constituted reference library for the 12S marker. Once this has been established, the eDNA results will be compared to visual fish diversity survey techniques.

Collins, R.A., Bakker, J., Wangensteen, O.S., Soto, A.Z., Corrigan, L., Sims, D.W., Genner, M.J., Mariani, S., 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods Ecol Evol* 10, 1985–2001. https://doi.org/10.1111/2041-210X.13276

Egeter, B., Veríssimo, J., Lopes-Lima, M., Chaves, C., Pinto, J., Riccardi, N., Beja, P., Fonseca, N.A., 2022. Speeding up the detection of invasive bivalve species using environmental DNA: A Nanopore and Illumina sequencing comparison. *Molecular Ecology Resources* 22, 2232–2247. https://doi.org/10.1111/1755-0998.13610

Kumar, G., Eble, J.E., Gaither, M.R., 2020. A practical guide to sample preservation and pre- PCR processing of aquatic environmental DNA. *Molecular Ecology Resources* 20, 29–39. https://doi.org/10.1111/1755-0998.13107

Wang, S., Yan, Z., Hänfling, B., Zheng, X., Wang, P., Fan, J., Li, J., 2021. Methodology of fish eDNA and its applications in ecology and environment. *Science of The Total Environment* 755, 142622. https://doi.org/10.1016/j.scitotenv.2020.142622

Keywords

EDNA; Fish Diversity Surveys; East African Coastline; Species Identification; 12S Marker; Illumina Next Generation Sequencing; Oxford Nanopore Technologies