A preliminary study showed that the digest enzymes used in dT-RFLP for terrestrial assemblages separated poorly the marine nematodes at taxonomic level for functional group analysis. A new digest combination was designed using the software tool DRAT (Directed Terminal Restriction Analysis Tool) to distinguished marine nematode taxa. Several solutions were provided by DRAT and tested empirically to select the solution that cuts most efficiently. A combination of three enzymes and a single digest showed to be the best solution to separate the different clusters.

Parallel to this, another tool is being developed to estimate the population size (qPCR). An improvement in qPCR estimation of gene copy number using an artificial reference is being performed for marine nematodes communities to quantify the abundance. Once developed, it is proposed to validate both methodologies by determining the spatial and temporal variability of benthic nematodes assemblages across different environments. The application of these high-throughput molecular approaches for benthic nematodes will improve sample throughput and their implementation more efficient and faster as indicator of ecological status of marine ecosystems.

DNA barcoding: an effective tool to overcome morphological identification constraints in the assessment of the ecological quality

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DNA barcoding has the potential to overcome taxonomic challenges in biological community assessments. However, fulfilling that potential requires successful amplification of a large and unbiased portion of the community. In this study, we attempted to identify mitochondrial gene cytochrome c oxidase I (COI) barcodes from 1024 benthic invertebrate specimens belonging to 54 taxa from low salinity environments of the Mira estuary and Torgal riverside (SW Portugal). Up to 17 primer pairs and several reaction conditions were attempted among specimens from all taxa, with amplification success defined as a single band of approximately 658 bp visualized on a pre-cast agarose gel, starting near the 5' end of the COI gene and suitable for sequencing. Amplification success was

achieved for 99.6% of the 54 taxa, though no single primer was successful for more than 88.9% of the taxa. However, only 68.5% of the specimens within these taxa successfully amplified. Inhibition factors resulting from a non-purified DNA extracted and inexistence of species-specific primers for COI were pointed as the main reasons for an unsuccessful amplification. These results suggest that DNA barcoding can be an effective tool for application in low salinity environments where taxa such as chironomids and oligochaetes are challenging for morphological identification. Nevertheless, its implementation is not simple, as methods are still being standardized and multiple species-specific primers are required at present to achieve amplification success.

Refaunation and the reinstatement of the seed dispersal function in Gorongosa National Park

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Seed dispersal is a vital step for plant reproduction and long-term vegetation dynamics, and many plants rely on animals for this process. Large animals are disproportionally important dispersers, however they tend to be under a higher extinction risk worldwide. There is compelling evidence that the global biodiversity crisis is leading to the deterioration of several ecosystem functions, including that of seed dispersal. However, there is virtually no information on how large-scale refaunation efforts can reinstate seed dispersal. We evaluated the effectiveness of a 62 km2 wildlife sanctuary, aimed at recovering large mammals' populations in the Gorongosa National Park (Mozambique), in restoring seed dispersal interactions. The sanctuary provided a unique natural experiment to test the effect of large-mammals' refaunation on this key ecosystem function. DNA barcoding was used to identify seeds, when it was not possible visually. The results reveal a higher diversity of dispersers inside the sanctuary that translates into a more diverse, larger and more complex seed-dispersal network. The higher number and diversity of seeds dispersed inside the sanctuary was explained mostly by the greater disperser's abundance, rather than by their identity. Overall, the seed dispersal network inside the sanctuary was less specialized (>H2') and there was a greater overlap on the plant species dispersed by all animals. Both networks were significantly modular and anti-nested. Our findings